QSAR STUDY OF IMMUNOTOXICITY IN ANTIBIOTICS

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THE FOLLOWING PUBLICATIONS, FIGURES, PLATES AND TABLES HAVE BEEN EXCLUDED ON INSTRUCTION FROM THE UNIVERSITY.

APPENDIX 8

FIG 2.12 P15, TABLE 2.1 P17, FIG 6.3 P72, FIG 6.4 P74. FIG 6.5 P75, FIG 6.7 P80, FIG 6.9 P85, TABLE 7.1 P89

"I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of science whatever the matter may be."

Lord Kelvin

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Abstract

Since their inception the β-lactam antibiotics have become one of the most important classes of pharmaceutical agents, both therapeutically and economically, in modern day usage for the treatment of a wide spectrum of bacterial infections. However, due to the versatility of bacteria many previously treatable species are developing resistance to the antibiotics currently available and so there is ever a need to develop more β-lactam antibiotics, which are effective and yet safe.

A major drawback to the β-lactams is the degree of immunologically adverse reactions they induce.

It was the aim of this study to develop both mechanistic and immunological methods to enable the prediction of a β-lactam's potential to induce an allergic response and to determine if a relationship between these responses and the molecular properties of the β-lactams was present.

In this study a database pertaining to frequency by which 70 β-lactams induce adverse reactions has been compiled and used to produce 27 QSAR models.

A highly sensitive assay for the quantitation of cross-reactivity between β-lactams and serum anti-benzylpenicillin antibodies has been developed and used to determine the cross-reactivity potential of 31 β-lactams and to develop 18 QSAR models.

All of the QSARs developed suggest that the shape and electron separation of the β-lactams are crucial to the development and extent of adverse response or cross-reactivity induced by a specific β-lactam antibiotic, new or old.

The QSARs developed will enable the design and development of new β-lactam antibiotics which present a significantly lower risk of inducing immunologically mediated adverse responses when used therapeutically.

Two sensitive assays for the quantitative determination of the cytokines IL2 and IL4 in lymphocyte culture supernatants have been developed, and have been shown to have a potential use in the prediction of the type of immunological response initiated following β-lactam stimulation of a sensitised individual.

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1.0 General Introduction

The β-lactam antibiotics remain the singularly most important class of antibiotics in modern day use. Despite a wide therapeutic index the β-lactams have a degree of toxicity [1] which manifests primarily as allergic reactions, with immediate hypersensitivity constituting the most dramatic and distressing form of allergic reaction. The development of new β-lactam antibiotics therefore, raises the following questions:

- Is the new drug potentially as sensitising as other drugs currently available?
- Will the new drug cross-react with other established β-lactams and so elicit allergic reactions in an already sensitised person? [2].

The answers to these questions lie in the evaluation of the immunotoxicity of existing and new β-lactam antibiotics.

Immunotoxicity is a consequence of the interaction of a chemical agent with the immune system, which leads to the development of a specific immune response. The manifestations of this may be through immunoglobulins (IgE), antibody or immune complex, cell-mediated tissue damage or cell-mediated reactions including contact sensitisation. All involve an immune response to the drug itself, to a bio-transformation product or to modified host tissue giving rise to neo-antigens [3]. The tissue damage mediated by these pathways should be recognised in pre-clinical studies and thus trigger the need for immunotoxicological function testing.

Recent advances in biotechnology coupled with those in molecular biology, have enabled the specific production of monoclonal antibodies, the use of which has vastly improved the assay systems available for immunological studies, and have led to a better understanding of the immune system and the role of cytokines in the induction and direction of immune responses. This has resulted in a large number of immunoregulatory cytokines and growth factors being identified. Thus, analysis of cytokine production by immune cell populations, following exposure to an allergen, could be applied in the testing of immunological function.

The competitive nature of the pharmaceutical industry demands methods of drug design and development that are quick and reliable, yet which produce safe and effective compounds. Initial strategies for the immunological assessment of the toxicity and safety of medicines have been to select a tiered panel of assays that will identify the immunotoxicological potential of a specific chemical agent [4].

Limited effort has been made within the field of immunotoxicology to compare, refine

and validate assays [4], so as to provide methodologies which ensure that reasonable compounds are developed, dubious compounds are tested more fully and undesirable compounds are eliminated from future testing. Thus leading to the production of common valid assays for use as screening tools, so leading to the conservation of the financial resources required for exhaustive testing, yet ensuring safety and toxicity are still investigated thoroughly [5].

Dewdney and Edwards [6] proposed that there are two general approaches for the pre-clinical assessment of a drug allergen's potential immunotoxicity, currently being evaluated. One is based on the expectation that it will be possible to develop animal models which mimic clinical adverse reactions. The second is a mechanistic approach, which assumes that a low molecular weight drug, such as a β-lactam antibiotic, possess one or more physico-chemical properties that are responsible for the induction of an immunotoxic reaction.

The EEC (1984) and OECD (Organisation for Economic Co-operation & Development) (1993) guidelines recommend that the assessment of the structural properties of allergens should be used where possible to assess potential immunotoxicity [7].

As immunotoxicity is a biological activity it should, in principle, be possible to utilise quantitative structure-activity relationship (QSAR) techniques in conjunction with immunological tests to provide a means of performing a mechanistic assessment of immunotoxicity. Thus permitting the identification not only of those structures responsible for the activation of an immunotoxic response but also the direction of the immune response and of those properties of the β-lactams themselves which are responsible for this directional switching [7].

This study has shown that it is possible to develop immunotoxicological assays and to generate QSAR models which can be used as screening tools. Extrapolation of the results attained and identification of their clinical relevance will enable the prediction of simple structural changes that will allow the pharmaceutical industry to design and develop new and safer β-lactam antibiotics [8].

2.0 β-Lactam Antibiotics

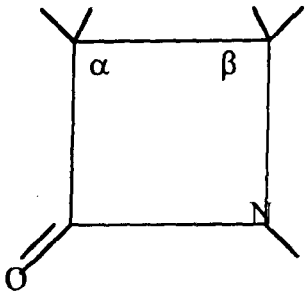
2.1 Introduction

Benedict and Langlykke [9] define an antibiotic as "a chemical compound derived from or produced by a living organism, which is capable, in small concentrations, of inhibiting the life processes of micro-organisms."

The β-lactam antibiotics are therapeutic agents used as the first line of treatment against bacterial infections and as such have become clinically and economically, the most important class of pharmaceutical agents in modern usage.

All β-lactam antibiotics contain, as the name suggests, a β-lactam ring structure (figure 2.1), this being a four membered ring which contains an amide functional group. The β suffix stems from the fact that the labelling of the lactam ring carbons (α and β), from the functional group means that the β carbon is that carbon attached to the nitrogen function.

Figure 2.1 The β-Lactam Ring



Naturally occurring β-lactam antibiotics are produced by a wide variety of micro-organisms, including numerous species of *Streptomyces*, *Nocardia*, a few filamentous fungi and some unicellular bacteria.

The natural and scientific interest in antibiotics, as well as their commercial success, has led to the identification and isolation of thousands of compounds. However, to be a useful antibiotic each compound must be significantly effective against a specific pathogen or group of pathogens without producing a significant toxic side effect. Each compound must also be stable so that it can be isolated, processed and stored for a reasonable length of time prior to use. Each processed compound must also be produced in an active dosage, amenable for absorption, detoxification and elimination, and which when administered at appropriate intervals will maintain therapy, but which upon cessation of administration is rapidly eliminated from the host. These constraints mean,

therefore, that of the thousands of likely antibiotics isolated only a few become medically available, but these few have been extremely successful and have made the antibiotics the most important products of the modern pharmaceutical industry.

The β-lactam antibiotics can be divided into five distinct classes: penicillins, cephalosporins, clavulanic acids, monolactams and novel β-lactam containing structures. The penicillins and cephalosporins are the most effective classes in current use for the treatment of infectious diseases.

2.2 Molecular Mode of Action

The majority of penicillin and cephalosporin analogues appear to employ an identical antibacterial mode of action in which they interfere with the biosynthesis of the peptidoglycan component of the bacterial cell wall [10]. The molecular mode of action of the β-lactam antibiotics is unique and well established [11]. Their action is to inhibit the peptidoglycan transpeptidation step of bacterial cell wall synthesis by inactivating the DD peptidase and DD carboxypeptidase enzymes involved in the process [11,12,13,14].

2.21 Normal Situation

Most prokaryotic cells are surrounded by a cell wall which is responsible for giving the bacteria their shape and for allowing them to survive in hypotonic environments. The bacterial cell wall of both Gram positive and Gram negative bacteria is composed of two layers of peptidoglycan (also called murein sacculus), a carbohydrate backbone, from which penta-peptide side chains of Ala-Gly-Lys-Ala-Ala extend. The wall is strengthened due to cross-linking between two adjacent murein layers (figure 2.2), in which the lysine of one penta-peptide attaches to the penultimate alanine of a penta-peptide in an adjacent murein backbone, with the removal of the terminal alanine. The reaction mechanism of cross-linking occurs in two phases (figure 2.3). This cross-linking gives a third dimension so strengthening the cell wall, holding the cell rigid and protecting it against osmotic rupture. The whole process is under the control of the transpeptidase enzymes.

Figure 2.2 Diagrammatic Representation of the Bacterial Cell Wall

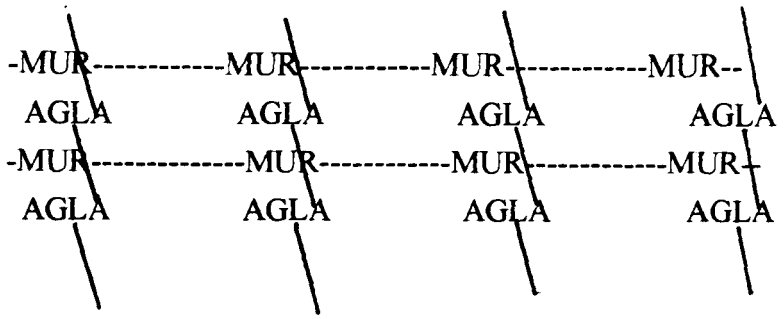
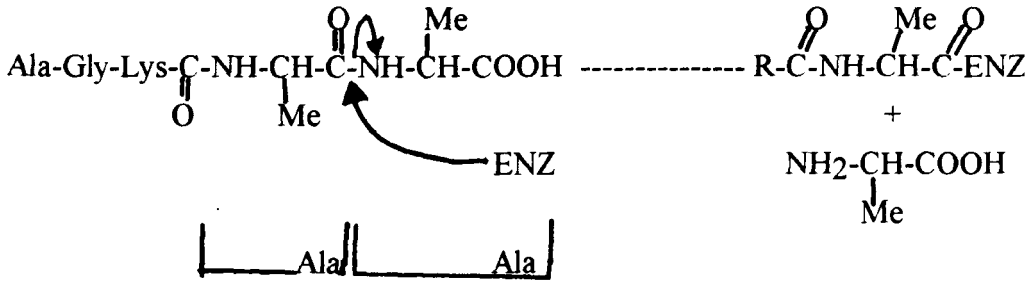


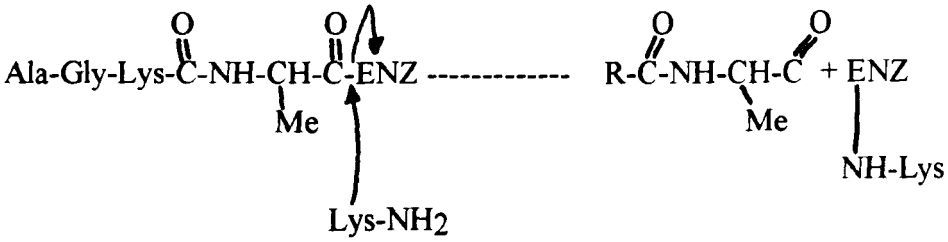
Figure 2.3 Mechanism of Normal Bacterial Cell Wall Synthesis

Phase 1:



Transpeptidase recognises two adjacent alanine amino acids and acts as a nucleophile, attaching to the carbonyl carbon atom of the penultimate alanine, thus causing the breakdown of the C-N linkage between the two alanines, so liberating the terminal alanine.

Phase 2:

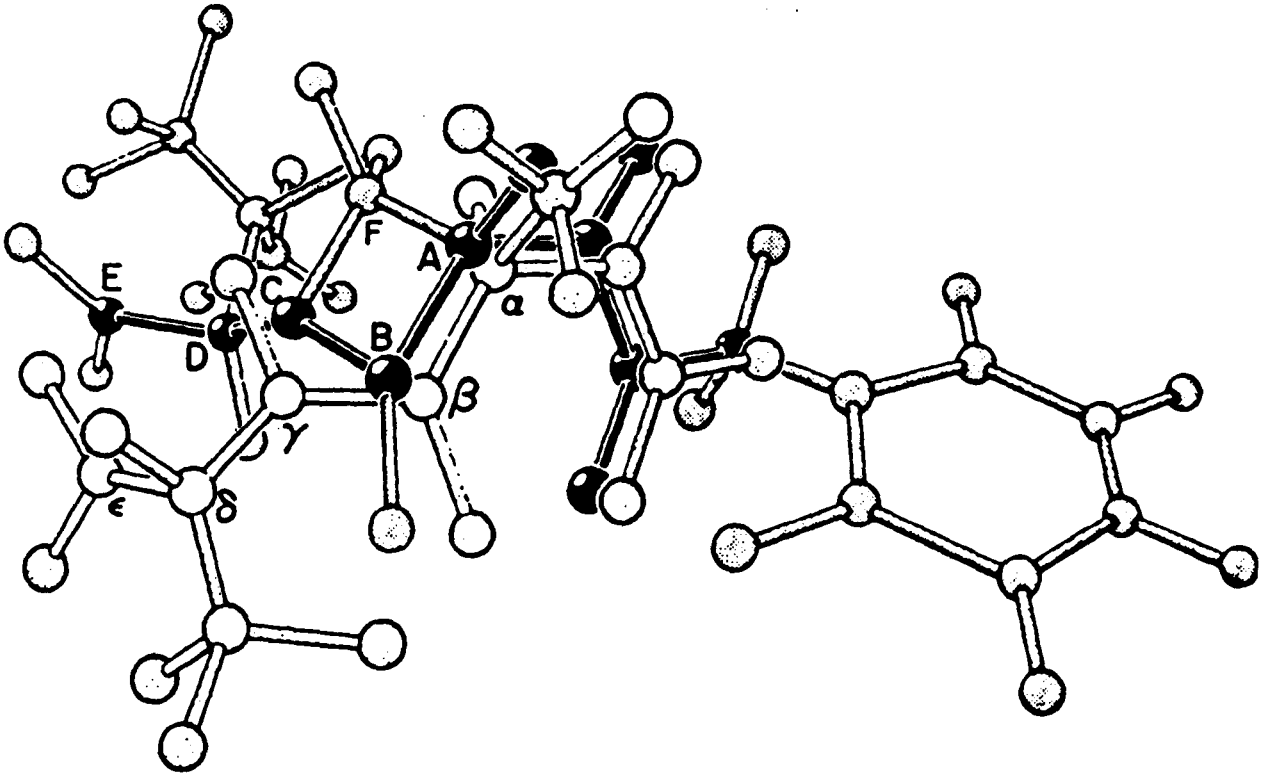


Lysine is a basic amino acid, the NH_2 group of which possess a lone pair of electrons, thus allowing it to behave as a nucleophile and form a linkage with the alanine carbonyl carbon atom on an adjacent pentapeptide. The enzyme is a better leaving group than lysine and so the linkage between alanine and the enzyme breaks readily with the free enzyme being displaced.

2.22 Action of the β-Lactams

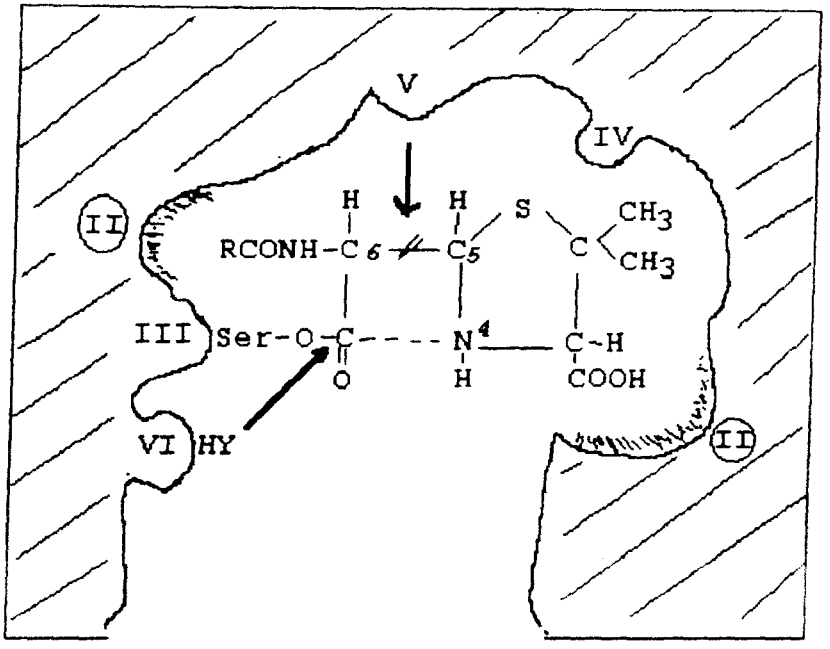
Tipper and Strominger [11] proposed that the β-lactam antibiotics are structural analogues of the C-terminal acyl-D-ala-D-ala component of one peptidoglycan strand. Park and Strominger [15] illustrated (figure 2.4) that the β-lactam antibiotics do in fact resemble the ala-ala couple present in the pentapeptide of the bacterial cell wall and that the carbonyl group of the β-lactam ring corresponds to the active carbonyl group in the centre of the pentapeptide ala-ala couple. Therefore, when a β-lactam antibiotic is introduced into the bacterial environment, the transpeptidase enzymes (DD-peptidases and DD-carboxypeptidases) are unable to distinguish between their natural substrate (the pentapeptide) and the β-lactam antibiotic. The β-lactams are very strained structures, and the effects of resonance within the structure cause the carbonyl carbon of the β-lactam to become more reactive than that of the natural ala-ala substrate couple. The enzyme attains a greater affinity for the carbonyl carbon of the β-lactam and thus reacts more readily with it than with that of the natural substrate. The hydroxyl group of a serine residue in the active site of the transpeptidase enzyme (figure 2.5) reacts with the carbonyl carbon of the β-lactam, the C-N bond within the β-lactam ring is cleaved and the enzyme binds to the carbonyl carbon atom liberated by the ring cleavage (figure 2.6). The bound β-lactam then forms a covalent bond with a serine residue at the enzymic active site and the enzyme becomes acylated and inactivated, becoming irreversibly bonded to the β-lactam and forming a β-lactam tetrahedral-acyl enzyme complex; hence the enzyme is rendered unable to play any part in further reactions and so bacterial cell wall synthesis becomes inhibited.

Figure 2.4 Diagrammatic Representation of the Similarity Between β-Lactams and the Natural Substrate of the Transpeptidase Enzymes
(Adapted from Rando [16])



The molecule labelled α,β,χ,δ,ε is the dipeptide and A,B,C,D,E is the corresponding portion of benzylpenicillin.

Figure 2.5 Diagrammatic Representation of Possible Enzymic β-Lactam Binding Sites
(Adapted from Ghuysen et al [17])



KEY:

II = Activation sites. Interact with β-lactam acyl side chain or peptide L-R_3 residue.

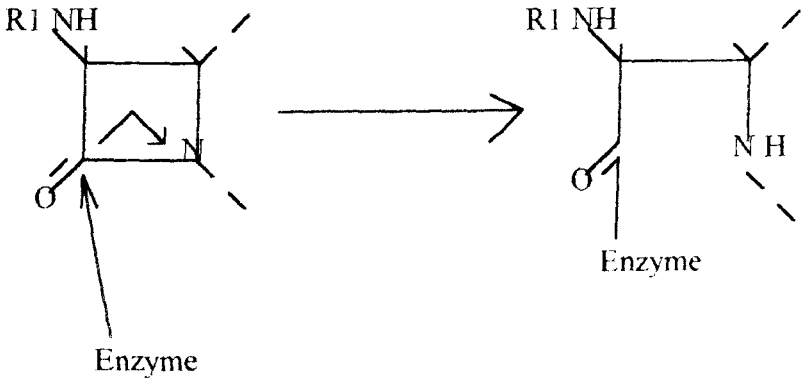
III = Fixation or acylation site. Nucleophilic attack of β-lactam C_7, or penultimate D-alanine residue carbonyl group by an enzyme serine residue.

IV = Stabilisation site. Interaction with the monocyclic thiazolidine part of molecule.

V = Fragmentation site. Catalyses cleavage of C_5-C_6 bond.

VI = Deacylation site. Catalyses the attack of penicilloyl-enzyme or substrate-enzyme complexes of serine ester linkage by water or nucleophile.

Figure 2.6 Illustration of the Molecular Mode of Action of the β-Lactam Antibiotics



2.3 Penicillins

2.31 Structure and Features

The penicillins are a class of β-lactam antibiotics with a common fused ring structure (figure 2.7) which comprises a β-lactam ring (A) fused to a sulphur-containing 5 membered ring, known as a 'thiazolidine ring' (B). An amide group with a variable group (R) is attached to the α-carbon of the β-lactam-ring. Two methyl groups and a carboxy group are attached to the thiazolidine ring.

2.32 History of Development

Penicillin was first discovered in 1928 by Alexander Fleming, who whilst working with *Staphylococci* bacteria noticed that a contaminating mould was actually killing the bacteria. This mould was identified as *Penicillium notatum*. In 1938 Florey, Chain and co-workers extended Fleming's initial findings by isolating, from surface cultures of *P.notatum*, a crude extract which contained antibacterial properties. Clinical applications of penicillin commenced in 1941, but use was limited due to problems in producing sufficient quantities of active extract. In 1943, with the discovery of 'Deep Culture' techniques, crude penicillin antibacterial mixes became widely available. The advent of X-ray crystallography allowed the structures of the antibacterial mix constituents to be determined. From the original antibacterial mix the four related compounds of penicillin X,F,G and K were isolated.

In November 1944, at a meeting of the Royal Society of Medicine, Florey made the prediction that "someday chemists will manipulate the penicillin molecule and improve its performance". Fulfilment of this prophecy came fifteen years later, with the isolation of the 6-amino-penicillonic acid (6APA) nucleus [18] and structural analyses which showed all penicillins as being derived from an enzymically catalysed hydrolysis reaction (figure 2.8) between 6APA and a carboxylic acid.

A drawback of the original isolate, penicillin-G, was its acid lability, which caused most of an oral dose to be destroyed in the stomach. The first acid-resistant penicillin discovered was penicillin-V, a natural product, first described in 1948, but not discovered as an antibiotic entity until 1953 [18]. Absorption was further improved with the production of the semi-synthetic phenoxy-penicillins; phenethicillin, propicillin and phenbenicillin. The next advance was the production of derivatives resistant to the destructive action of *Staphylococcus* penicillinase, e.g. methicillin, which therefore were effective in penicillin-resistant infections. This was followed by the production of the ixazole-penicillins; oxacillin, cloxacillin, dicloxacillin and flucloxacillin, which combined penicillinase and acid resistance.

Figure 2.7 Common Penicillin Structure

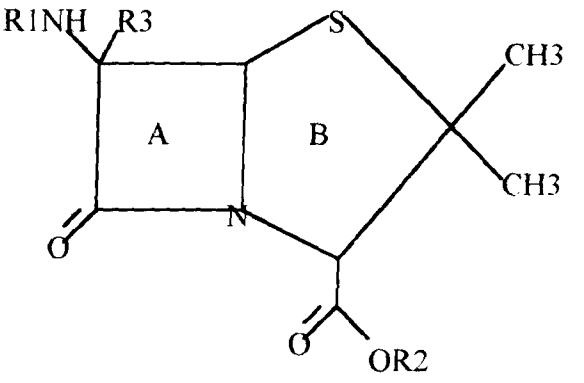
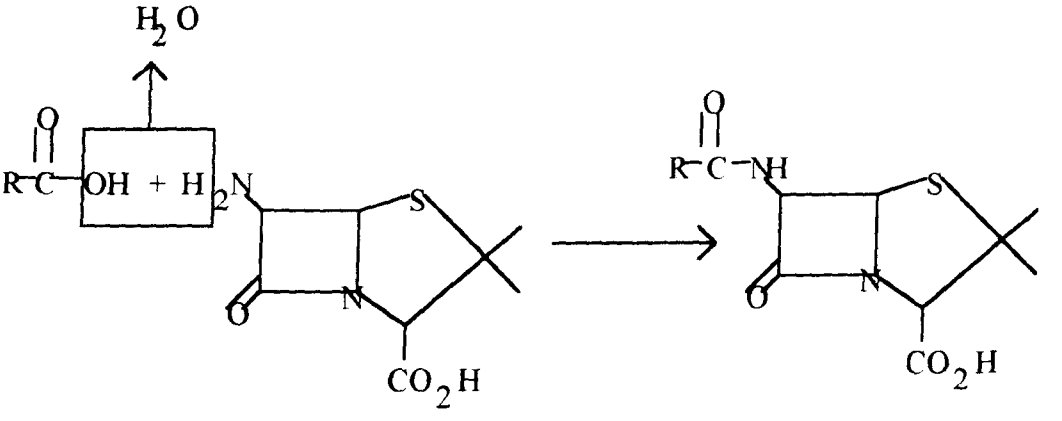


Figure 2.8 Formation of a Penicillin via Hydrolysis



Carboxylic acid

6APA

NEW penicillin

Another disadvantage of penicillin-G was its low degree of activity against Gram negative organisms, except *gonococci* and *meningococci*. The development of ampicillin represented a great advance in that its action against Gram positive organisms (including susceptibility to penicillinase) was similar to penicillin-G but it showed considerably greater activity against many Gram negative organisms.

Attachment of different acid chloride moieties to the acyl side chain of the 6APA nucleus coupled with advances in fermentation technology, has led to the semi-synthetic production of thousands of new penicillins, all of which possess different physico-chemical and antibacterial properties, including stability and activity. An illustration of the time scale of penicillin development is given in figure 2.12.

2.4 Cephalosporins

The discovery of cephalosporin antibiotics has provided a means of producing semi-synthetic β-lactam derivatives which are effective against bacterial species showing resistance to penicillin antibiotics.

2.41 Structure and Features

The cephalosporins are a class of β-lactam antibiotics which comprise a β-lactam ring fused to a sulphur-containing 6 membered ring, known as a 'dihydrothiazine ring' (figure 2.9). An amide group is attached to the α-carbon of the β-lactam-ring, and a methyl and a carboxy group are attached to the dihydrothiazine ring. Upto 4 variable side chains (R_1, R_2, R_3 and R_4 respectively) may be attached to the core structure, each of which affects cephalosporins' properties.

2.42 History of Development

The first cephalosporins were isolated from a mould *Cephalosporium acremonium*, cultivated in 1945 from a sewage outfall in Brotzu, Sardinia [18]. Florey found that the crude extract consisted of several antibacterial compounds including, cephalosporin-N (a penicillin) and a cephalosporin which resembled a steroid. Due to its occurrence in such low concentrations the presence of cephalosporin-C (a natural cephalosporin) was not discovered until 1953 [18].

Cephalosporin-C was shown to be resistant to *Staphylococcus* penicillinase (no penicillin at this time showed β-lactamase resistance) but it only had 10% of the antibacterial potency of the penicillins.

Twenty years elapsed before *Cephalosporium acreonium* was induced to give an adequate yield of cephalosporin-C, which could be used in further research and development.

It was discovered that all true cephalosporins comprise a common nucleus of 7-amino cephalosporanic acid (7ACA) (figure 2.9). However, production of new cephalosporins on an industrial scale, using processes similar to those used for the penicillins, was expensive. Eli-Lilly discovered it was possible to expand chemically the penicillin 6APA nucleus (which could be produced in vast quantities and cheaply) to produce the 7ACA nucleus for the semi-synthetic production of cephalosporins. Reaction of 7ACA and an acid chloride allowed the industrial production of a wide range of semi-synthetic cephalosporins (figure 2.10).

To date more useful semi-synthetic modifications of 7ACA nucleus have resulted from acylation of the 7 amino group with different acids or from nucleophilic substitution or reduction of the acetoxyl group. All semi-synthetic cephalosporins possess penicillinase resistance, have a wide range of antibacterial potency, differing spectra of activity, and a generally low toxicity, so making them a favourable therapeutic for use against bacterial infections in penicillin-allergic subjects [19].

Cephalosporins in clinical use can be placed into one of five categories (figure 2.11), based on β-lactamase resistance, metabolic stability and means of administration [20].

An illustration of the time scale of cephalosporin development is illustrated in figure 2.12.

Figure 2.9 Cephalosporin Common Structure and Structure Activity Relationships

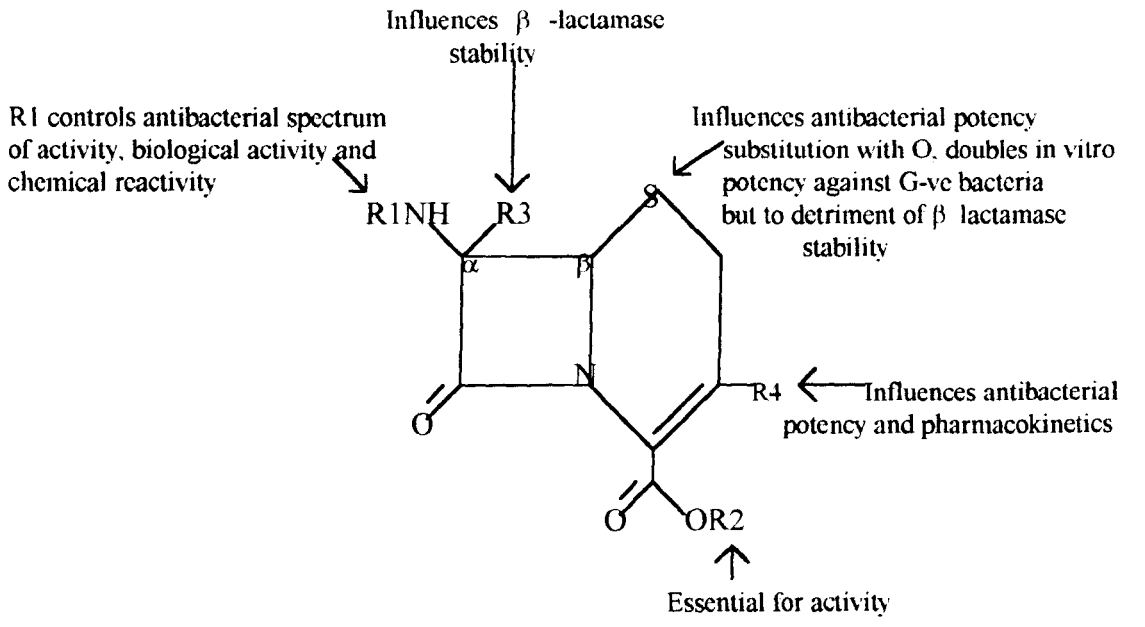
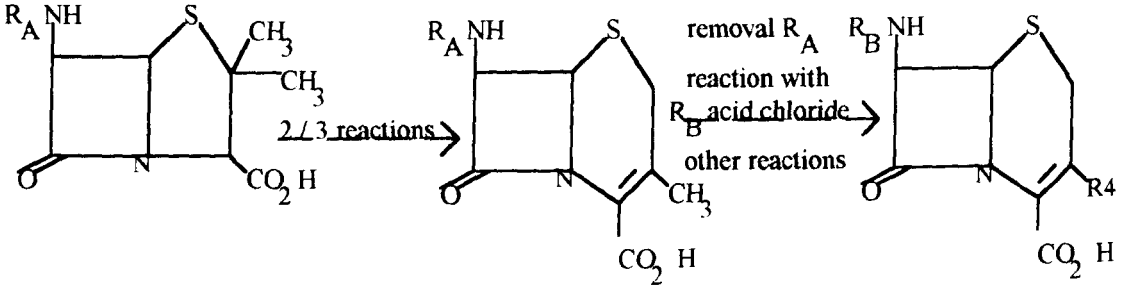


Figure 2.10 Outline of Cephalosporin Production



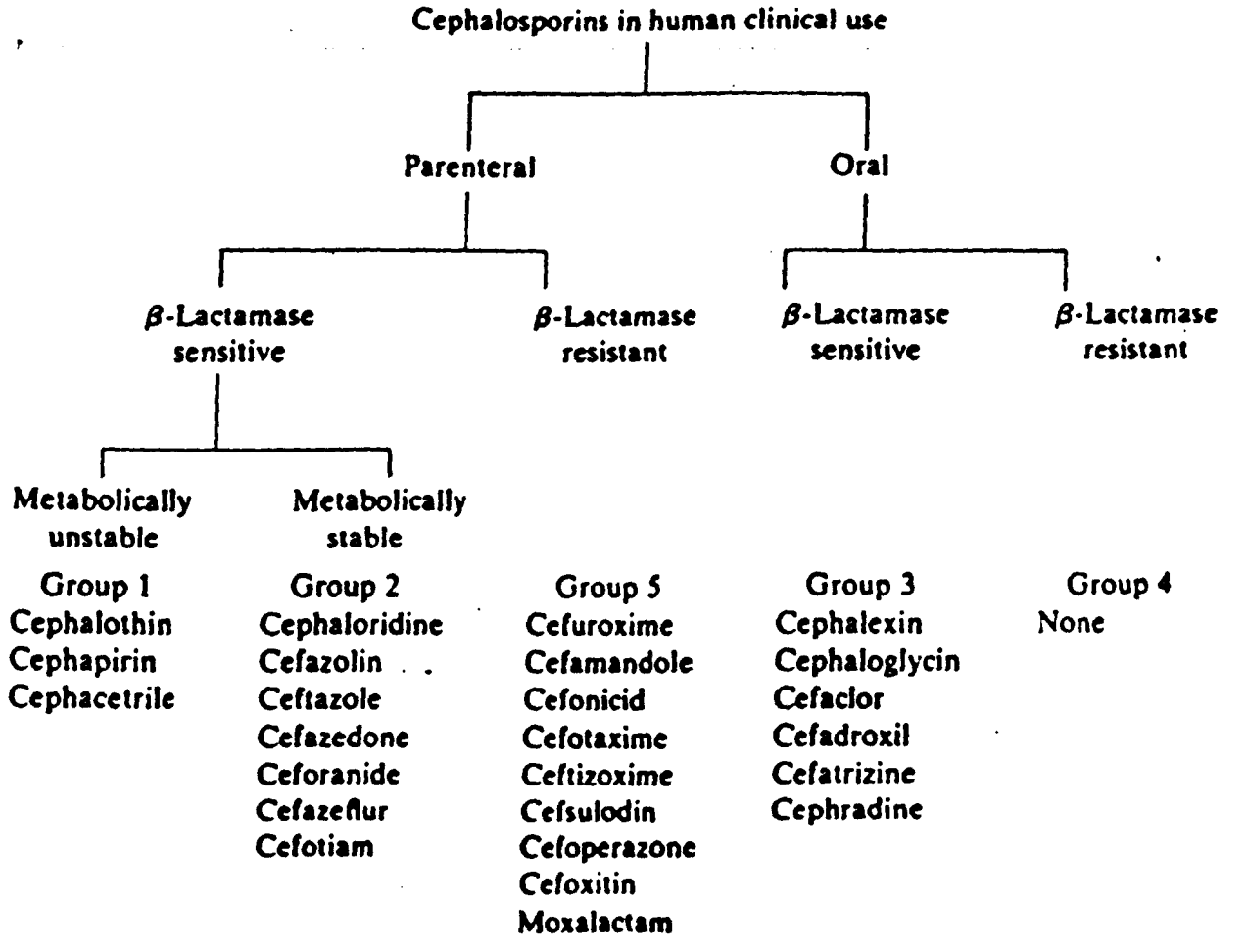
PENICILLIN

EQUIVALENT CEPHALOSPORIN

NEW CEPHALOSPORIN

Figure 2.11 Cephalosporin Classification
(Adapted from O'Callaghan [21])

NOTE: It was ruled in 1975 that new compounds with names derived from 'cephalosporin' should be spelt with 'f' instead of 'ph'



2.5 Drawbacks of β-lactams

2.51 Immunological Adverse Effects

Since their initial discovery the β-lactams have rapidly become one of the most commonly prescribed drug types in the world [23].

Compared with other groups of pharmaceutical agents the β-lactam antibiotics have a wide therapeutic index, a relatively low toxicity and are rarely associated with serious adverse reactions. However, the use of β-lactams in human medicine is not without its problems, namely the incidence of immunologically related adverse effects which they induce.

An adverse reaction can be described as 'any response to medication, which is undesired or unintended by the physician' [23].

The β-lactams are very potent immunogens, in that they are low molecular weight compounds and as such function as haptens irreversibly and covalently binding to tissue macro-molecules, such as proteins, forming an immunogenic multi-valent hapten-protein complex which acts to induce an immune response [1,25,26,27]. These responses range in severity from minor symptoms such as dry mouth, cutaneous rash or sleepiness to anaphylaxis, drug fever and potentially fatal damage to the bone marrow or liver. Table 2.1 lists some of the types of adverse reactions which have been reported following the therapeutic use of β-lactam antibiotics. Many of these adverse reactions are unlikely to involve immunologic reaction mechanisms, but of those which do, urticaria and maculopapular rashes are the most frequent and it is suspected that they are brought about through allergic reaction mechanisms. The incidence of immunologically mediated adverse reactions to β-lactam antibiotics constitutes a major clinical problem which complicates their medical management and hence limits their use, as a significant proportion of the general population is believed to be pre-sensitised to them [1].

2.521 Allergic Reactions

An allergic reaction is the process through which a xenobiotic provokes a specific immunological response which results in the development of a tissue damaging hypersensitivity reactions [28], and discussed in more detailed in 6.7.

Frequency of Allergy:

During the early years of penicillin therapy, allergic reactions generally affected the skin. As early as 1943, urticaria was observed in 2-3% of patients treated with penicillin. After 1944 the incidence increased to 6 to 8%. Following the introduction of crystalline penicillin, in 1947, the frequency seemed to level off at 1-2% [30].

Anaphylactic reactions are the most worrying type of allergic reaction, particularly when associated with drug administration, as they have the potential to be fatal [1]. Anaphylaxis due to penicillin was first reported by Cormia et al [31] in 1945, and the first reported death due to penicillin induced anaphylaxis occurred in 1949 [1]. Today, anaphylactic reactions occur in 0.004 to 0.015% of the patients receiving penicillin, with the incidence of fatal anaphylaxis being 0.0015 to 0.002% [1].

It is estimated that today between 0.7 and 10% of the population are allergic to the β-lactam antibiotics. Arreaza [32] proposed that possible reasons for this high prevalence of allergy can be attributed to: the widespread and sometimes indiscriminate use of the drug, to highly reactive degradation products which when combined with tissue proteins act as very powerful sensitising haptens, to extensive cross-reactivity among the β-lactam drugs which include semi-synthetic penicillins and to a lesser degree the cephalosporins, and to the frequent and yet inadvertent sensitisation of the population by "hidden" sources of antibiotics, such as milk (breast and animal), soft drinks, occupational hazards, mycotic infections, vaccines and *in utero* placental transfer.

2.52 Cross-Reactivity

The β-lactams have a high degree of structural similarity, both in their core nucleus and in the side chain structures. It is believed that it is because of these similarities that some β-lactams cross react with serum antibodies previously raised against anothers [33]. That is, that the antibodies raised against a β-lactam in pre-sensitised patients recognise and react with similar β-lactams, as if they were the sensitising antibiotic, thus giving rise to an allergic type reaction (as explained in 2.51 and 7.231).

The phenomenon of cross-reactivity between β-lactams in allergic patients is a complex problem involving many factors [34]. Although the frequency of allergic cross-reactivity amongst β-lactams, is not generally extensive and the extent differs between individuals, the clinical significance of cross-reactivity between different β-lactams is of concern and constitutes a serious problem with regard to the choice of a secondary antibiotic for administration to β-lactam-sensitive patients [35,36].

2.6 Future Developments

Bacteria are well known for being extremely versatile in their ability to circumvent the mechanisms by which antibiotics bring about their demise, e.g. penicillinase resistance. In the 1990's there has been a resurgence in the number of antibiotic resistant bacterial infections observed clinically [37]. There is therefore a continual need to design and develop new β-lactam antibiotics, for the effective treatment both of old and new forms of an infection. Increasing efforts are being made to develop new active compounds of both wider antibacterial spectra and potency, and diminished levels of toxicity [38].

The use of quantitative structure-activity relationship (QSAR) models pertaining to antibiotic potency, as developed by Irwin et al [39] and Hansch et al [40], in conjunction with those pertaining to toxic and cross-reactive potential (as derived in this work 9.0 and 10.0), will provided a means of identifying any relationships existing between the structural and / or physico-chemical properties of the β-lactams and their potential potency and toxicity Thus enabling the design and development of a new β-lactam antibiotics by modification of specific molecular features, in such a way as to enhance potency and limit its toxicity, whilst also, greatly reducing the use of animal tests and development costs.

3.0 Computational Chemistry

3.1 Concepts and Applications

In the 1980s a vast increase in the availability of computing power led to an increased use of computational chemistry in the area of drug design. Computational chemistry is basically the use of computer programmes to give quantitative predictions of chemical structure, properties and molecular behaviour, from a knowledge of the molecular structure. When allied with the growth in molecular graphics, computational chemistry has proved to be a valuable tool for the estimation of a synthesised or unsythesised molecule's three dimensional properties [41]. Information generated by computer is provided at a fraction of the time and a fraction of the cost needed, than if it were to be determined experimentally.

The assessment of molecular structure commences with the input of the chemical structure of the compound under investigation into the chosen software package, in a suitable format e.g. SMILES code, Cartesian co-ordinates or manual graphical entry. Calculations are then performed using mathematical models derived from the classical theories of bonding and atomic behaviour, to produce the most geometrically stable arrangement of the atoms within the molecule and to calculate the energies associated with the 'minimised' conformation of the molecule. The energy calculation is the basis of all molecular modelling as energy is inversely proportional to stability (equation 3.1).

$$\text{Energy} \propto {}^{1}/_{\text{Stability}} \qquad (3.1)$$

There are two major advantages in the use of computational chemistry;

1- it provides the ability to produce meaningful results easily on not yet synthesised chemicals or even on chemicals that can not exist under real conditions, as well as for those chemicals that are common-place in the laboratory.

2- vast amounts of information can be produced in relatively short periods of time, at minimum expense.

There are two basic approaches for the prediction of chemical structure and properties for a given molecule, Molecular Mechanics and Molecular Orbital Theory.

3.11 Molecular Mechanics (MM) [42,43]

MM or free field calculations regard the molecule as a collection of atoms (balls) held together by elastic or simple harmonic forces (springs). The forces are described in terms of potential energy functions of the bond lengths and angles, which when considered together describe the total potential energy of the molecule. Each potential energy function in combination with force field energy describes changes in the energy due to the deformation of structural features from the 'natural' condition.

Different computer programmes employ different sets of potential energy functions. In its simplest form the energy of a molecule in a force field, E can be represented in the form of equation 3.2.

$$E = E_l + E_a + E_t + E_{nb} \qquad (3.2)$$

Where E_l is the energy of a bond stretched or compressed from its natural length.

E_a is the energy due to bond deformation.

E_t is the torsional energy due to twisting about bonds.

E_{nb} is the energy due to non-bonded interactions, including steric hindrance, electronic and steric repulsion or attraction and hydrogen bonding.

There are no strict rules regarding which or how many potential energy functions should be used or included in force field calculations. However, there are two classes (as given in table 3.1), which comprise five potential energy functions common to most methods.

It is important to realise that the energy of a molecule has no physical meaning on its own, but is purely a measure of intra-molecular strain, relative to a hypothetical situation. The relevance of energy lies in its variation, in different structural geometries of a molecule, which thus allows predictions regarding to molecular behaviour.

Table 3.1 Two Classes of Potential Energy Functions.

Bonded Interactions	**Non-bonded Interactions**
Bond Length	van der Waals interactions
Bond Angle	(represents steric interactions occurring between non-bonded atoms)
Torsion Angle	Electrostatic interactions

3.22 Molecular Orbital Theory (MO)

Molecular orbital theory uses quantum mechanical calculations in the prediction of molecular properties. Quantum mechanics lies at the heart of all modern science, its equations describe the behaviour of very small objects, i.e. atoms, and provide the only understanding of the world of the very small. Without these quantum mechanic equations there would not have been the ability to design nuclear power stations, lasers or to understand DNA and genetic engineering. Schrodinger described quantum mechanics in terms of waves (ψ), familiar features in the world of physics, and proposed that particles are guided by the wave and the strength of the wave at any point in space and so in order to solve the problems of quantum mechanics one should measure the probability of finding the particle at a particular point [44]. In principle the solution of the Schroedinger Wave Equations should provide answers to every chemical problem. Unfortunately, a perfect solution of the equations is at present possible only for the hydrogen atom, with only approximate solutions for slightly more complicated but still small entities. The advent of computing technology has allowed the inclusion of various major approximations into the Schroedinger equations, which have allowed for their solutions, at an arbitrary level of accuracy, for larger molecules. The techniques for solving the Schroedinger Wave Equations are grouped together under the title Approximate Molecular Orbital Theory, a summary of available techniques is given in table 3.2. As the methods become more approximate, the less computer time they require, thus enabling larger molecules (20 to 50 atoms) to be analysed. However, it must be remembered that results will become less reliable and so must be treated with due caution.

Table 3.2 Summary of the Various Approximate Molecular Orbital Methods
(Adapted from Cronin [42])

Increase in Accuracy and Computer Time ↓	Hartree-Fock limit ab initio
	Extended basis set ab initio
	Minimum basis set ab initio
	Minimum Neglect of Differential Overlap (MNDO)
	Complete Neglect of Differential Overlap (CNDO)
	Iterative extended Huckel Theory (IEHT)
	Extended Huckel Theory (EHT)

3.2 Computational Chemistry in Quantitative Structure-Activity Relationships (QSARs)

The ability to calculate the minimum energy conformation of a molecule, which is assumed to represent the most active conformation, is of extreme value as a tool in quantitative and qualitative analysis. Parameters calculated through computational chemistry techniques have been shown to be of great value in the development of QSARs.

There are now commercially available complete molecular modelling packages, which will find the minimum energy state of a molecule and calculate physico-chemical and structural descriptors.

Examples of such packages are:

- Chem-X from Chemical Design Ltd
- SYBL from Tripos
- CHARMm from Polygen.

Furthermore, the pharmaceutical industry itself has developed software tailored to its own requirements, such as;

- COSMIC from SmithKline Beecham.
- Profiles from Wellcome Corporation.

4.0 Quantitative Structure-Activity Relationships (QSAR)

4.1 Definition

Correlation's between structure and activity are important for the understanding and development of pharmaceutical agents and for the investigation of chemical toxicity [45]. The basis of all QSAR studies is the belief that any change in the molecular structure of a compound will alter the physical, chemical and biological properties of that compound [46,47]. A QSAR study can therefore be defined essentially as 'the development of a statistically significant mathematical model, which relates the observed biological activities of a series of compounds to the physico-chemical and structural properties of those compounds'. A QSAR will thus aid the understanding of the underlying mechanisms of the biological response and may lead to the ability to predict the biological activity of new, untested or even hypothetical compounds [45,48].

4.2 Historical Background and Developments

The idea that a relationship exists between the chemical structure of a compound and its pharmacological or toxicological properties can be traced back to the nineteenth century [49]. In 1858 the Russian chemist and composer, Borodin wrote that 'by comparing poisonous substances with each other, one came to realise that their toxicological properties and chemical make up are closely related. The first thing to be noted was the fact that many substances consist of the same element or take part in similar reactions and also exert similar actions in the organism' [49].

In 1868, whilst investigating the narcotic effects of analgesics, Crum Brown and Fraser [50] postulated that 'the physio-logic action of the analgesic (ϕ) is a function (f) of its chemical structure (c) [51,52] as presented in equation 4.1.

$$\phi = f(c) \qquad (4.1)$$

In 1878, Langley first proposed that drugs act on specific components within the body [49] and in 1893, whilst studying a number of simple polar compounds, Richet [53] determined that toxicity is a function of the aqueous solubility of the compound itself, thus giving credence to the work of Crum Brown and Fraser [50].

By the start of the twentieth century a dichotomic approach to structure-activity relationships was in existence, with Erlich, Landsteiner and Clark [54] investigating the idea that 'chemical constitution was the basis for understanding the biological activity of

compounds', whilst Overton, Meyer and Ferguson followed the alternative idea that 'the physical properties of organic compounds are of central importance in predicting biological activity' [55].

In 1901, Overton [56] and Meyer [57] independently correlated the biological activity of narcotics to molecular properties. Overton observed that for a series of simple compounds, the toxicity to tadpoles was actually a function of each compound's partition coefficient and not the aqueous solubility directly, which is itself governed by the partition coefficient. Overton also experimented with alkaloids and commented that the difference in the toxic effects of morphine to humans and to tadpoles was due to differences in the structure of the proteins in the two organisms and that these proteins would form salt-like complexes with morphine. He then suggested that variations in toxicity would result from differences in the solubility of these complexes. Although Overton did not have the benefit of modern statistical methods, his reported data are as reliable as any aqueous toxicity data in modern day literature. His monograph 'Studien uber die Narkose' [58] is viewed in numerous scientific fields as the turning point in the understanding of the relationships between chemical structure, cell permeability, potency and other biological activities. Since its publication this monograph has provided impetus and acted as a stepping stone for the work of many researchers in many disciplines, and has been of enormous value to modern toxicologists, particularly those involved in QSAR development [59].

In 1939, Ferguson [55] formulated a concept linking narcotic activity, partition coefficient and thermodynamics. He reasoned that in a state of equilibrium, simple thermodynamic principles could be applied to drug activities and so the important parameter to consider for the correlation of narcotic activity was the concentration relative to saturation.

In 1942, Bell and Robin published results of a quantitative study of *in vitro* activity of 46 sulphonamides. Plotting potency at pH 7.0 against pKa resulted in a biphasic curve. They interpreted the curve to rationalise the enhanced potency of the sulphonamides at pKa 7.0 and explained that the observed variations were due to the ionisation state of the sulphonamide [52].

Modern QSAR, in which the physical, chemical and structural properties of a molecule are used to predict its biological activity, has more recent origins, taking its present form approximately thirty years ago, with the pioneering work of Hansch and his co-workers. Developments have been vast and due impart to advances in computing technology.

In 1962, a number of events occurred which led to the birth of modern QSAR;

1- Hansen [60] attempted to correlate bactericidal effects with Hammett constants.

2- Zharadnick [61] attempted to develop a correlation of biological activity without physico-chemical parameters, known as the *de novo* approach, in which pseudo physical numerical values related to biological activity are generated.

3- Hansch and Fujita [62] developed the concept that the physico-chemical properties of a compound influence its biological activity. Their work with herbicides showed that the factors of hydrophobicity and electronic effects control the herbicidal activity of the compound. They published this relationship as a QSAR (equation 4.2), in which the biological activity is represented as the $\text{Log}^1/_C$ term. Where C represents the concentration of the meta or para substituted phenoxyacetic acid which induces a 10% growth of *Avena coleoptiles* in a 24 hour period. The logarithm of the reciprocal biological activity data is used as it best represents a normal Gaussian distribution, thus the data is more amenable for statistical modelling.

$$\text{Log}^1/_C = 4.08\pi - 2.14\pi^2 + 2.78\sigma + 3.36 \qquad (4.2)$$

This work is very important in that it made four major breakthroughs;

a- π the hydrophobic substituent constant (π) as defined by equation 4.3 and discussed in more detail later in 4.3213.

$$\pi = \text{Log} \frac{(\text{partition coefficient of the derivative})}{(\text{partition coefficient of the parent compound})} \qquad (4.3)$$

b- in order to measure the partition coefficient (P), they used an octanol-water system. There is no perfect system to predict partitioning, but the use of octanol provides a model for lipid membranes, as octanol has a long hydrocarbon chain and has a polar head group. Today, the n-octanol/water system is the most widely used system for the determination of partition coefficient, when experimentally necessary, with relatively pure supplies of octanol being readily available.

For all its numerous advantages this solvent system does have some drawbacks:

- octanol acts as an acceptor and a donor of hydrogen bonds, i.e. it is amphiprotic. This presents problems in the interpretation of what is actually occurring in the biological system. It is possible to distinguish between donor and acceptor activities of a compound by using an additional solvent pair, which acts only as a proton acceptor e.g. propylene glycol dipelargonate (PGDP) or as a proton donor e.g. chloroform. The ability to distinguish between acceptor and donor activities enables a full picture of drug partitioning to be obtained.

- Experimental determination of partition coefficient is tedious, in that there must be mutual saturation of the phases i.e. mixing for five hours, and care must be taken to maintain constant temperature and pH and to limit the presence of impurities. Wang and Lien [63] have shown also, that the partition coefficient of a compound is dependent on the nature and concentration of the buffer used in its evaluation.

c- the use of more than one parameter (in the final equation 4.2) to describe the biological activity ($Log^1/_C$) had not been attempted before. This was thus the start of the use of multiple regression analysis in the determination of modern QSARs.

d- the use of π and π^2 terms together (i.e. a quadratic equation) to describe a biphasic relationship between biological activity and LogP was novel and justified by the assumption of a normal Gaussian distribution of the biological activity data with LogP.

4.3 Objective of QSAR

The overall objective of any QSAR is to develop a quantitative (mathematical) relationship between the biological activities of a series of compounds and the physical, chemical and structural properties of the compounds, by the use of statistical techniques [48,52]. Such correlations between biological activity and chemical structure are of use in assisting the understanding of the underlying mechanisms of the biological activity and should enable the biological activities of other, similar, compounds to be predicted [48]. In order to establish a QSAR, three components are essential; biological activity data for modelling, physical, chemical and structural descriptors and statistical techniques which can be used to correlate the biological data and the descriptors.

4.31 Biological Activity Data

The prediction and explanation of biological data are the crux of any QSAR analysis. Prior to the formation of a strong model, accurate, reliable, high quality data of a consistent form and pertaining to the biological activity on which to base the relationship, are essential, as a good QSAR correlation will not be found if there is excessive error in these data. Biological data usually take the form of a measured response to a fixed dose or molar concentration of xenobiotic, or a fixed response (e.g. LD_{50}) generated over a range of molar concentrations. The data generated maybe quantitative (e.g. x M or y %), semi-quantitative (e.g. +++, -,+,--) or qualitative (e.g. response, no-response). Each type of data has its use in QSAR analysis, but requires the use of different statistical techniques. The choice of biological data is important and the model derived is only as good as the original biological data utilised.

4.32 Physical and Chemical Descriptors

The physico-chemical descriptors can, as a rule, be assigned to one of three categories [5], Hydrophobic, Electronic, Steric parameters

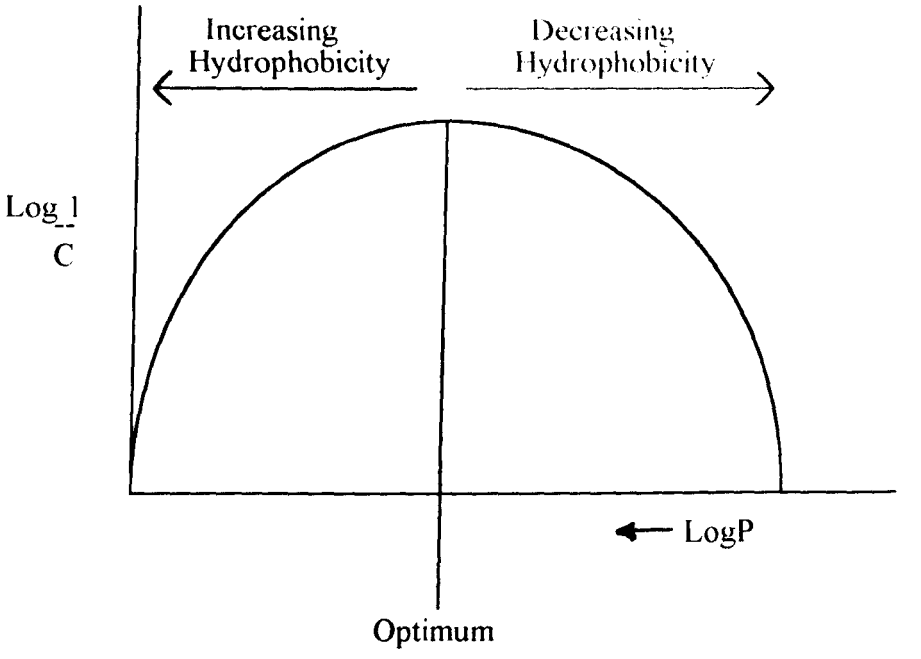
4.321 Hydrophobic Parameters

The term 'hydrophobic interaction' refers to the tendency of the non-polar portions of molecules to associate together when placed in an aqueous environment. It is important to realise that this effect is due to the structural properties of water itself [49]. Hydrophobicity is therefore, a measure of the relative affinity of a molecule for a non-polar environment versus its affinity for an aqueous environment. Hydrophobic forces are probably the most important when considering the provision of the driving force for the non-covalent intermolecular interactions of a molecule in an aqueous solution [64]. Several hydrophobicity parameters have been used as predictors of biological activity, some of the most common being; partition coefficient, chromatographic parameters and solubility.

4.3211 Logarithm of Partition Coefficient (LogP)

Partition coefficient is the most important and the most extensively utilised hydrophobic parameter. It was first defined by Berthelot and Jungfleisch [65] as "the ratio of concentration at equilibrium of a solute distributed between two immiscible phases".
Partition coefficient determines the equilibrium between an external phase, such as water and a biophase such as lipid membranes, and is represented in QSARs as LogP. The logarithmic term is used because it represents the free energy of the partitioning process. Hansch suggested that molecules which are highly hydrophilic will not readily partition from water into lipid molecules, so if the receptor lies within or beyond that lipid membrane, a hydrophilic molecule will have a low probability of reaching the receptor within a set time. Conversely, molecules of high hydrophobicity will readily partition into the first lipid membrane but will be reluctant to pass out of the lipid membrane into an aqueous phase and so will be held there, thus slowing the rate of movement of the compound to the receptor site. Therefore, there must be some optimum value of LogP which would correspond to a maximum probability of the compound reaching the desired receptor in a set time interval. That is to say biological activity induced by receptor binding is biphasically dependent on LogP, as illustrated in figure 4.1.

Figure 4.1 The Biphasic Relationship Between Biological Activity and LogP



4.3212 Chromatographic Hydrophobic Parameters

Petrauskas [66] and Tomlinson [67] have both utilised chromatographic techniques in evaluating of values pertaining to a compounds partitioning ability and subsequently used their results in structure-activity studies.

A- Thin Layer Chromatography

In reverse phase chromatography the partition coefficient is proportional to Rm, which equals $^1/_{RF}$-1. Thus LogP is directly proportional to Log($^1/_{RF}$-1).

B- High Performance Liquid Chromatography

LogP is directly proportional to LogK, where K is the capacity factor of the column as defined in equation 4.4.

$$K = \frac{t - t_0}{t_0} \qquad (4.4)$$

Where t = time taken for a specific compound to elute from the column

t_0 = time taken for an unretained compound to elute from the column.

4.3213 Substituent Hydrophobic Parameters

An important feature of the partition coefficient is that it is to a first approximation additive. Thus the contribution of a single group to the overall hydrophobic character of the molecule can be determined from the partition coefficient of the parent molecule and that of the corresponding derivation [51]. Hansch and co-workers [68] in this way devised a hydrophobic parameter for substituent π, as defined by equation 4.3. They observed that the measured partition coefficients of compounds of the type $C_6H_5.CH_2CH_2CH_2X$, where X is a polar group or atom, were lower than values calculated by summation of the π values (equation 4.3). They attributed this to back folding of the side chain in such a way as to allow substituent X to interact with the aromatic ring. Hansch [69] since proposed that as π values are additive, it is possible to make good estimates of partition coefficients by summing the relevant published π values.

Rekker [70] suggested that the Hansch system makes no distinction for the hydrophobic contribution of hydrogen and hence no distinction is made between πCH_2 and πCH_3 groups. Using a large database of LogP values he devised an analytical / statistical approach for the evaluation of partition coefficient values on the basis of additivity,

which utilises fragmental hydrophobic constants (f values), in conjunction with correction or interaction factors (F) which allow for proximity effects, the attachment of hydrogen to an electronegative centre and cross-conjugation. Values of F are multiples of what Rekker termed his 'magic number' 0.28.

Leo and co-workers [71] also accepted the wisdom of fragmentation and have developed a synthetic approach to the calculation of partition coefficients. They firstly defined, very clearly and exactly, what constituted a fragment. They then determined the partition coefficients of a number of small molecules, including hydrogen, from which they were able to obtain fragmental constant values. They also found it necessary to introduce correction factors, for such things as chain branching, fragments attached to aromatic rings and numbers of bonds between fragments.

The development and improvement of fragmental constant methods has provided a means of calculating a compounds LogP values, which has inturn increased the knowledge regarding LogP and π values. Rekker's approach has since become generally accepted as the most practical way of calculating LogP values [72].

4.3212 Solubility

Solubility generally is simply a reflection of the partitioning process. As such aqueous solubility is inversely proportional to partition coefficient, for liquids (equation 4.5), and for solids is a function both of the partition coefficient and the melting point of the respective solid [73] (equation 4.6).

Aqueous solubility has been used as a parameter in numerous QSARs.

$$S(aq) \propto {}^{1}/_{P} \qquad (4.5)$$

$$LogS = aLogP - bMP \qquad (4.6)$$

Where a and b are constants.

4.322 Electronic Parameters

All properties of a molecule are related to the electron distribution within the molecule itself and the behaviour of these electrons. Therefore, any change in the electron distribution of a compound will alter the physical, chemical and biological properties of that compound. It is, therefore, not suprising that electronic properties have been used in quantitative descriptions of biological activity [74].

The electronic effects within a molecule control bond order and govern the susceptibility of a given bond to metabolic attack. The inter-molecular interaction forces control both the extent and the strength of xenobiotic-receptor interactions [74]. Interaction types such as ion-ion, ion-dipole, ion-induced dipole, dipole-dipole, dipole-induced dipole and hydrogen bonding, all depend on the electronic distribution within the molecule or substituent and the ease with which the distribution can be modified [74].

Electronic descriptors are physico-chemical parameters which quantify the electronic effects of a molecule or its substituent [52] and are derived from the electronic configuration of the molecule [5].

Electronic descriptors can be divided into substituent parameters used with a congeneric series of compounds, or whole molecule parameters used with a mixed collection of compounds.

4.3221 Substituent Electronic Parameters

4.32211 Hammett Substituent Constant (σ)

Hammett showed that the acid dissociation constants of meta and para substituted benzene derivatives could be correlated by equation 4.7. This parameter is applicable to many types of reactions or interactions which depend on the degree of electron release or withdrawal by the substituent [74].

$$\rho\sigma_X = \text{Log} \frac{(K_X)}{(K_H)} \qquad (4.7)$$

Where ρ is a series constant pertaining to the type of compound and type of reaction / interaction

K_H is the rate or equilibrium constant for the unsubstituted parent compound,

K_X is the rate or equilibrium constant for the substituted derivative,

σ_X is the substituent constant and refers to the electron directing effect of the substituent relative to hydrogen.

The Hammett sigma constant (σ) can be used to assign a numerical value for electronic effects, as it reflects the electronic effect of a substituent on the electron density of the reaction centre of the parent compound. σ is also, a reliable descriptor of the electronic nature of the different substituents. However it should be borne in mind that σ values are not necessarily additive, as constitutive effects (e.g. steric effects, hydrogen bonding) may cause deviation from additivity [64]. The Hammett relationship provides a quantitative equation which allows comparisons of the relative sensitivity of a reaction to electronic or polar effects. Conversely, it provides a method to examine the relative electronic effects of different substituents on a parent molecule [64].

4.32212 Swain and Luptons Parameters

In 1968, Swain and Lupton introduced two parameters -F, the field effect or inductive parameter and R, the resonance effect, derived by fractionation of the Hammett constant into its inductive and resonance components [52].

4.32213 Hydrogen bonding and molar refractivity parameters may also be treated as substituent electronic descriptors. These two parameter types are discussed in depth later in 4.3223 and in 4.3214,4.32223, 4.32316 respectively.

The main drawback of substituent electronic parameters is the basic assumption that the substituent has an additive effect with regards to other properties of the whole molecule and that any changes are due to the substituent. This assumption has yet to be proven and may not in fact operate, so any substituent electronic parameters must be viewed with some degree of scepticism. Also, substituent parameters can be used only with congeneric series.

4.3222 Whole Molecule Electronic Parameters

Whole molecule electronic parameters overcome the problems of additivity as seen with substituent electronic parameters, and also can be used with non-congeneric groups of compounds. It is therefore, intrinsically better to utilise whole molecule electronic parameters, where possible, as they account for every change occurring in the molecule.

4.32221 Dipole Moment

Dipole moment (μ) is a measure of the partial charge separation occurring within a molecule. μ is described as the magnitude of charge (e) multiplied by the distance between the charge centres (d). However, there is a problem with the use of μ in that it is a vector quantity, and different molecules have different dipole moments acting in three different directions. One of these vector directions may be more relevant that the total vector quantity. This problem may be overcome by calculating dipole moment using say the MOPAC software package, which gives X, Y and Z vector values, as well as the total dipole values.

4.32222 Electron Polarisability

Electron polarisability (P_E) reflects the ability of the electrons in an atom, group or molecule to be polarised in the presence of an electric field. This being an implication for intermolecular interactions [75]. Polarisability is a constitutive property and so is dependent on individual molecular components, as defined by equation 4.8 and in a manner similar to molar refractivity (4.32316).

$$P_E = \frac{(n^2-1)}{(n^2+2)} \times \frac{Mw}{\rho} = \frac{4\pi N\alpha E}{3} \qquad (4.8)$$

4.32223 Molar Refractivity (MR)

Molar refractivity is calculated from refractive index, and thus partly constitutive. The units of MR are those of molar volume, and thus it can be considered as a measure of molecular bulk (4.32316). However, since it is obtained from refractive index (equation 4.9), which is itself dependent on electron polarisability (4.32222 and equation 4.8), it can also be considered as a measure of polarisability [74]. It has been suggested that if the MR term in a QSAR has a positive coefficient, it is modelling polarisability, and if it has a negative coefficient, then it is modelling bulk [74]. This begs the question of how to interpret a QSAR that contains both MR and MR^2. Since in most QSARs MR can be replaced by a size parameter, it seems likely that it generally models bulk (4.32316).

4.32224 pKa

pKa can be considered as a dual parameter; firstly it reflects the electron directing ability of substituents and as such is equivalent to the Hammett constant, and secondly it controls the extent of ionisation of a compound, which affects the partition coefficient and so reflects the hydrophobicity of a compound.

It is now possible to calculate pKa with reasonable accuracy using available computer software, e.g. HazardExpert.

4.32225 Electrostatic Potential

Electrostatic Potential defines the ease with which the electron cloud about a molecule may be moved or polarised. Weinstein has produced a computer package which enables the calculation of the electrostatic potential around a molecule and thus enables the creation of a contour map of the molecule, in which each contour is represented by lines of equal energy of interaction with unit positive charge.

4.32226 Chemical Shift (NMR)

The chemical shift of a proton or other nucleus is dependent on the electron density around it. Chemical shift is thus a sensitive probe of atomic environment, and can throw light on specific interactions with solvent and receptor alike. It has the disadvantage that it has to be obtained experimentally using the technique of NMR spectroscopy.

4.32227 Molecular Orbital Parameters

Molecular orbital theory (3.12) is complex and not easy to understand, so use of parameters derived from M.O. theory without a full understanding of their meaning can lead to problems in their interpretation with respect to biological activity.

Some parameters which fall into this group are;

A- Atomic charge, a whole molecule parameter generally used only in the study of a congeneric series of compounds, so the charges on specific common atoms may be related.

B- E_{HOMO} (the energy of the highest occupied molecular orbital) is the energy required to remove an electron from the outermost orbital, and is thus the negative of the ionisation potential.

C- E_{LUMO} (the energy of the lowest unoccupied molecular orbital) is the energy required to accept an electron into a vacant orbital and is thus a measure of electrophilicity.

D- Heat of formation, representative of the total energy of the molecule.

4.3223 Hydrogen Bonding Parameters

Hydrogen bonding is an important interaction in biological activity, affecting processes such as drug-receptor reactions, solubility and partitioning. Hydrogen bonding can be classified as both a substituent and a whole molecule electronic parameter. However, there is a major problem with the use of hydrogen bonding parameters, this being that no one parameter has been developed which quantitatively models hydrogen bonding and at the same time is easy to use. Over the years, there have been a number of attempts to devise suitable hydrogen bonding parameters.

4.32231 Sieler's I_H Parameter

Seiler [76] was the first to attempt to develop a hydrogen bonding parameter. He used the difference between LogP values measured in an n-octanol/water system and those evaluated in a cyclohexane/water system, in order to produce a numerical parameter (I_H), which is representative of the group contributions to hydrogen bonding. However, since octanol acts both as a proton donor and acceptor, the I_H value does not distinguish between proton donor or acceptor capabilities. A further problem with I_H is that it is essentially the difference between LogP values in two solvents and as such must reflect polarity as well as hydrogen bonding.

4.32232 Moriguchi's Ew Parameter

Moriguchi [77] attempted to overcome the problem encountered by Seiler's parameter, by assuming that LogP contained volume and polarity components and by calculating the polarity component (Ew). This is the difference between an n-octanol/water system LogP for a polar compound and that of a non-polar compound of the same molecular volume. But, as LogP can be factored into volume, polarity and hydrogen bonding terms, it is clear that Ew contains both polarity and hydrogen bonding terms.

4.32233 Fujita et al's Indicator Variable

Fujita et al [78] devised a hydrogen bonding indicator variable, which takes a value of unity if the molecule or substituent under investigation is capable of hydrogen bonding and a value of zero if it is not capable of doing so. It is possible to modify this method to distinguish between proton donor and acceptor capabilities (4.32234). This is a very crude measure of hydrogen bonding ability, but since it is easy to use, has been widely applied in QSAR studies.

4.32234 Charton and Charton's Parameter

Charton and Charton [79] modified Fujita's approach (4.32233), by using the number of hydrogen bonds that the molecule under investigation was capable of forming, in place of the simple 1 or 0 approach of Fujita et al.

4.32235 Yang et al's Parameters

Yang et al [80] devised two hydrogen bonding parameters HB1 and HB2. HB1 being very similar to Charton and Charton's parameter. HB2 is defined as the number of atoms able to form hydrogen bonds multiplied by the value of the hydrogen-bond energy, the whole being scaled by a factor of 0.1. Average enthalpy values for each type of hydrogen bond are used [80].

4.32236 Solvatochromic Parameters

Kamlet, Taft and Abraham [81,82] have developed a set of parameters known as 'Solvatochromic parameters', because they were originally developed using spectroscopic techniques. They hypothesised that essentially any interaction is a function of molecular size (υ), polarity (π^*), hydrogen bond acceptor ability (α) and hydrogen bond donor ability (β).

These parameters show excellent correlations with physico-chemical and biological properties, such as solubility and toxicity. However, they have a major disadvantage in that they require experimental derivation, although some attempts have been made to calculate them.

Dearden [83], examined the various types of hydrogen bonding parameters and concluded that Fujita's parameter was relatively easy to determine and hence is the most widely used parameter, but that Yang's parameters appear to be the least problematic and so are currently the best indicators of hydrogen bonding and with HB2 values for 144 substituents having already been evaluated, it is relatively easy to determine values for a range of compounds.

4.323 Steric Parameters

Steric parameters fall into one of two types; size and shape.

Both the size and shape of a molecule or of a specific substituent are important in the control of biological activity [49,74]. Bulky substituents adjacent to the reactive site may shield the active site and thus hinder binding and metabolism, or the substituent may shield a polar region within the molecule and thus act to reduce the molecule's affinity for water or increase its affinity for lipid phases [74].

4.3231 Molecular Size Parameters

4.32311 Molecular Diameter

This parameter is measured in terms of van der Waals radii and is a measure of size. However, it is only an approximation, being accurate only for spherical molecules.

4.32312 Molecular Weight (Mw)

Mw is directly proportional to the size of a molecule hence is an accurate and easy means of enumerating the size of a whole molecule of substituent, for a homologous series of compounds. Molecular weight is also directly proportional to LogP.

As a measure of volume however, it is unlikely to reflect precisely the group size.

4.32313 Molar Volume

Molar volume is a measure the volume occupied by one mole of compound, which takes into account any intermolecular interactions, such as hydrogen bonding, occurring within the molecule.

4.32314 Intrinsic Volume

Intrinsic volume is simply a measure of the volume of a molecule, usually based on van der Waals radii.

4.32315 Molar Refractivity (MR)

MR is a crude steric term (see 4.32223) [75,84], and is measured using the Lorentz-Lorenz equation as detailed in equation 4.9 [55,62,64].

$$MR = \frac{(n^2-1)}{(n^2+2)} \times \frac{\text{molecular weight}}{\text{density}} \qquad (4.9)$$

Where n is the refractive index

$\frac{\text{molecular weight}}{\text{density}}$ is a molar volume term

4.32316 Surface Area

Surface area is regarded as the surface of a molecule which is available to make contact with other molecules, prior to the initiation of a response and so is a very useful parameter. Surface area can be measured in a number of ways, the most common are;

4.323161 The summation of the atomic surface areas is calculated from the van der Waals radii. However, since the individual atoms of the molecule may overlap or shield regions of the surface, the summation procedure does not give a good indication of the effective surface area.

4.323162 The accessible surface area (ASA) as proposed by Connolly. The ASA value is evaluated using a computer program, which effectively rolls a water molecule over the calculated van der Waal surface of an energy minimised representation of the molecule under investigation [74]. ASA is a very useful parameter, as it gives a true representation of the area of the molecule which is actually available for interaction. It should be noted that ASA has a constitutive component; for example, the ASA of 1,2-dichlorobenzene will be smaller than that of 1,3-dichlorobenzene.

4.3232 Substituent Size Parameters

4.32321 Taft's Steric constant (Es)

Es was originally derived for a series of esters by experimental determination. It is a measure of the relative hydrolysis constant of the molecule under investigation. The bigger and more bulky the substituent on the molecule the more difficult it is to hydrolyse the adjacent bond. Es is therefore directly related to the van der Waals radii [5,74]. Es is then an effective molecular descriptor for improving those correlations in which σ is not adequate.

4.32322 Charton's Upsilon Parameter (γ)

γ describes the difference between the published substituent van der Waals radii and the van der Waals radius of hydrogen (1.20) [74].

4.32323 Parachor (PA) [85]

$$PA = \frac{Mw \times \gamma^{1/4}}{\rho_L - \rho_V} \qquad (4.10)$$

γ = surface tension ρ_L = liquid density ρ_V = vapour density

(ρ_V can be ignored as $<< \rho_L$)

If measurements are carried such that $\gamma = 1$, then $PA = \frac{M}{\rho_L}$ molar volume

PA is very seldom used nowadays.

4.32324 van der Waals Radius

The approximate volume of a molecule or substituent can be derived from a modelled structure and is defined as the van der Waals radii. Although the calculation of van der Waals volume is accurate, its use is limited by its difficulty to reflect the various shapes of groups, which means therefore that the results attained inevitably contain errors [5].

4.3233 Shape Parameters

Shape refers to the distribution of molecular 'bulk' according to the molecular conformation or the relative position of the constituent atoms. The role of shape in determining the biological activity of a compound is implicit in the stereo selectivity of biological receptors [52].

4.32331 Sterimol Parameters

These multi-dimensional parameters were introduced by Verloop, Hoogenstraaten and Tipler in 1976 [86]. Since then they have become known as 'Sterimol parameters', after the computer program which was developed to perform their calculation. The approach arose from a consideration of how to fit a molecule to its receptor site [49].

The calculation of Sterimol parameters uses van der Waals radii, standard bond lengths and angles evaluated from a reasonable conformation of the molecule, in order to define the molecular spatial arrangement, for a given substituent and generates five measured values of substituent size (figure 4.2); L corresponds to the length of the substituent group, measured along the axis of the bond between the first atom of the substituent and the parent molecule; B_1 to B_4 are width values and are calculated starting with the minimum, B_1, which is measured of the distance from the length axis to the perpendicular surface of the substituent group. B_4 is a measure of the distance along the B_1 axis, in the opposite direction. B_2 and B_3 are measured perpendicular to the B_1 and B_4 axi, respectively. B_1 to B_4 can describe non-symmetrical substituents and emphasise the directional character of steric effects, whilst simultaneously providing additional information regarding the actual conformation of the receptor surface [86].

In 1987, a new parameter B_5, which corresponds to the maximum width of the substituent group, was introduced.

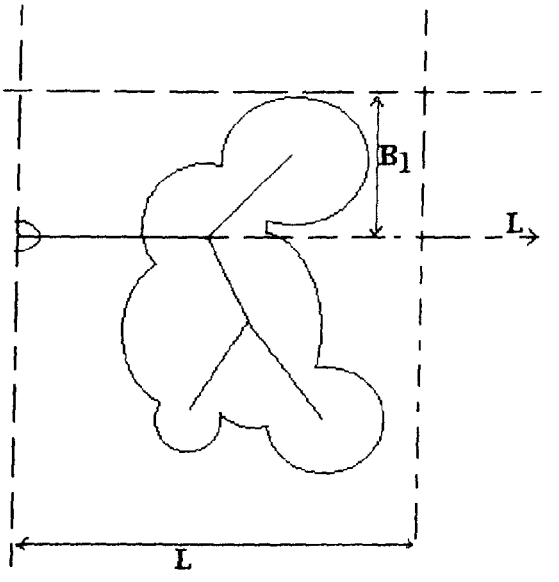
There are a few criticisms of these parameters, in that they cannot be used as 'true' whole molecule parameters, as there is a need to measure L in a specific direction from a specific reference point. It is however, possible to evaluate Sterimol parameters for virtually whole molecules in a congeneric series. One appoints a common terminal atom or group, e.g. hydrogen, to represent the compound and thus the remaining structure becomes the substituent. Sterimol parameters are then evaluated from the compound (hydrogen atom) along the bond attaching it to the substituent (whole molecule).

It is important that when determining Sterimol parameters that the molecules under investigation are in the same orientation, thus ensuring that comparable measurements are attained.

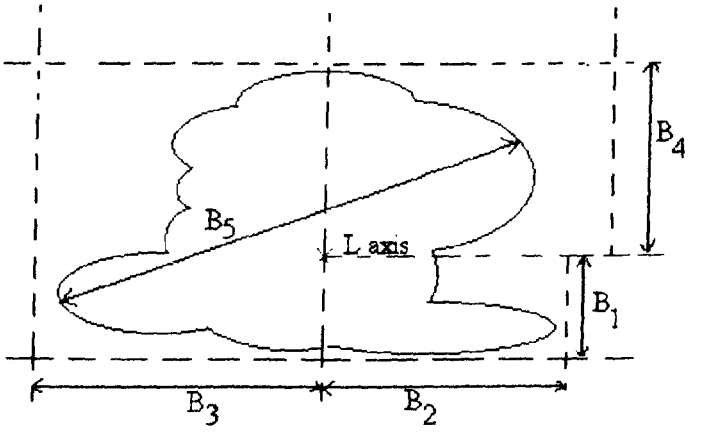
Figure 4.2 Diagrammatic Representation of Verloop's Sterimol Parameters

(Adapted from Hansch and Leo [72])

i) Perpendicular cross-section of the substituent along the L axis



ii) - Vertical section viewed 'down' the L axis



4.32332 Molecular Shape Analysis (MSA)

MSA was devised by Hopfinger [87], and is used in the analysis of a congeneric series of molecules. MSA takes the molecule which shows the most activity or is believed to fit the receptor best, and superimposes all other molecules of the series onto this standard. The area of common overlap is then measured, this being the molecular area (or common overlap volume, Vo) which corresponds to the steric requirement of the receptor.

This methodology is not widely used, as it has been superseded by calculation of the probable overlap volume.

4.33 Structural or Topological Descriptors

The topological or geometric features of a molecule can be used to characterise the structure of that molecule in a topological analysis. Features can be generated by following certain rules of algorithm, starting with single atoms or bonds as the centres of atom or bond centred fragments [88]. The simplest possible descriptors are counts of atoms or bonds of specific types within the molecule or fragment. These descriptors however, contain very little information regarding the topology of the molecule and different molecules may have the same descriptor value [88].

In order to differentiate between different molecules and obtain more topological information, many different topological descriptors have been devised [88]. The one which is by far the most widely used is Randic's Branching Index, refined, developed and extended by Kier and Hall as molecular connectivity.

4.331 Randic's Branching Index

Randic devised his topological index in 1975 [89], his idea being to assign each atom with a numerical value, corresponding to its 'degree of connectivity', this being simply the number of other atoms to which it is attached, other than hydrogen. Each bond is given a value which is the reciprocal square root of the product of the atomic numerical values of the two respective vertices. The major problem with this index is that there is no differentiation between carbon and heteroatoms or between single and multiple bonds and therefore, the index has limited use in the correlation of biological activities [52].

4.332 Molecular Connectivities

Kier and Hall [90] extended the work of Randic to yield what they term 'Molecular Connectivity Indices'. Molecular connectivity parameters (χ) contain information about the molecular structure and the way in which atoms are connected to one another. Molecular connectivity parameters can be readily calculated manually, but are most conveniently determined by computer software, such as MOLCONN-X. A simplified version of the calculation of molecular connectivity parameters is given in figure 4.3. It will be noted that the method of calculation shown in figure 4.3 is independent of the nature of the atoms. That is, the method does not discriminate between carbon and heteroatoms. In order to overcome this problem Kier and Hall [90] introduced modifications that allowed the presence of heteroatom, double and triple bonds to be taken into account. The connectivity values thus obtained are termed valence-corrected molecular connectivities (χ^V).

Since their introduction molecular connectivity indices have met with much scepticism in the QSAR field. The main reason for this is that although they often yield good correlations with biological activity, the actual physical significance of the indices is not known. Thus no conclusions regarding mechanisms of action can be drawn from them. Furthermore, it is extremely difficult to use a QSAR containing χ terms to predict other bioactive molecules. Nevertheless there is undoubtedly much structural information contained within χ indices, and it is to be hoped that methods will be developed to elucidate it.

4.333 Kappa Index (κ)

The Kappa Index was devised by Kier [91] to describe different molecular shapes. κ is based on a count of two bond fragments in a hydrogen suppressed graph i.e. a molecular structure with hydrogen atoms removed, relative to the maximum number possible in the isomeric star graph. As with χ values, κ values can be represented in two forms; the minimal steric distance (κ) and the minimal topological distance (κ_α).

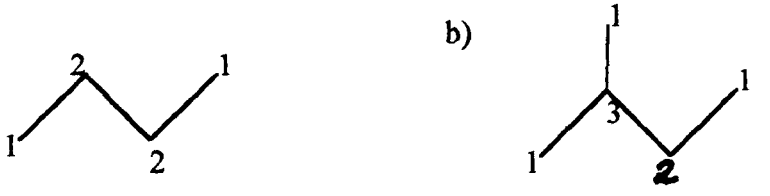
Figure 4.3 Simplified Representation of a Molecular Connectivity Calculation

A- The molecules under investigation are represented as a hydrogen suppressed graph.

a) $CH_3CH_2CH_2CH_3$ b) $CH_3CH(CH_3)CH_2CH_3$

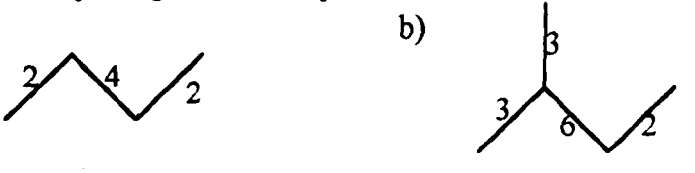
B- A numerical value is assigned to each atom, which corresponds to the number of non-hydrogen bonds formed by that atom.

a) b)



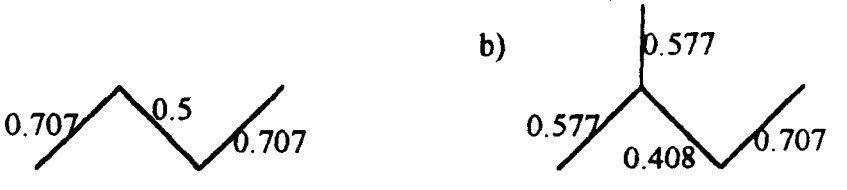
C- In order to show how the relationship of these atoms varies in different molecules, multiply the adjacent atom values together and place the new numerical value alongside the bond adjoining the two respective atoms.

a) b)



D- Determine the reciprocal square root of each bond value.

a) b)



E- Sum the reciprocal square root values to obtain a numerical value for the first order connectivity ($^1\chi$).

a) $^1\chi = 1.914$ b) $^1\chi = 2.269$

Calculations across two or more bonds enables the calculation of higher orders of connectivity.

4.334 Degree of Flexibility (Φ)

It is possible by mathematical manipulation of the Kappa indices (equation 4.11) to determine a numerical value which corresponds to the flexibility of the molecule under investigation.

$$\Phi = \frac{{}^1\kappa_\alpha \times {}^2\kappa\alpha}{A} \qquad (4.11)$$

Where ${}^1\kappa_\alpha$ is the first order minimal topological distance kappa value,

${}^2\kappa_\alpha$ is the second order minimal topological distance kappa value,

A is the number of atoms in the molecule.

4.335 Minimum Topological Difference (MTD)

MTD is similar to MSA (4.32332), in that MTD is the number of non-superimposable atoms, when a molecule is overlaid onto a standard reference molecule, which is hypothesised to represent the receptor cavity. Different cavity assumptions will give different sets of MTD values [52].

4.34 Statistical Techniques SEE SECTION 5.0

4.4 Principal steps in the Development of a QSAR

1- Select a group of compounds which are believed to act in a similar fashion and which have a known type of biological activity (these should have a good range of activity and descriptor space).

2- Select a range of chemical descriptors, so all the appropriate properties of each compound are represented, as all may be important to activity.

3- Collect or evaluate, using appropriate protocols and dedicated computational software, quantitative values for all biological activities and chemical descriptors.

4- Input all descriptor and biological activity data into a dedicated statistical database and determine by analysis of variance, using a suitable statistical technique (as detailed in 5.0) if a correlation exists between the biological activity and any combination of the descriptor variables.

5- Validate the resultant QSAR by testing its ability to predict the activities of compounds not in the training set.

6- Use the QSAR to assist in the optimisation of the biological activity, i.e. to maximise any desired effects, and / or minimises any undesired biological effects [88].

4.5 Restrictions of QSARs

The success of any QSAR depends on the quality of the data used and the suitability of the physico-chemical and structural descriptors utilised [5], and upon the statistical techniques used in the analysis [74]. Also, no QSAR can be expected to give reliable predictions outside the parameter range of the training set, or reliably to predict the activity of compounds which are unlikely to act via the same mechanism as the original training set. It is vital that any QSAR be effectively validated.

QSARs enable the optimisation of specific biological activities by modification of the relevant physical, chemical or structural properties of a type of compound for a specific pre-determined activity, but they do not enable the design of a new type of compound for a new usage. That is QSAR is a lead optimising not a lead generating technique.

4.6 Advantages and Uses

The real value of QSAR development has been in the order which it has brought to structure-activity data, the understanding of the relationships between physico-chemical and structural properties and the biological activity of a compound and the concepts which have thereby been developed and which are now being used for rational drug design [52]. QSARs have the potential to allow the extrapolation of knowledge from agents of known activity to untested or new, yet related, compounds [92].

Due to the demands of time, the high cost of testing new compounds for toxicity using test animals, and the intrinsic problems that test animals differ from humans in their genetic and environmental characteristics, it would be an advantage to be able to estimate the toxic response of chemical agents using non-animal approaches [93]. The use of QSAR as a screening tool provides such an alternative to animal testing, as QSAR can show when further synthesis would yield compounds of equivalent or greater toxicity, thus suggesting the cessation of synthesis and any associated animal or *in vitro* testing [52,94].

QSAR is an aid to the conservation of available financial resources for the testing of compounds, yet provides a methodology which helps to ensure that questionable compounds are either discarded or tested more thoroughly, thus enhancing safety [5].

QSARs have been used in a wide range of fields, some of these being; the prediction of bactericidal activity, anti-inflammatory activity, analgesic activity, carcinogenicity, the prediction of mutagenicity, drug toxicity, environmental toxicity, α_2-antagonism, odour, and biodegradability. They are also used to the predict physical, chemical and structural properties of compounds, although such correlations are more properly referred to as quantitative structure-property relationships (QSPRs).

4.61 Examples of some QSARs

1- Quantitative relationship between the *in vitro* antibacterial activities (the minimum concentration of the antibiotic that inhibits bacterial growth, MIC) of cephalosporins and their n-octanol/water partition coefficients [40]

a) in *Staphylococcus aureus* $MIC = 0.888(LogP)^2 + 6.414\ LogP + 11.575$

$n = 7 \quad r = 0.905 \quad s = 1.447$

b) in *Streptococcus pneumoniae* $MIC = 0.174(LogP)^2 + 1.234\ LogP + 2.165$

$n = 7 \quad r = 0.717 \quad s = 0.545$

2- Bacterostatic activity of trans-3-benzoyl acrylic acids against *Staph. aureus* [46]

C is the concentration of the compound producing the desired biological response.

$$Log\,{}^{1}/_{C} = 0.770\pi - 0.755\sigma - 0.032$$

$n = 11 \quad r = 0.951 \quad s = 0.219$

3- Toxicity of phenols to fathead minnows [46]

LC_{50} is that concentration of compound which results in the death of 50% of the test population.

$$Log({}^{1}/LC_{50}) = 0.666LogP - 0.00721E_{LUMO} + 2.73$$

$n = 34 \quad r = 0.960 \quad s = 0.192$

4- Inhibition of human dihydrofolate reductase by pyrimidines [46]

K_I represents the lowest concentration of pyrimidine which inhibits enzyme activity.

$$Log({}^{1}/_{KI}) = 0.59\pi_{3,5} - 0.63\,(\beta \cdot 10\pi^{3,5}+1) + 0.19\pi_4$$

$n = 38 \quad r = 0.879 \quad s = 0.266$

5- Olfactory threshold concentration of alkanes [47]

C is the lowest concentration of a compound which can be nasally detected.

$$Log({}^{1}/_{C}) = 2.57LogP - 0.24(LogP)^2 + 1.36$$

$n = 7 \quad r = 0.970 \quad s = 0.39$

In each model:

- n represents the number of compounds in the training set
- r is the correlation coefficient of the QSAR.
- s is the standard error of the QSAR.

The latter two values, are discussed in further detail in 5.4 and reflect the statistical significance of the QSAR with regards to how well the model fits the biological data used in its generation.

5.0 Statistical Techniques

5.1 Introduction

In order to develop a QSAR, it is essential to establish a relationship between the biological data and the physico-chemical and structural descriptors of the compounds. With the careful application of statistical methods, much more information than is at first apparent may be gleaned from the correlation.

A variety of statistical techniques for use in QSAR development are available, which can be divided into two broad categories, depending upon the type of biological activity end-point used; a quantitative end point which yields numerical values, and statistical methods such as multiple regression analysis are used to correlate the biological data with the physico-chemical parameters, and a qualitative end point which yields, for example a positive or negative response rather than numerical data, and pattern recognition techniques are used to obtain relationships.

5.2 Linear Relationships

5.21 Simple Graphical Plots

If only one descriptor is used in the development of a QSAR, its relationship to the biological activity can be expressed as a simple linear graph and the QSAR equation 5.1 is obtained as:

$$y = mx + c \qquad (5.1)$$

Where y represents the biological activity value

x represents the descriptor value

m represents the slope of the graph

c represents the intercept of the line of the graph and the axis.

5.22 Regression Analysis

Historically, in the early 1960's, two sets of workers, Hansch & Fujita [62,69,95] and Free & Wilson [96], independently first applied the use of statistical analysis to QSAR studies. Each of their respective methods being based on regression analysis. Today, regression analysis is the most widely used statistical technique in QSAR [97,98].

Regression analysis can be explained in simple terms as 'a search of data for a relationship between the dependent variable, Y (biological activity) and one or more independent variables, X (descriptors)' and by equation 5.2, which is essentially a modified version of equation 5.1.

$$Y = \beta o + \beta_1 X \quad (5.2)$$

Where β_1 is the slope of the regression line

βo is the intercept

The relationship may not be truly rectilinear, but can appear as such due to the segment of the data windowed, i.e. the data chosen may be a part of a larger curve.

The regression line may not initially pass through any of the data points, this is overcome with the addition of an error term (figure 5.1) to the general equation 5.2 giving the new equation 5.3

$$Y_i = \beta o + \beta_1 X + \varepsilon_i \quad (5.3)$$

Where ε_i is the mean residual of the observed value Y minus the predicted value Y_i.

When a regression line is fitted to a set of data points the aim is to minimise the distances between the points and the regression line, using typically the method of least squares, i.e. minimising $\Sigma\varepsilon_i^2$. When fitting a mathematical equation to the regression line, it is assumed that the population at large all behave in the same fashion. That is, a confidence interval based on ε_i is assumed in which the error terms all have a normal distribution (N), as depicted by equation 5.4. If over a range of means the variance is homoskedastic (constant) and the regression has effectively modelled the data. In some instances the model does not display normal distribution and is said to be heteroskedastic. In such cases it may be possible to achieve normal distribution by mathematically transforming the data to a logarithmic, square-root or inverse ($^1/_y$), for example.

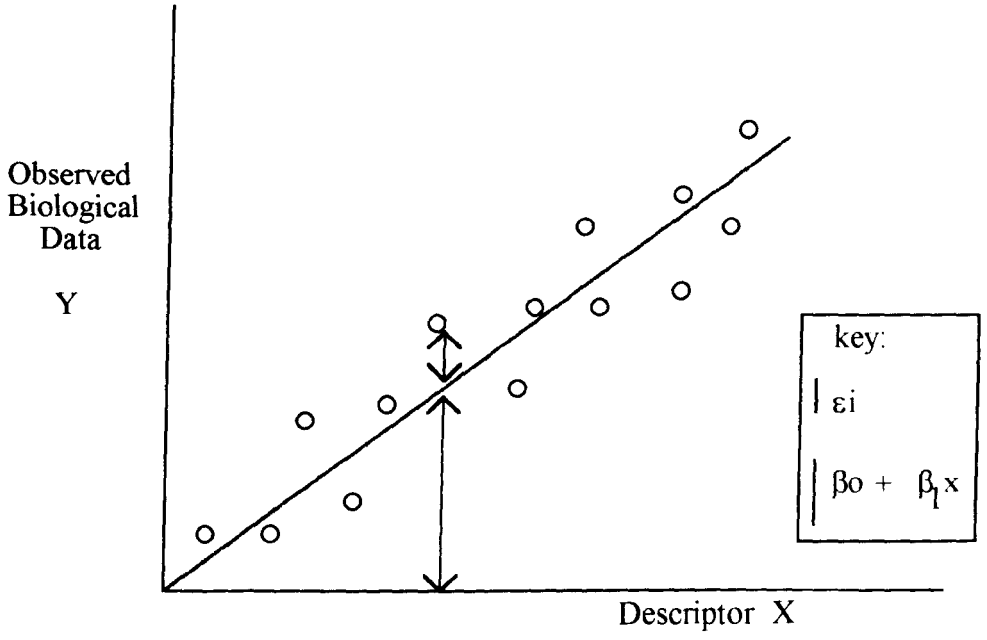
$$\varepsilon i \sim N(\mu, \sigma^2) \quad (5.4)$$

Where μ is the mean value of y in regression analysis

σ^2 is the variance

Regression analysis has a number of advantages, in that it is predictive, easy to understand and highlights any outliers. However, it also, has some disadvantages, in that the technique needs quantitative data, there are risks of chance correlations and collinearity between descriptors can lead to instability. Although regression analysis relies heavily on continuous parameters, that is to say quantitative data, it is possible to incorporate some qualitative responses in to the analysis (i.e. yes or no responses) in terms of numerical indicator variables, for example let 1 represent a positive and 0 a negative response).

Figure 5.1 2D Representation of a Linear Regression Relationship



5.221 Hansch or Linear Free Energy Approach [99,100]

Hansch *et al* [69,95], postulated that the biological activity of a molecule is a linear function of one or more of three different properties of that molecule, namely its hydrophobic, electronic and steric effects. The influence of hydrophobicity is related to the probability of the molecule moving between biological environments in order to reach the required site of action. The electronic properties of the molecule control the ability of the molecule to interact with an appropriate receptor through hydrogen bonding, dipole forces etc., and also, in the case of ionisable compounds, control the extent of ionisation. Steric properties affect the ability of a molecule to fit the receptor site and can also affect the rate of metabolism.

Model generation is based upon 3 assumptions;-

1- that one can quantitatively measure or calculate molecular physico-chemical and structural properties which will determine the biological activity of a molecule,

2- that one can quantitatively measure the appropriate biological response,

3- that one can mathematically describe the relationship between the physico-chemical and structural descriptors and biological properties [99].

Using these assumptions Hansch et al [69] were able to derive a model (equation 5.5) which describes the relationship of the biological activity with the physical, chemical and structural properties for a set of compounds.

$$\text{Log } {}^{1}/_{C} = a(\text{LogP})^2 + b\text{LogP} + cE + dS + e \qquad (5.5)$$

Where C represents the molar concentration of the compound under investigation which elicits a constant biological response from a given organism.

LogP represents hydrophobic effects (octanol/water partition coefficient),

E represents electronic effects,

S represents steric effects,

a,b,c,d,e are constants obtained through multi-linear regression analysis.

The $(\text{LogP})^2$ term was introduced to allow for the observed biphasic dependence of biological activity on hydrophobicity.

The Hansch approach, also known as multiple linear regression, to QSAR is widely used within the pharmaceutical industry and other fields of science for the investigation of a range of biological effects [99] and also allows quantitative predictions of activity to be made, but it suffers from the drawback of the possibility of chance correlations [99].

5.222 The Free-Wilson Approach

Free and Wilson [96] developed a method for deriving *de novo* biological substituent constants, to be used when for example, physico-chemical parameters are unavailable. The method is based on the assumption that in a series of related compounds each particular activity is independent of the presence or absence of substituents at other positions within the same molecule. Each compound yields an equation, in one of two forms, equations' 5.6 or 5.7, and the group contributions are found by solving a set of multiple simultaneous equations [101].

$$\text{Biological Activity} = \sum A_{ij} \times S_{ij} + k \qquad (5.6)$$

Where A_{ij} represents the activity contribution of a substituent i at position j.

S_{ij} 1 or 0 according to the presence or absence of substituent, i at position j.

k is a constant, representing the average activity of a series of compounds [96].

$$\text{Biological Activity} = Sa + Sb + \ldots + u \qquad (5.7)$$

Where Sa,Sb, etc. are the contributions added by the substituents a,b, etc. respectively.

u is the contribution of a hypothetical parent compound to the bio-activity.

An advantage of the Free-Wilson approach is that there is no need to determine physico-chemical or other properties, as the *de novo* constant (the additive group constant) generated, combines information on all the properties of the substituent. However, a disadvantage is that predictions are limited to compounds comprising the parent molecule and the substituents contained within the 'training set'. Also, the numerical value attained gives no direct information as to the mechanism of action and so can only be used to give interpolative predictions. Furthermore, the method assumes that each substituent has a constant contribution to activity, irrespective of other substituents. This is not necessarily so: For example a chlorine substituent could increase the polarity of a derivative of low hydrophobicity, but could decrease the activity of a hydrophobic derivative (see figure 4.1).

5.23 Pattern Recognition or Multi-Variate Analysis [99]

Pattern recognition methods are being used increasingly more in QSAR [102]. The term 'pattern recognition' serves to describe any mathematical or statistical method which may be used to detect patterns which lie within data sets. Pattern recognition is used to seek qualitative correlations between a set of atomic or molecular structure descriptors and the presence or absence of specific biological activities, usually in a series of structurally related compounds. To appreciate fully the action of pattern recognition, the data matrix should be considered as a multi-dimensional information space. For n parameters there are n dimensions. Data for each compound are projected into the information space, so each is represented by a discrete point.

The Hansch approach (5.221) requires that the biological activity be expressed in terms of hydrophobicity, electronic and steric properties, which requires the use of a number of independent variables. Any representation of the relationship would therefore, need to be in several dimensions, which is impossible to achieve for more than two such variables graphically. The use of statistical computer software is therefore imperative. The increased use of computational chemistry and molecular modelling in QSAR has led to the development of many software packages which enable the generation of a great number of independent variables for each compound (50 to 100 as a matter of routine). Hyde and Livingstone [103] termed the subsequently generated data matrices as 'Over Square'. Pattern recognition methods are intended for use with data sets containing many variables and usually consider all variables simultaneously, and so are suited to the statistical manipulation of the 'over-square' matrix.

Some pattern recognition methods have their origins in artificial intelligence research, which resulted in the establishment of rules which can be used to classify a compound into a data set based on its biological properties. Two types of data sets are essentially involved, a **training set** for which classification is known and which is used to develop a predicitvely capable QSAR and a **test set** for which the classification is unknown and on which predictions are made. A third type of data set, the **evaluation set** which consists of compounds of known classification but which are not used in the model development process may also be used to check the validity and credibility of the developed model [100]. This concept of training or learning allowed Livingstone [103] to classify pattern recognition techniques into the two categories, **supervised** and **unsupervised** techniques (table 5.1), the major differences being that supervised techniques require the involvement of biological data.

Supervised Learning requires the use of biological data in the process of model construction, i.e. the training set is used to produce the rules of classification.

In supervised learning techniques, if n >/= m then the technique may not work, due to failure to invert the matrix or due to mis-classification of data.

Unsupervised Learning methods do not need any form of classification to be assigned in order for the model creation, i.e. the known activity categories of the 'training set' are not utilised.

In both supervised and unsupervised learning techniques extra variables which do not contain useful information may act to obscure meaningful patterns and chance correlations also present problems. Chance correlations may present themselves, due to the fact that there are many independent (physico-chemical) variables and it is inevitable that descriptors may describe the same molecular feature. In such cases statistical manipulation, such as multiple linear regression analysis may incur the phenomenon of collinearity between different independent variables [84], which will result in spurious results. Topliss and Costello [104] evolved the 'rule of thumb' for QSAR, that a minimum of thirty observations be used to test five variables, in order to minimise the risk of chance correlations and that at least five compounds should be included for every parameter in the final model.

Table 5.1 Pattern Recognition Methods used in QSAR.
(Adapted from Livingstone [105])

Supervised Learning Techniques	Unsupervised Learning Techniques
Multiple regression	Non-linear mapping
Discriminant analysis	Principal components
Linear learning machine	Factor analysis
Canonical correlation	Cluster analysis
Adaptive least squares	K nearest neighbour

Some pattern recognition techniques, such as K-nearest neighbours, discriminant analysis and SIMCA utilise the properties in the information space to enable quantitative distinctions to be made [106]. This is especially beneficial when activity is categorically classified, e.g. active or inactive, because the position in the information space of a compound of unknown activity relative to compounds of known activity may enable estimation of the unknown activity.

5.231 Supervised Learning Techniques

5.2311 Stepwise Multi-linear Regression Analysis (SMRA)

SMRA is used widely in QSAR. The Hansch approach [95] to QSAR may be performed with a few selected parameters or the procedure can employ the process's of stepwise and best subsets regression analysis, in order to develop the predictive model of biological activity. SMRA is based on measured, predicted or calculated physico-chemical descriptors and relies on the availability of quantitative biological data. It is useful when large numbers of variables are being considered.

As with all techniques SMRA has its advantages and disadvantages, as outlined in table 5.2. The empirical nature of SMRA is both advantageous and limiting. The empirical equations derived do not require the prior development of a hypothesis of the mechanism of action. Those parameters identified as important in the modelling will or could lead to the development of a hypothesis. Martin [64] stated that the empirical nature of the equations is limiting, as they provide little feedback to a theoretical understanding of the problem and conversely, any hypothesis developed is of no help if the equation fails to explain a new data set.

Table 5.2 Advantages and Limitations of SMRA
(Adapted from Livingstone [105])

Advantages	Limitations
empirical	empirical
predictive	chance correlations
outlier recognition	continuous parameters required
cheap	

SMRA can be performed in one of two directions, namely forwards and backwards.

In the **forward selection method**, the calculation commences with the best predictor variable and adds successively to the equation those variables with the largest F-to enter values.

In the **backward elimination method**, the starting point is an equation comprising of all the variables and those variables with the lowest F-to enter values are then successively removed from the equation.

Once an equation has been developed it is essential to assess how well the equation fits the data used in its generation. This is achieved by attaining the residual value by subtracting that value of Y calculated by the equation from the actual value of the dependent variable (Y), for each compound in the data set.

Examination of the residual values for a complete data set enables the detection and identification of any 'outliers', these being any residuals which are far greater, in absolute value than the other ones and so which lie three or four standard deviations from the mean of all the residuals.

Since, the function of QSAR is to develop robust and predictive models, it is valuable to try and gain insight into reasons why any particular compound behaves as an 'outlier'.

Devillers and Lipnick [97] ascribed 'outlier' behaviour to one or more of the following;

1- the biological data represent a lower degree of precision, reflecting one or more error sources not associated with other studied compounds,

2- the biological data reflect a systematic error,

3- the bio-data represent a different biological end-point than that for other compounds,

4- there may be errors in the independent (descriptor) variables,

5- the 'outlier' has one or more physico-chemical property values which places it outside the spanned substituent space of the other compounds,

6- the 'outlier' may yield one or more metabolites.

An understanding of the cause of 'outlier' behaviour may be of great value in gaining a more fundamental insight into the underlying chemical processes governing biological action.

5.2312 Discriminant Analysis (DA)

Discriminant analysis (DA) is a pattern recognition technique, which has proven to be the most general technique available for dealing with semi-empirical or qualitative biological data and relating it to 'mixed variables'. It was first used in SAR studies by Martin *et al* [107] in a study of the weighting and statistical significance of various physico-chemical properties which could distinguish between agonists and antagonists.

In discriminant analysis the user has advanced knowledge as to the identity of each group, e.g. active or inactive, and sets out to explain and maximise the separation between each group using specific predetermined vectors. The data for the individual compounds under investigation are projected as definite points into a 'pattern space', which is a derived orthogonal co-ordinate system, with the system axes being those of the physico-chemical descriptors. In the 'ideal' situation related groups or classes of compounds will cluster together in different regions within the 'pattern space' so

separating active and inactive compounds into two distinct groups. The regions of 'pattern space' where related compounds have grouped are then defined using one of two basic methods.

Hyperplane methods are the most widely used and involve the derivation of an equation for a plane that will separate the pattern space into distinct regions, each representing a region of space where one of the groups or classes of compounds is most likely to occur. The optimal linear discriminant function (LDF), a function line originating from the origin and which gives minimal overlap between the two clusters [93,108]. Solution of the derived equation with respect to each test compound is then determined and then used to assign each untested compound to the appropriate group.

Distance methods involve the allocation of the untested compound (Q) to the nearest group or population, where 'nearness' is a measure of the probability of Q belonging to a specific group or class, rather than a metrical measurement. The general distance used is the Malhanobis distance.

5.2313 SIMCA - Linear Learning Machine

SIMCA is a classification method, which seeks to describe each activity class separately by generating a principal component model which describes the individual activity class (see section 5.2321). These models can be thought of as 'hyper-boxes' in the n-dimensional parameter space, that act to enclose all of the compounds of a particular class [64].

5.2314 K-Nearest Neighbour (KNN)

KNN makes use of the same matrix of interpoint distances as non-linear mapping and cluster analysis (5.31 and 5.2322 respectively) to classify compounds based upon their position in a multi-dimensional space [109]. Compounds are assigned to membership of a particular group or class on the basis of the class membership of their nearest neighbours, in terms of inter-point distances (K). K is normally selected *a priori* and taken as being an odd number, so that a majority vote will decide the class of assignment.

5.2315 Canonical Correlation Analysis (CCA)

CCA is a multi-variate technique which enables the simultaneous analysis and correlation of two data sets [102], the size of which need not be the same. CCA calculates a linear combination of responses (q) and a linear combination of descriptors (p), such that a pairwise correlation between the two vectors is at a maximum. The next

best correlation, which is orthogonal to the previous, is then calculated. The process is repeated until the number of correlations is equal to the number of data points in the smallest set. Comparison of the correlation scores enables the characteristics of the molecular properties to determined and biological data to be assigned relative to the data points.

5.232 Unsupervised Learning Techniques

5.2321 Principal Component Analysis (PCA).

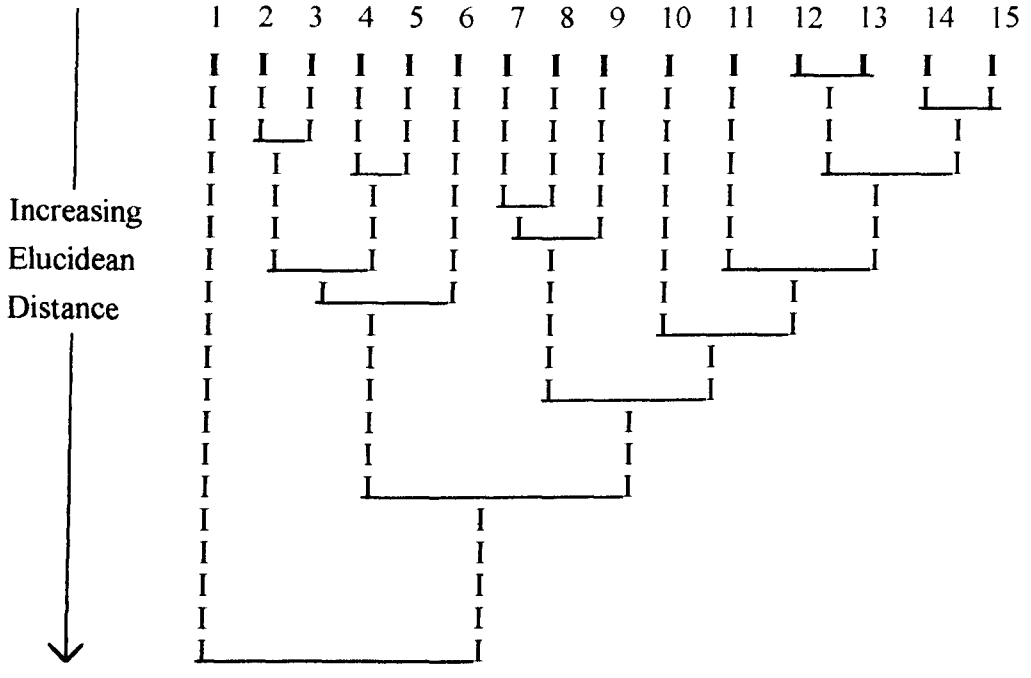
PCA is not actually a statistical technique but is a data display technique, which is particularly useful at the start of an analysis, where it serves to confirm that a data set contains useful information [102]. The process involves the transformation of the original data set into a new set of fewer uncorrelated variables, which are linear combinations of the originals and are created in such a way as to account all the original variation. The effect of the new data set creation is to reduce the total number of variables and thus reduce the likelihood of information redundancy, as seen with the original data set. The first principal component (PC1) is a linear combination of the values of those descriptors whose variability is as large as possible. Therefore, it is PC1 which contains the largest part of the variance of the data set, and subsequent components contain progressively smaller amounts of variance, some latter PCs may infact contain zero variance. PCs have disadvantages in that they are often difficult to interpret, since they are composed of a combination of variables and it is not possible to transfer the co-ordinates of a point on a PC plot to values of individual variables.

5.2322 Cluster Analysis (CA)

CA is a classification scheme rather than a procedure allowing the prediction of biological activity. CA is used to identify those factors which are of importance in classifying a compound due to its activity or lack of it [110]. The basis of CA is to define a space of k descriptors and to define n compounds within that space in terms of their respective descriptor values. Then to calculate the Euclidean distances (d_{ij}) between each pair of points in the descriptor space. The Euclidean distances for each compound within the data set and their respective similarity to other compounds can be displayed pictorially in the form of a dendogram, figure 5.2. Analysis of the dendogram enables identification of compounds which are most similar, of the number of groups or classes contained within a data set and of the presence of any outliers.

Hansch and Unger [110] have used CA to identify redundancies amongst substituent parameters within a given data set.

Figure 5.2 Dendogram Presentation of Cluster Analysis Results



In the above case it can be observed that the data falls into three classes;

1- compounds 7,8 and 9,

2- compounds 10 to 15 inclusive, and

3- compounds 2 to 6 inclusive.

Class 1 (compounds 7,8 and 9) is more similar (closely related) to class 2 (compounds 10 to 15) than to class 3 (compounds 2 to 6), due to the shortness of the elucidean distance between the respective classes.

The two most similar compounds are 12 and 13, due to the extreme shortness of the distance between them.

Compound 1 is not similar to any of the other compounds and so must be considered to be an outlier.

5.3 Non-Linear Relationships

In some cases a linear regression model is inadequate to model the dependence of biological activity on an independent variable, e.g. the case of partition coefficient (P) in the equation derived by Hansch (4.3211). True non-linear relationships are those which cannot be described by any line, straight or not. Sometimes there is a non-linear dependence of biological activity on one or more parameters. In such cases appropriate transformations of the parameters (biological and / or descriptors) may serve to model the data, e.g. by squaring or inverting.

Kubinyni [111] was able to improve Hansch's model by modifying it in order to account for the fact that biological activity and P initially vary in a recti-linear fashion, increasing on a Log scale to an optimal value after which the values decrease in a recti-linear fashion. That is the model is bi-linear.

The new model being expressed in terms of equation 5.8.

$$\text{Log}\,{}^{1}/_{C} = a\text{LogP} - b\text{LogP}(\beta P+1) + d \qquad (5.8)$$

Where β is a non-linear term calculated by an iterative procedure.

P is the n-octanol/water partition coefficient

a,b and d are linear terms calculated by linear regression.

5.31 Non-Linear Mapping (NLM)

NLM is a non-linear technique for the projection of points from an n-dimensional space to a two or more dimensional space. NLM operates by comparing the distances between pairs of points in the n-space (Dij*) and in the two dimensional space (dij) and seeks to minimise the differences between these interpoint distances using an error function (E). This has the effect of making similar compounds group together in tighter clusters.

Analysis of the non-linear map simply suggests that the physico-chemical data contain information that will be useful in the classification of the compounds. NLM has the advantage that it does not impose a linear combination on the variables, in order to produce the two-dimensional display. But it does suffer from a number of disadvantages. Since the method relies on the minimisation of a set of interpoint distances, it is not possible simply to plot a new compound onto the map; the whole map must be recalculated and in some cases the inclusion of a new compound may alter the map considerably, although useful patterns are generally preserved [111].

5.4 "Goodness of Fit" of Statistical Models

The "goodness of fit" of the derived statistical equation, with regard to the biological data used in its generation, is expressed by the statistical criterion of 'total sum of squares' (SS_{TOT}), and can be defined in terms of equation 5.9.

$$SS_{TOT} = \Sigma \ (y_i - y)^2 \qquad (5.9)$$

Where y_i is the observed value of biological activity,
y is the mean value of biological activity.

Sokal and Rohlf [112] presented a detailed description of the partitioning of TS in which all variations present in the sample can be separated into two parts, in which most variation is attributed to differences between groups and the remaining variation can be attributed to differences within groups (different individual Y and group mean values). Thus, 'total sum of squares' (SS_{TOT}) can be factored into two parts; i) that part attributed to the model, termed **MS** and ii) that part attributed to error or the residual part, termed **RS**. (**TS** has n-1 degrees of freedom, where n is the number of observed values of Y and is continuous and so ever increasing. **MS** has p degrees of freedom, where p is the number of independent variables and **RS** has n-p-1 degrees of freedom). Further terms of importance in the estimation of a statistical models 'goodness of fit' have been derived, and some are detailed below.

A - Fischer and then Hayslett and Murphy [113] determined that the ratio of RS and the number of degrees of freedom, yielded a term for variance (equation 5.10), known as **'the error mean square'** (S^2 or s^2):

$$s^2 = \frac{RS}{n-p-1} \qquad (5.10)$$

Martin [64] determined that the square root of s^2 would give a value (s) which corresponds to the **standard error of the estimate.**

B - The global measurement of the quality of a model is the '**coefficient of determination**' (r^2), which is described in equation 5.11.

$$r^2 = \frac{MS}{TS} \quad \text{(the regression or model sum of squares)} \quad (5.11)$$

Values of r vary between 0 and 1, with 1 being representative of a perfect fit. The square root of r^2 gives a value, r which is the 'correlation coefficient'.

C - Houlton [113] suggested that the '**correlation coefficient of determination**' should be adjusted for the number of degrees of freedom, the resultant value being termed 'r^2(adj)'.

D - The Fisher statistic or variance ratio (F value), as described in equation 5.12, allows the estimation of whether the obtained relationship is statistically significant. The larger the value of F the greater the probability that the derived equation is actually modelling the data and is not due to chance correlations. Analysis of the 'F' distribution allows a choice of confidence limits to be established. If F = 0 then there is no relationship between x and y, and the larger F the greater is the significance of the realtionship between x and y.

$$F(\text{variance ratio}) = \frac{MS}{s^2} \quad \begin{matrix}\text{(model /regression mean square)} \\ \text{(residual/ error mean square)}\end{matrix} \quad (5.12)$$

E - Standard error s of coefficients of each variable, as well as r^2 and F are used in initial tests (prior to validation), to allow determination of whether the relationship determined is strong or weak. Strong relationships have high r^2 values (preferably over 0.9), low s values (preferably less than 0.3) and highly significant F values. If such a strong relationship is indicated, it can be concluded that the QSAR derived has modelled the relationship well and that with careful use, it may provide useful information with regard to the biological mechanism of molecular action and may allow predictions of biological response to be made for new related compounds. However, QSARs with weak relationships present low r^2 values, high s values and less significant F values. In such a case it can be concluded that the model is unsuccessful and interpretations made using the model should not be made.

F- Validity plots [98]

Plot 1 is a plot of the difference between the observed biological effect and the value of biological activity predicted by the model (Ei) against the values of biological activity predicted by the model.
If the points are evenly distributed about a central zero line then the plot is termed homoscedastic and signifies that error is reasonably consistent throughout all the biological data used in the generation of the model and the model is said to be valid. If the points appear to diverge from or converge upon the zero line then the plot is termed heteroscedastic and signifies that any change in stimulus will proportionately affect the response, that is to say that a large scope for error exists within the biological data and therefore the model is invalid. If the data are heteroscedastic it means that if the overall variance is based on all of the data, then for the regions where the variance is small, the variance will be overestimated, in regions where the variance is large, it will be underestimated by the overall variance.

Plot 2 is a plot of the difference between the observed biological effect and the value of biological activity predicted by the model. The plot illustrates the probability that all the error terms will have an equal distribution about a regression line through the data points and so is a test of normality.
A linear plot shows the error to have a normal distribution about the linear line and therefore suggests the model is valid. A skewed plot shows that the distribution of error is not normal and therefore suggests that the model is not valid.

Analysis of the graphical plots in conjunction with the statistical properties, such as r^2, produced during regression analysis show how well the model fits the data used in its development, but they do not provide any information as to the predictive powers of the model, this is attained by assessment of the models validity.

5.5 Validation of Statistical Models

The statistical validation of models has been increasingly recognised as a vital step in the development of a QSAR. Currently there are two methods of validation in common use. The first method and possibly the more appropriate, involves the separation of data into two data sets. One the 'training set', which is used to develop the QSAR model, and the other is an 'evaluation set', which is not used in the model development, but is used to validate the model. The predictability of the model is tested by calculating the biological activity of the 'test set' and comparing the resultant calculated activities with actual measured activities of those same compounds.

The second method of validation is known as 'leave-one-out' or as 'cross-validation'. The model is developed using the entire data set. Validation is performed by excluding one or more compounds from the data set and the model is recalculated. The recalculated model is used to predict the biological activity of the excluded compound(s). The process is repeated until each compound has been omitted from the data set and its biological activity been calculated using the alternative model. The deviation of the calculated biological activities from those evaluated in the original model, gives an indication of the predictability of the original model [114].

Wold [115] proposed that a model's **'predictive significance'** can be evaluated from the values of Predictive REsidual Sum of Squares (PRESS) and Sum of Squares of response values (SSY), as generated through 'leave-one-out' cross-validation procedures, in accordance with equation 5.13.

$$\frac{PRESS}{SSY} = \text{Predictive significance} \qquad (5.13)$$

Where values < 0.4 indicate predictive significance.

Baroni et al [116] have shown, using equation 5.14, that it is possible from the predictive significance to calculate a value indicating the model's predictive validity called the **'cross-validated coefficient of determination'** ($r(CV)^2$).

$$r(CV)^2 = 1 - \text{Predictive significance} \qquad (5.14)$$

In accordance with the above proposal of Wold and the equation of Baroni (1 - 0.4) a threshold of predictive validity would be a $r(CV)^2$ value of 0.6. Below which the model is not valid. Clemetis [46] has proposed that a $r(CV)^2$ value of 0.1 indicates predictive validity. Clearly the value of $r(CV)^2$ chosen as the threshold of predictive validity is open to variation and currently is ultimaltely set at a level determined by and at the discretion of the model generator.

6.0 Immunology

6.1 Introduction

The immune system is a multifunctional adaptive system which is classically concerned with maintenance of host defence mechanisms, is responsible for the process's of self and non self recognition, homeostasis, immuno-surveillance, specificity and memory [117] and as such is a tightly regulated, highly organised complex network of interacting organs, tissues, cells and molecules which act in concert in an ordered and regulated fashion in order to give rise to a specific adaptive immune response following the recognition of materials within the system as foreign or changes in the antigenic environment [7,118,119].

The delicate balance of the immune system is regulated by a network of events which in turn are controlled by the differential secretions of cytokines from specific cells and by the genetic capabilities of the host itself [118].

An ideal immunologic response is one that adequately deals with insults whilst protecting the host in such a way as not to overreact and bring about adverse immunologic responses (immunotoxicological responses), such as allergy, immunosuppression, reduced immunocompetance or autoimmunity.

6.2 History of Immunological Ideas

The history of immunological ideas must have begun with the wives' knowledge that some recognisable childhood infections only struck once, and later when people began to recognise that persons with pock marked faces did not develop a second case of smallpox. Hence, the eighteenth century epidemic of smallpox, throughout Europe gave immunological ideas' pre-eminence [120].

The history of virolisation as a deliberate attempt to provoke immunity and the first effective immunisation performed by Edward Jenner in 1796 [120], in which a vaccination with cowpox was utilised as a means of protecting against smallpox, is known to all. The first general scientific method of immunisation against infectious diseases was provided by Pasteur in 1880 [120], when he protected fowl against chicken cholera by inoculating them with attenuated virus. He explained his results by suggesting that "immunisation had 'exhausted' something necessary for the proliferation, *in vivo*, of the virulent virus".

Also, around this time a new problem became universal recognised that of bacteria and pathogenic resistance.

In 1882 Elie Metchinkoff (a Russian zoologist) [120], performed a series of experiments in which he identified the role of motile cells within transparent starfish larva in the process of protection from foreign intruders. He showed that the mechanism of this protection was through leukocytes of the host engulfing the invading micro-organism, a process he termed 'phagocytosis' [120]. This work provided the starting point for the study of cellular immunity.

In 1894 Pfeffier and Isaeff [120] described the importance of the humoral defence mechanism, known as the 'Pfeffier phenomenon', and concluded that this was the main defence mechanism of an organism.

Controversy between the cellular and humoral theories of immunity existed until 1903 when Almorth Wrights' ideas on opsonins lead to the recognition of the complexity of the processes and cellular types involved in immunity [120].

Further immunological theory developed as something concerned with a wider field than just immunity to infection [7]. In 1898, Bordet [120] recognised the immune lysis of foreign red cells, and in 1904 Landsteiner [120] discovered the ABO blood groups. From these works came the realisation that only foreign material was antigenic and thus added new problems to the understanding of the immunological processes, and the main stream of immunological thought became concerned with the antibody. By 1930 it was clear that antibodies were associated with serum globlins. From this Lederberg developed the 'instructive' theory of antibody formation. Landsteiners work in the 1930s was of importance in establishing a chemical basis for the specificity of the immune pattern and the suggestion that antibodies were produced by the body, by some impression of a complementary pattern on normal serum globin during its synthesis.

In the 1940s Pauling [120] began to relate immunological ideas and the developing biochemical principles, and the development of quantitative biochemical techniques in place of the medical immunological techniques of titration. Between 1940 and 1955 there was a rapid technical advance in immunology, with Tiselius' simple electrophoresis, Ouchterlonys' visualisation of Antigen-Antibody precipitation reaction on agar and with Williams and Grabar's combination of the latter in the development of immunoelectrophoresis [121].

Over the past 40 years, numerous advances in technology and theory have enabled vast amounts of information on the immune system, and its actions in response to infection to be amassed and have provided the current knowledge. However, to date not all the problems regarding the exact structure, function or relationships of all the immunological components, have been solved and much work still needs to be performed if all the answers are to be found.

6.3 Immunity

There is a number of biological defence mechanisms that protect an individual from potentially harmful material and micro-organisms [122]. These mechanisms can roughly be divided into two types: Innate immunity which is the first line of defence a foreign molecule encounters on entering the bodily environment, such as the physical barriers of the skin, mucus, enzymes and lysozymes. Adaptive immunity which develops in response to contact with and repeated challenge by an infectious agent, it comprises components which have the ability to recognise variations in the structure of foreign material and to remember them thus eliminating foreign material initially and on subsequent occasions.

The two types interact and regions of commonality exist, but it is adaptive immunity, because of its ever changing and adaptive nature, which is of the most interest to the immunologist.

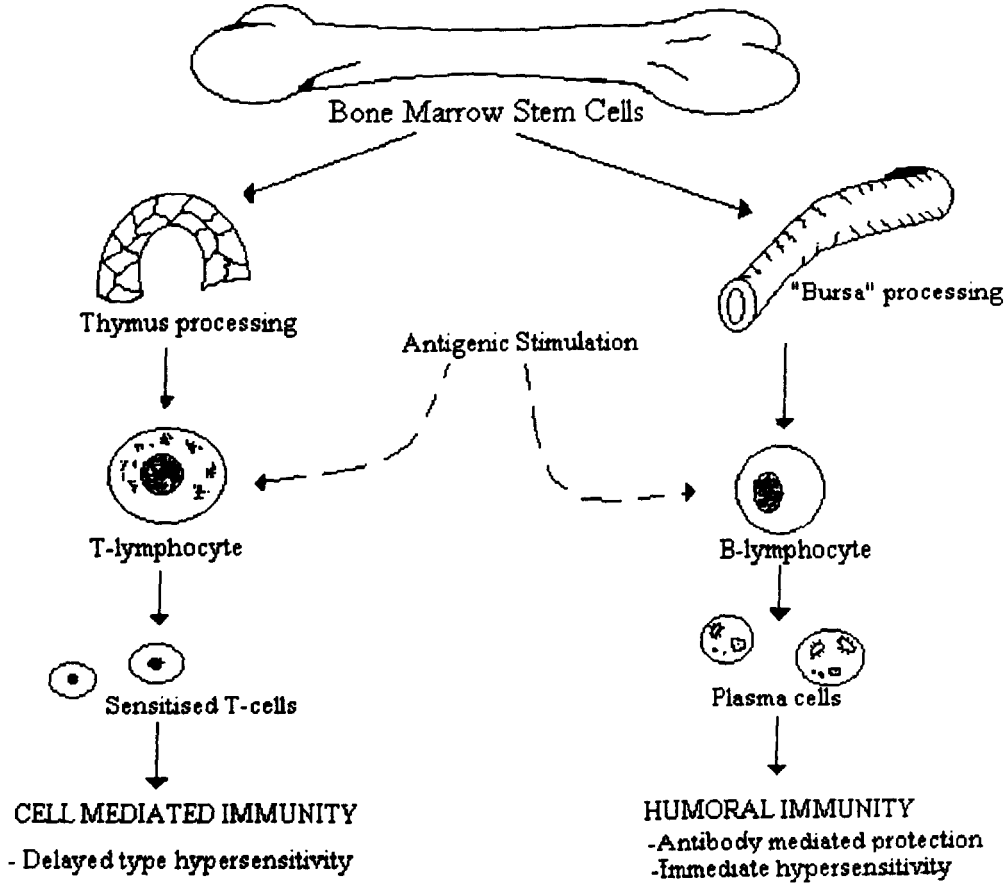
6.31 Adaptive Immunity

The adaptive immune system continually develops throughout life and comprises two distinctly derived but not entirely independent systems (figure 6.1): Cell-mediated Immunity, in which the presence of foreign matter stimulates cytotoxic cells to produce sensitised lymphocytes which act against the invading matter and Humoral Immunity, in which specific antigen stimulated B lymphocytes produce plasma cells which subsequently produce specific immunoglobulins (antibodies) that act to eliminate the specific foreign agent, via a number of mechanisms, including the neutralisation of toxins and viruses, the assistance of cell lysis and opsonisation and chemotaxis.

6.4 Immunohistology

Both anatomically and functionally the immune system comprises three main compartments; the stem cell pool which is distributed evenly throughout the blood and bone marrow, the primary lymphoid organs which are involved in the development and normal ontgeny of the T and B lymphocytes, and the secondary lymphoid organs which comprise the centres of cell mediated humoral responses the spleen and the lymph nodes, as well as the mature functioning lymphocytes and accessory cells [122].

Figure 6.1 Illustration of the Differential Derivation of the Two Systems of Adaptive Immunity
(Adapted from Vos [123])



6.41 Lymphoid Organs

The lymphoid system comprises those bodily tissues or organs in which lymphocytes reside, which are responsible for the production of the immune cells and maintenance of their presence within the circulatory systems of blood and lymph, these being primarily the thymus, the spleen and the lymph nodes .

6.411 The Thymus

The thymus is an epithelial organ, originating from the third bronchial pouch during embryogenesis and which has migrated to the mediastinum which comprises two regions (figure 6.2) the cortex and the medulla. The cortex is densely honeycombed with pockets of proliferating lymphocytes and can be sub-divided into outer and deeper regions. The outer cortex is composed of specialised nurse cells which contain the primary lymphocytes. The deeper cortex contains dendritic cells which express the major histocompatablity class II molecules (MHC II), essential for T cell activation. The medulla is irregularly infiltrated by lymphocytes and other cells associated with blood vessels, i.e. macrophages. Within the medulla are thymic epithelial cells or interdigitating cells responsible for the secretion of several hormones which act to influence T-lymphocyte development and differentiation, the most prominent hormones being; four types of Thymopoietins, twenty-five types of Thymolysins, Thymielin and Thymic humoral factor.

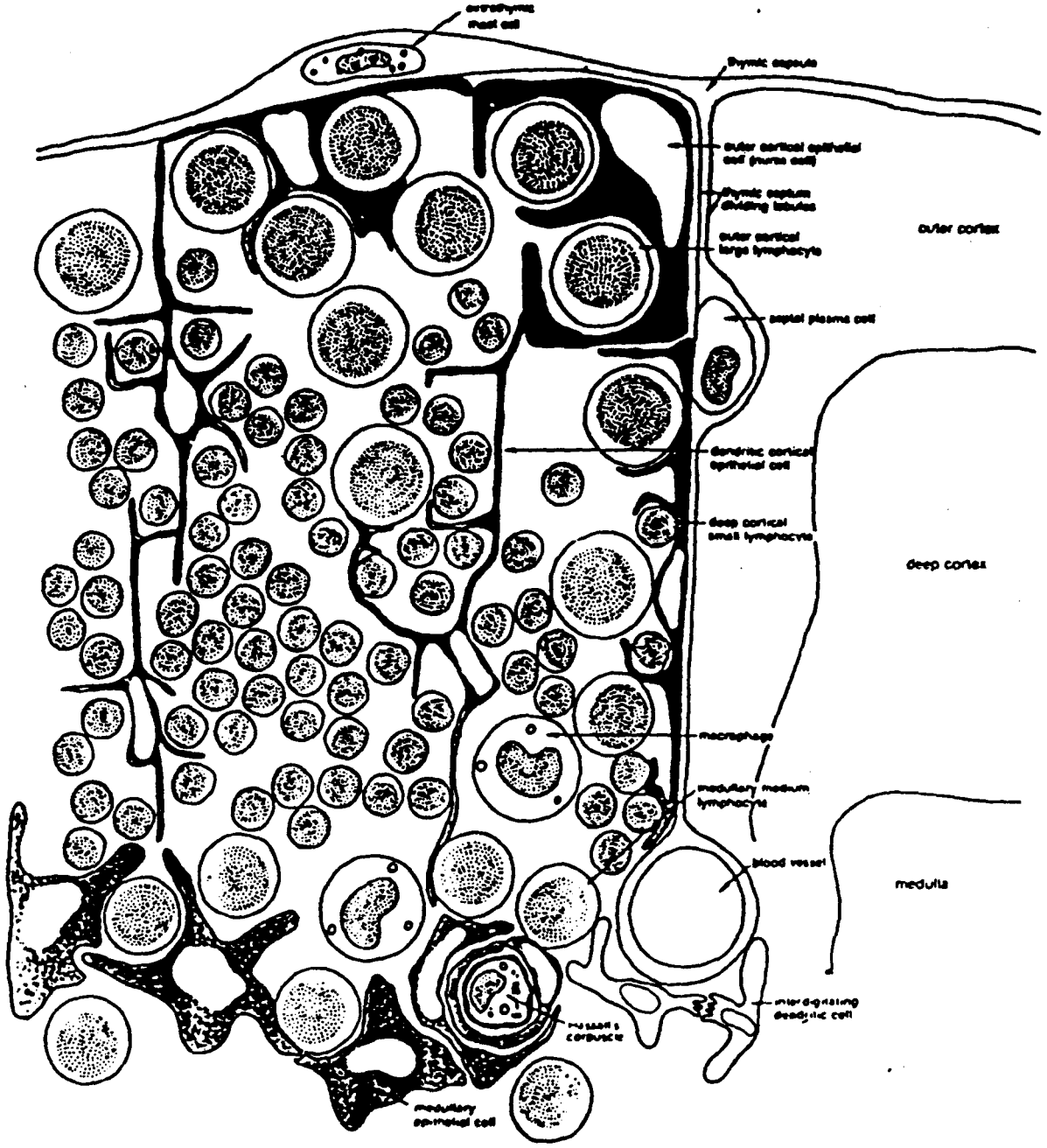
Precursor T cells from the bone marrow enter the thymus nurse cells which then migrate down through the thymus cortex and medulla, so enabling, differentiation and development of the precursor T cells to into different types of virgin T-lymphocytes; T_{helper}, $T_{suppresser}$ and $T_{cytotoxic}$ cells.

Only a small proportion (1%) of the virgin T-lymphocytes produced in the thymus will leave via the blood system and migrate to the lymph node. The remainder are eliminated / degraded by the Hassells' corpuscles of the thymus medulla.

6.412 The Spleen

The spleen is a highly vasculated organ, comprising two regions (figure 6.3), the red which acts as a storage reservoir for surplus red blood cells and breakdown damaged cells, and white pulp which represents the aggregation of the white blood cells around the afferent blood vessels, creating a sheath known as the Periarteriola lymphatic sheath, which acts to screen the blood for the presence of any foreign matter and if necessary generate an immune response.

Figure 6.2 Diagram of the Internal Structure of the Thymus



6.413 The Lymph Node

Lymph nodes are the small rounded dynamic structures, found in the lymphatic system, which serve as a centre for complex processes of local immunity [122].

Each lymph node has a classical kidney shape (figure 6.4) with an internal structure comprising three distinct regions; the cortex, the paracortex and the medulla, and consists of a 3D stromal network which forms an intricate lattice which supports the migratory cells responsible for the production and secretion of lymphocytes and also serves as a filtration unit removing waste products and bacteria.

The cortex is populated by primary follicular cells which contain large numbers of lymphocytes. It is in these follicular cells that the lymphocytes (mainly the T_{helper} cells) replicate by clonal expansion. The paracortex is the primary site of T lymphocyte and macrophage localisation within the lymph nodes. Whilst the $T_{cytotoxic}$ and $T_{suppresser}$ cells are found mainly in the medulla.

Lymph fluid enters the nodes via the afferent lymphatic vessels which communicate with the subcapsular sinuses, any antigens in the lymph fluid are recognised and taken up by the Langerhan cells of the lymph node and are presented to the macrophages and lymphocytes, thus initiating an immunological response.

6.5 Cellular Components of the Immune System

An individual immune response to a particular antigen is the result of interacting factors mediated through numerous different cell types, each with its own particular function. All cells of the immune system develop from a common precursor, the pluripotent stem cell, present in the bone marrow (figure 6.5), via one of two differentiation pathways [122], giving rise to myeloid and lymphoid cell lineages respectively. The myeloid lineage gives rises to cells such as the mononuclear and polymorphonuclear leukocytes, mast cells, platelets, basophils, eosinophils and neutrophils. Whereas the lymphoid lineage differentiates into two distinct cell lines producing B and T lymphocytes [122].

6.51 Pluripotential Stem Cell

The Pluripotential Stem Cell also known as the Haemopoietic cell, is found in the bone marrow and is the source of almost all the cells involved in immunological responses and also, gives rise to cells of the haematopoietic system, including erythrocytes and platelets and families of inactive cells [127].

6.52 Macrophages

The macrophages or blood monocytes arise from the common myleoid progenitor the mononuclear phagocyte. Macrophages are phagocytic cells present in abundance in the liver, lung, in the lining of the spleen sinusoids, and the lymph node medullary sinuses, and are also found throughout the connective tissue and around the basement membranes of blood vessels [128]. The major role of the macrophage is to provide defence against bacteria, viruses and protozoa, through the phagocytosis of foreign cells, antigen presentation in order to stimulate lymphocyte activity, the secretion of over sixty mediator substances which act to modulate the immune system, and in aid the activation of the cells of the immune system.

6.53 Polymorphnuclear Leukocytes

Polymorphonuclear leukocytes or granulocytes constitute 70% of the circulating population of leukocytes, and can be sub-divided into neutrophils, eosinophils, basophils and mast cells. Neutrophils are the most abundant and are involved in the digestion of micro-organisms. Eosinophils are phagocytic cells which collect at the sites of allergic reactions, the granules of which are lysosomal. Basophils are analogous to the tissue mast cells in that their granules contain histamine and heparin. Mast cells can be sub-divided into those found in the connective tissue and those associated with the mucosa, and are responsible for the release of chemical mediators following antigenic stimulation, i.e. histamine which initiate the classical symptoms of drug induced hypersensitivity.

6.54 Lymphocytes

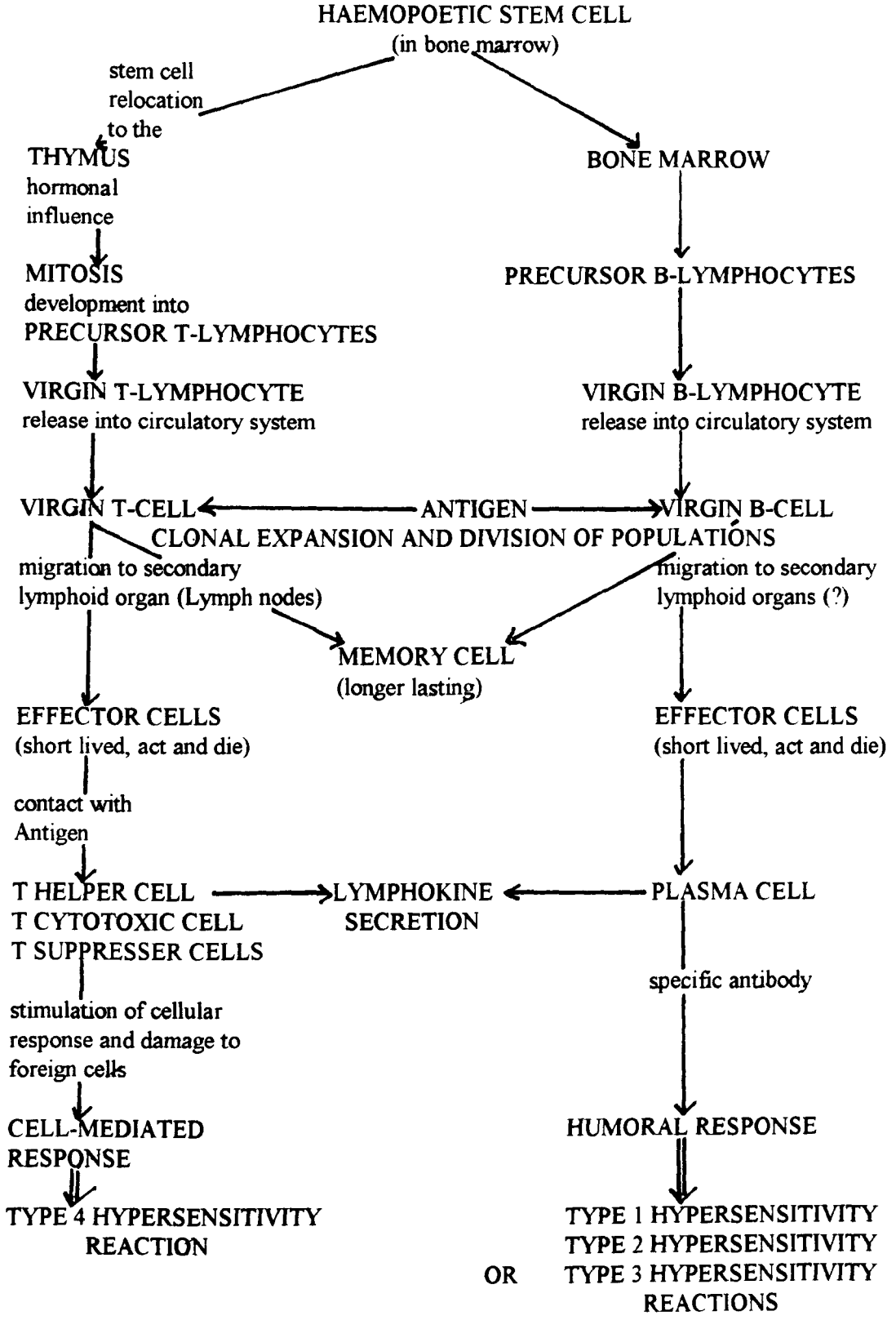
Lymphocytes are the most significant cells of the immune system, being of central importance to the functioning of the immune system. Lymphocytes constitute 20% of the leukocyte population and occur in three main sizes; small, medium and large. Lymphocytes are all morphologically identical, but it is possible to differentiate them into T and B lymphocytes (figure 6.6), on the basis of their differing routes of production and their extremely different functions / effects. Both types continuously circulate within the body, passing out of the blood into the lymphatic circulation, through the lymph nodes and the spleen, following preferential migration pathways:

T-lymphocytes preferentially migrate to the peripheral lymph nodes and are vital for the effective functioning of the immune system as they are responsible for the continuous surveillance of antigenic insult, communication and co-operation between cell types, controlling the proliferation and differentiation of antigen specific cells, selecting the distribution of effector cells to the sites of antigen exposure, and for effecting the recirculation of generated memory cells.
B-lymphocytes preferentially migrate to mucosa associated lymphoid tissue (MALT) found in the lining of the gastro-intestinal tract, tonsils, etc.
Both B and T lymphocytes work in conjunction, via multiple molecular communication mechanisms, to bring about the immunological processes and to regulate the production and activation of other immunological cell types such as the macrophages, mast cells, platelets, eosinophils and basophils [127].

6.541 T-Lymphocytes

The T cell line of lymphocytes originates from the thymus and develops into three morphologically identical classes of T cell, the helper, cytotoxic and suppresser cells. It is possible to distinguish between the different classes due to unique surface molecules present on the respective cells. These surface markers are termed CD markers and are defined by a cluster of monoclonal antibodies all reacting with the same molecule, e.g. CD3 marks the antigen receptor on all classes of T-cell, CD4 marks T_{helper} cells and CD8 marks $T_{cytotoxic}$ cells.
Each different T-cell class has a specific immunological function; T_{helper} cells (Th) aid cell amplification, $T_{cytotoxic}$ cells(Tc) destroy target matter and $T_{supressor}$ cells (Ts) act to switch off cells within the immune system.
The T cell population can be sub-divided into two distinct functional types, CTL which proliferate and directly kill antigen-bearing target cells following stimulation, and HTL which secrete cytokines and proliferate in response to antigenic stimulation, with the assistance of T helper cells [129].

Figure 6.6 Diagram of Lymphocyte Production, Activation and Immune Response Initiation



T-lymphocytes are antigen specific and therefore have a receptor for antigen recognition (figure 6.7), which is a heterodimer consisting of an acidic amino acid chain (45-55 KDa) and a basic amino acid chain (40-50 KDa) linked by a disulphide bond on the T cell surface [127]. The receptor is unable to recognise the antigen alone but recognises the antigen in association with MHC II, so prior to recognition the T lymphocytes must be activated, the antigen modified and presented

Mature T-lymphocytes are inactive, once the presence of an antigen has been detected it is necessary to activate the mature lymphocytes, for this to occur the presence of a macrophage is essential. The macrophage, acts as an antigen presenting cell, taking up the antigen phagocytically and degrading it into particles, known as antigenic determinants. Some of these antigenic determinants, when excreted, adhere to the outer membrane of the macrophage, where they are expressed along with MHC II as an antigenic complex. The antigenic complex is recognised by circulating T_{helper} lymphocytes, which in turn bind to the antigenic determinants expressed on the macrophage, and initiate T cell activation.
T_{helper} cells stimulate the macrophage to secrete a lymphokine, known as Interleukin-1 (IL1), which acts to enhance the metabolism of other T_{helper} cells in the surrounding area, which in response secrete the lymphokine Interleukin-2 (IL2) which enables lymphocyte recognition of the foreign (antigenic) matter. Upon antigen recognition the lymphocytes proliferate and differentiate into one of two cell types; an effector cell which produces a variety of antigen sensitive and regulatory cells which act by secreting specific substances known as lymphokines, which act to bring about a specific cell mediated immune response (figure 6.6) and memory cells which enhance recognition and reaction should a second infection occur.

6.5411 T Helper Cells

T_{helper} cells are defined by the possession of the membrane determinant CD4 [7]. Mosmann et al [131] categorised CD4+ T_{helper} cells into two subclasses; Th1 and Th2 clones, which are in turn responsible for the development of two different types of immune response [132]. These cell clones probably represent the most differentiated forms of T lymphocytes derived from a common precursor during an immune response. Fernandez-Botran et al, [133] showed that following antigenic stimulation the T_{helper} clones (Th1 and Th2) proliferate synergistically, with a marked difference in the respective responses showing within a few days of stimulation, Thus, distinction between Th1 and Th2 was possible on the basis of the cytokines secreted following activation (figure 6.8) [131,132,134].

Th1 clones secrete the cytokines, Interleukin-2 (IL2) a T cell growth factor which plays an important role in the clonal expansion of the T cells and Interferon-gamma (IFN-γ) which has numerous actions including anti-viral activity, macrophage activation, B cell activation and stimulating the differentiation and secretion of polyclonal IgM, inhibition of IgG1 and IgE secretion and the enhancement of IgG2 secretion. Together IL2 and IFN-γ act to initiate the cell mediated delayed hypersensitivity reactions.

Th2 clones secrete the cytokines, Interleukin-5 (IL5), Interleukin-6 (IL6) and Interleukin-4 (IL4) which is involved in the activation, proliferation and differentiation of B and T cells, in inducing resting B cells to increase their expression of MHC II and of low affinity receptors for the Fc portion of IgE, and act as co-stimulator of B cells in the presence of anti-immunoglobulin so inducing the production of IgG and IgE antibodies.

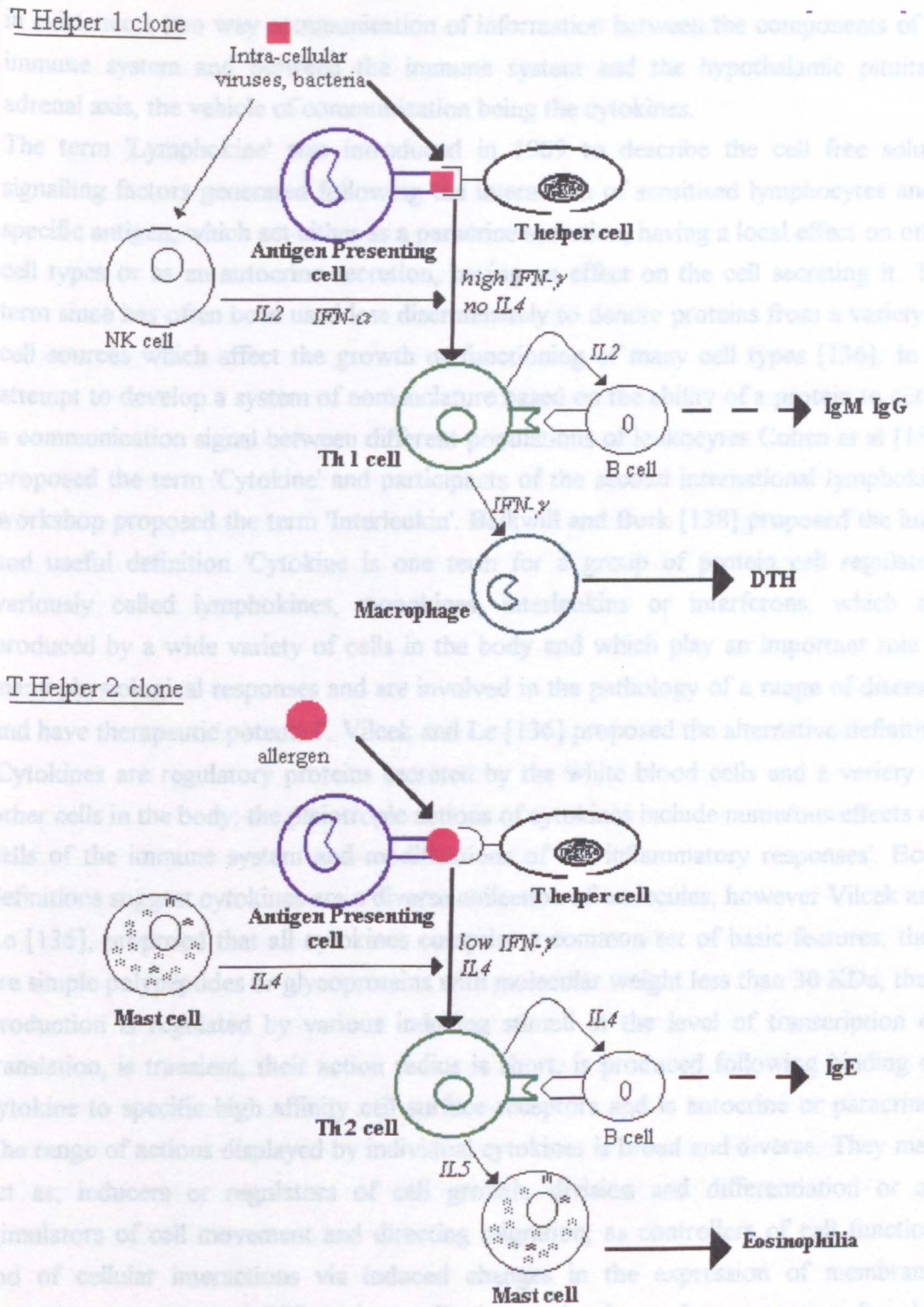
IL4 is the only cytokine that regulates IgE production and the initiation of humoral immediate hypersensitivity reactions.

Both clones secrete the cytokines Interleukin-3 (IL3), Granularcyte macrophage coloney stimulating factor (GM-CSF), Tumour necrosis factor (TNF) and Preproenkephlin and other specific induction proteins.

The nature and properties of IL2, IL4, IFN-γ and some other cytokines are discussed more fully later in 6.6.

The observation that IL4 and IL2 are secreted by distinct T_{helper} clone suggests that different activation requirements for the two T clones may play an important part in the mechanism by which the relative production of IL4 and IL2 and ultimately the selection of the Ig isotypes secreted in humoral immune responses are regulated [129,131]. The concentration of the cytokines IL4 and IL2 may provide a particular selection pressure and so direct the type of cells stimulated and the form of the immune response initiated [130].

Figure 6.8 Diagram of the Differential Cytokine Secretions by Th1 and Th2 clones



6.6 Cytokines

The pioneering work of Besedosky, Sorkin and co-workers [135] showed there to be in existence a two way communication of information between the components of the immune system and between the immune system and the hypothalamic pituitary-adrenal axis, the vehicle of communication being the cytokines.

The term 'Lymphokine' was introduced in 1969 to describe the cell free soluble signalling factors generated following the interaction of sensitised lymphocytes and a specific antigen, which act either as a paracrine secretion, having a local effect on other cell types or as an autocrine secretion, having an effect on the cell secreting it. The term since has often been used less discriminately to denote proteins from a variety of cell sources which affect the growth or functioning of many cell types [136]. In an attempt to develop a system of nomenclature based on the ability of a protein to act as a communication signal between different populations of leukocytes Cohen et al [137] proposed the term 'Cytokine' and participants of the second international lymphokine workshop proposed the term 'Interleukin'. Balkwill and Burk [138] proposed the lucid and useful definition 'Cytokine is one term for a group of protein cell regulators variously called lymphokines, monokines, interleukins or interferons, which are produced by a wide variety of cells in the body and which play an important role in many physiological responses and are involved in the pathology of a range of diseases and have therapeutic potential'. Vilcek and Le [136] proposed the alternative definition 'Cytokines are regulatory proteins secreted by the white blood cells and a variety of other cells in the body; the pleiotropic actions of cytokines include numerous effects on cells of the immune system and modifications of the inflammatory responses'. Both definitions suggest cytokines are a diverse collection of molecules, however Vilcek and Le [136], proposed that all cytokines comprise a common set of basic features, they are simple polypeptides or glycoproteins with molecular weight less than 30 KDa, their production is regulated by various inducing stimuli at the level of transcription or translation, is transient, their action radius is short, is produced following binding of cytokine to specific high affinity cell surface receptors and is autocrine or paracrine. The range of actions displayed by individual cytokines is broad and diverse. They may act as; inducers or regulators of cell growth, division and differentiation or as stimulators of cell movement and directing migration, as controllers of cell function and of cellular interactions via induced changes in the expression of membrane determinants, such as MHC antigen adhesion molecules and as receptors for the cytokines themselves [119]. Another action is that they either lead to an increase or decrease in the levels of production of other cytokines.

The cytokines of interest in this work are those secreted by the T_{helper} lymphocytes, following drug administration and so play an important role in the initiation of the immune response. The T_{helper} cells secrete a number of cytokines, which can be classified into two groups, the interleukins (IL) and the interferons (IFN).

6.61 Interleukins

Interleukin means 'between leukocytes'. The term Interleukin was coined in order to free the nomenclature of soluble communicating from the constraints associated with the definitions derived from a single bioassay.

Over the last 20 year's knowledge pertaining to the activity of the T-lymphocytes has expanded significantly to include information regarding the direct effects of cytokines on the growth and differentiation of T & B lymphocytes, Natural Killer Cells, Monocytes, Macrophages, LAK cells and Oligodendrocytes. This increased knowledge base has thus, lead to more investigations to be performed and more detailed information regarding the actual responsible cytokines to be gleaned. The main interleukins released by the T_{helper} cells are IL2, IL3, IL4 and IL5, each of which has a different function or effect.

6.611 Interleukin 2

IL2 was first identified by Morgan and co-workers in 1975 [139] as a bone marrow derived T cell growth factor. IL2 is a 15.5 to 17 KDa soluble sialoglycoprotein, comprising a 133 amino acid polypeptide chain which contains a single intramolecular disulphide bridge between amino acids 58 and 108. This bridge is important in maintaining the active conformation of the molecule [140,141]. The overall arrangement of the amino acids within the IL2 structure (figure 6.9) has generated a molecule comprising of 6 alpha-helical domains (A to F). IL2 is produced by the resident T cells of the lymphoidal organs drained at nonmucosal tissue sites, the axillary, inquinal and brachial lymph nodes and the spleen [140,141]. The main roles of IL2 are the propentiation of T cell proliferation via binding to a specific IL2 cell-surface receptor [142,143,144], the amplification of activated T cells, the proliferation of T cytotoxic cells, the induction of NAT killer cells, of lymph killer cells and of B cell proliferation and differentiation.

6.612 Interleukin 3

IL3 in humans is a 133 amino acid heavily glycosylated polypeptide chain, which acts on the broadest range of target cells within the haematopoietic system of any cytokine promoting the formation of erythroid, megakaryocyte and mast cell colonies. IL3 also has a special ability to stimulate growth of early stem cells and progenitors of mast cells and megakaryocytes [145].

6.613 Interleukin 4

IL4 was identified in 1982 and is a 144 amino acid soluble glycoprotein produced by the resident T cells of the lymphoidal organs, drained at mucosal tissue sites such as the Peyers patches, cervical, periartic and parathymic lymph nodes [146]. IL4 is secreted in response to allergen-induced cross-linking of IgE at the Fc receptor and acts as a B cell growth cell factor initiating the synthesis of IgE and IgG1 type immunoglobulins and plays a part in mast cell degranulation.

6.614 Interleukin 5

IL5 is a 40-45KDa glycoprotein, unique with regards other cytokines in that it has a disulphide linked homodimer. IL5 is a B cell growth factor which initiates the synthesis of IgA type antibodies and also stimulates amplification of the eosinophillia cell lineage and possibly activates the basophil cells [147].

6.615 Interleukin 6

IL6 is a multifunctional cytokine which is believed to play an important role in host defence, through regulating various aspects of immune and inflammatory responses, in the regulation of B lymphocyte differentiation, acute-phase protein synthesis and haematopoiesis and may also be an important co-factor in T-cell function [148].

6.62 Interferons

Interferons are a second sub-group of cytokines. They are the major class of cytokines which contribute to the first line of anti viral defence. There are three main interferons α, β & γ. IFN α & β play a major role in the host resistance to viral infections prior to the activation of the immunological mechanisms [149].

6.621 Interferon - Gamma

IFN-γ is released by T-helper lymphocytes following sensitisation by anti-viral agents. Following its release it augments phagocytosis, activates monocytes, enhances NK activity, stimulates T-cytotoxic cells, MHC II expression, B cell growth and the synthesis of IgG2a type antibodies.

7.0 Immunotoxicity

7.1 Introduction

In the main drugs are designed to prevent or ameliorate disease; it is, however, a well recognised and accepted fact that no drug is completely safe and that some form of subjective adverse reaction will be a feature of any pharmaceutical therapy. It is therefore the premise of toxicological investigations to enable the development of safer compounds, whether they be therapeutic, industrial or agrochemical [150].

Toxicologists have only recently come to appreciate that the dynamics of the immune system with the ongoing processes of cell proliferation and differentiation [151] make it a major target for a xenobiotic [152] and thus of the resulting chemically induced toxic damage. The recognition of the immune system as a significant target organ of xenobiotic toxic insult and in the realism of the need to examine the modifications within the immune system in response to therapy, in conjunction with routine toxicity studies and the efforts of scientists such as; Drs Amos, Bees, Berlin, Dean, Gibson, Hubbard, Luster, Mullen, Parke, Smith and Sprecifico, [153] has lead to the development a new discipline termed Immunotoxicology [154]. The term immunotoxicology was coined in order to distinguish the new immunotoxicologists from the pre-existent immunopharamacologists. Immunotoxicology is therefore a relatively new discipline which marries together the fields of immunology and toxicology and draws from other relevant areas of science in order to analyse the way in which the immune system functions in health and in disease states and attempts to construct a framework for the interpretation of the effects of a broad spectrum of xenobiotics on the immune system [117]. In general immunotoxicology provides important information, which gives an additional dimension to the safety assessment and also, provides major input into the development of our basic knowledge of the immune system through the use of xenobiotic probes [155,156].

The favoured definition is that 'immunotoxicology is the study of the interaction of a xenobiotic with the immune system' [157], this accommodates the two main areas of the discipline, the ability of a xenobiotic to compromise an immune function or 'classical immunology' and the provocation by a xenobiotic of specific tissue damaging immune responses or allergens.

7.2 Immunotoxic Drug Reactions

The interaction of drugs with the immune system may result in various forms of toxicity [157] arising from tissue damage via immunological mechanisms. In susceptible individuals a variety of xenobiotic agents may stimulate an immunotoxic response, resulting in the generation of the undesirable effects associated with immunosuppression, autoimmunity or allergy [157].

7.21 Immunosuppression

The presence of a parasitic infection or of a specific drug may generate antibodies within the host which interfere with different process's across the spectrum of immunity, causing disruption of the lymphoid tissues or organs directly in such a way as to cause reduction of normal immuno-activity, by increasing the number and activity of $T_{suppresser}$ cells or inhibiting the proliferation and / or activity of T_{helper} cells and hence antibody generation. Thus, any invasion of the host by another foreign agent will meet limited resistance or may even go unchallenged, so causing exaggerated disease states [122]. An example of a disease state due to immunosuppression is AIDS, where the HIV virus acts to suppress the immune system components in such a way that other diseases, which are normally effectively combated by the immune system, such as the pneumonia's, or some rare illnesses are able to infect the patient, in most with a fatal outcome.

7.22 Autoimmunity

The immune system contains an extensive repertoire of specific B and T cells that are directed against self components. A breakdown or malfunction in the mechanisms controlling their activity, and their tolerance of self, may lead to the host producing large quantities of antibodies direct against itself, thus resulting in autoimmunity [158]. The earliest example being 'Hashimoto's Thyroiditis' where the thyroid gland has become excessively infiltrated with inflammatory lymphoid cells, which produce auto-antibodies. These auto-antibodies, may or may not be organ specific, and act to produces a range of disease states (table 7.1), however, overlaps do exist. Common target organs affected in organ specific disorders include the thyroid and adrenal glands, the stomach and the pancreas. Non-specific organ disorders affect the skin, the kidneys, the muscle and the joints.

7.23 Allergy

In 1906 von Piquet [159] proposed the term 'allergie' to denote a deviation of immunity from the original state or a 'changed reactivity' of an individual. Allergic diseases are a diverse group of pathological conditions characterised by immunological mediated adverse or tissue damaging responses [160] following exposure of a susceptible individual to a sensitising industrial, environmental or pharmaceutical xenobiotics [161] by inhalation, ingestion, injection or skin contact, as opposed to a response due to pharmacological idiosyncrasy, direct toxicity overdose, interaction between drugs, [162,163] and which induces a specific immune reaction [154] localised in a particular organ or tissue.

7.231 Hypersensitivity Reactions

Hypersensitivity reactions are amongst the most common allergic drug reactions occurring in humans [6], the severity of which are of great concern to both the health authorities and pharmaceutical companies.

The term hypersensitivity denotes an immunologic sensitivity to a specific allergen in which the expression of the beneficial adaptive immune responses occurs at excessive levels or in inappropriate forms, causing irreparable tissue damage, disease or pathology to host tissue [119,159].

Coombs and Gell classified hypersensitivity reactions into four different classes, on the basis of their immunologic pathogenesis, Type I (Immediate), Type II (Antibody dependent), Type III (Immune-complex mediated) and Type IV (Delayed).

In practice, as with most immunologic responses, there is a degree of overlap between these reaction types, such that no one reaction type generally occurs in full isolation. The two types of chemically induced allergy which pose the most concern and are therefore of greatest significance to the toxicologist are immediate and delayed hypersensitivity [129], with delayed hypersensitivity being the most frequently encountered health problem. Whilst both immediate and delayed hypersensitivity result from the sensitisation of a specific immune response by a chemical allergen followed by its subsequent provocation by the same or a structurally similar allergen, they are mechanistically very different [129].

7.2311 Immediate Hypersensitivity Reactions

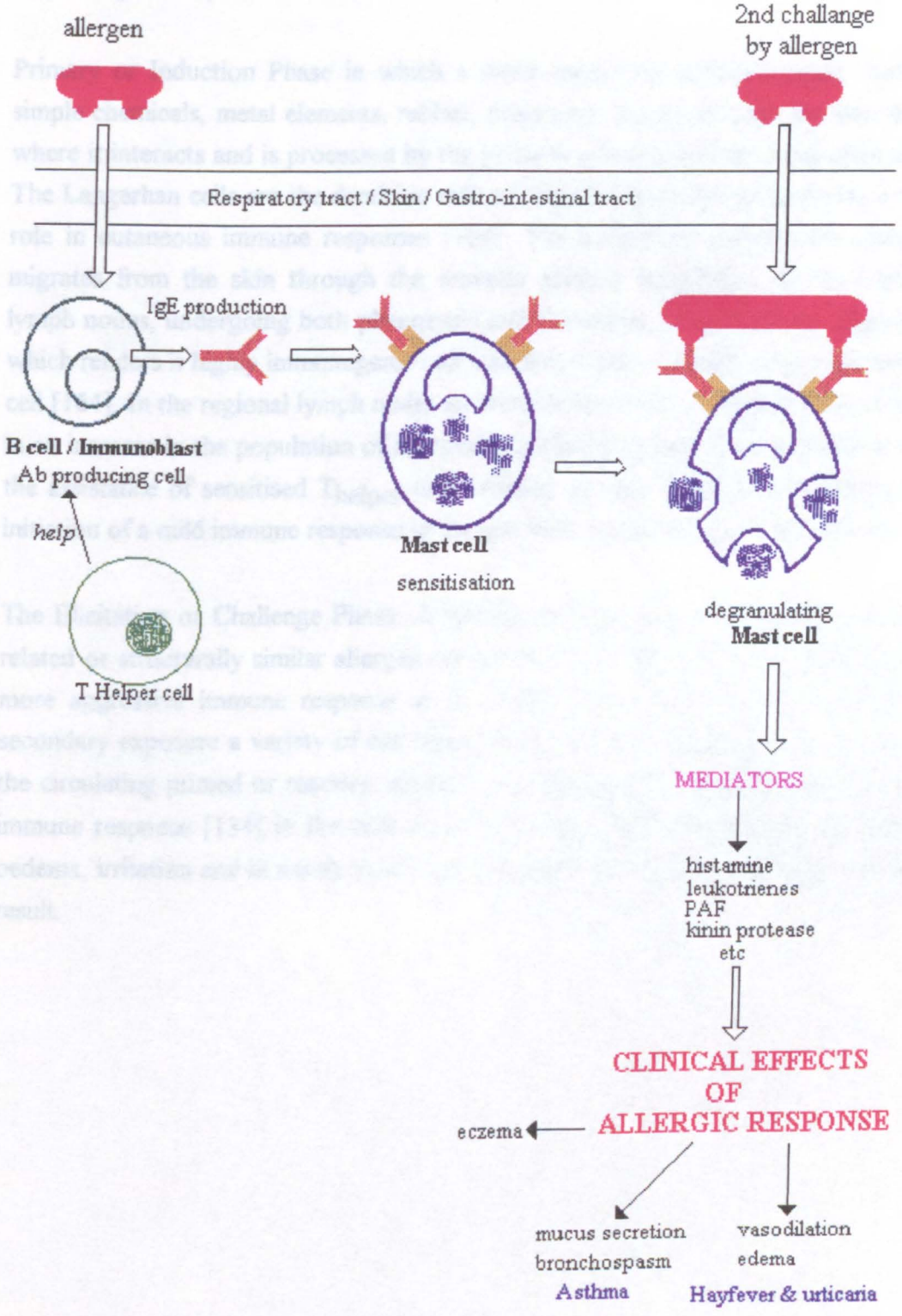
Immediate hypersensitivity reactions are humoral reactions in which an IgE antibody response is directed against an innocuous allergen, resulting in the release of pharmacological mediators such as histamine from IgE sensitised mast cells and which manifest themselves within 15 - 20 minutes. Immediate hypersensitivity is a haptenic system occurring in three phases [134] (figure 7.1):

Phase 1: The sensitisation of mast cells by the drug (allergen).
Mast cells are storage cells for granules containing potent inflammatory, repair materials and strong pharmacologically active materials, such as heparin, histamine, serotonin and kinin protease [158]. Sensitisation occurs as a result of an allergen stimulating B-lymphocytes, under the assistance of T_{helper} cells and IL4 secretions, to synthesise and secrete IgE type immunoglobulins active against the allergen. These IgE antibodies upon secretion bind via their Fc region to the mast cells thus causing sensitisation.

Phase 2: (Mast cell activation) A second challenge by the allergen (drug) leads the IgE immunoglobulins, adhered to pre sensitised mast cells, acting as cellular receptors for the multi-epitope allergen [161]. Upon binding of the allergen it may cross-link with another IgE immunoglobulin receptor causing stabilisation and subsequent degranulation of mast cells, [163] leading to the release of the cellular mediators into the circulatory system.

Phase 3: (Effector Phase) The release of pre-formed or newly synthesised mediators (such as histamine, heparin, eosinophil-chemotatic factor, leukotrienes, platelet activating factor, arachidonic acid metabolites, high molecular weight neutrophil-chemotatic factor, kinin generating protease's, prostaglandin's and thromboxane's), causes vasodilation, increases endothelial cell permeability, leading to inflammation and redness and also causes constriction of bronchiole smooth muscle cells, promoting the shortness of breath, all of which are associated with the clinical symptoms of an allergic response.

Figure 7.1 Diagrammatic Representation of the Cellular Events in an Immediate Hypersensitivity Reaction



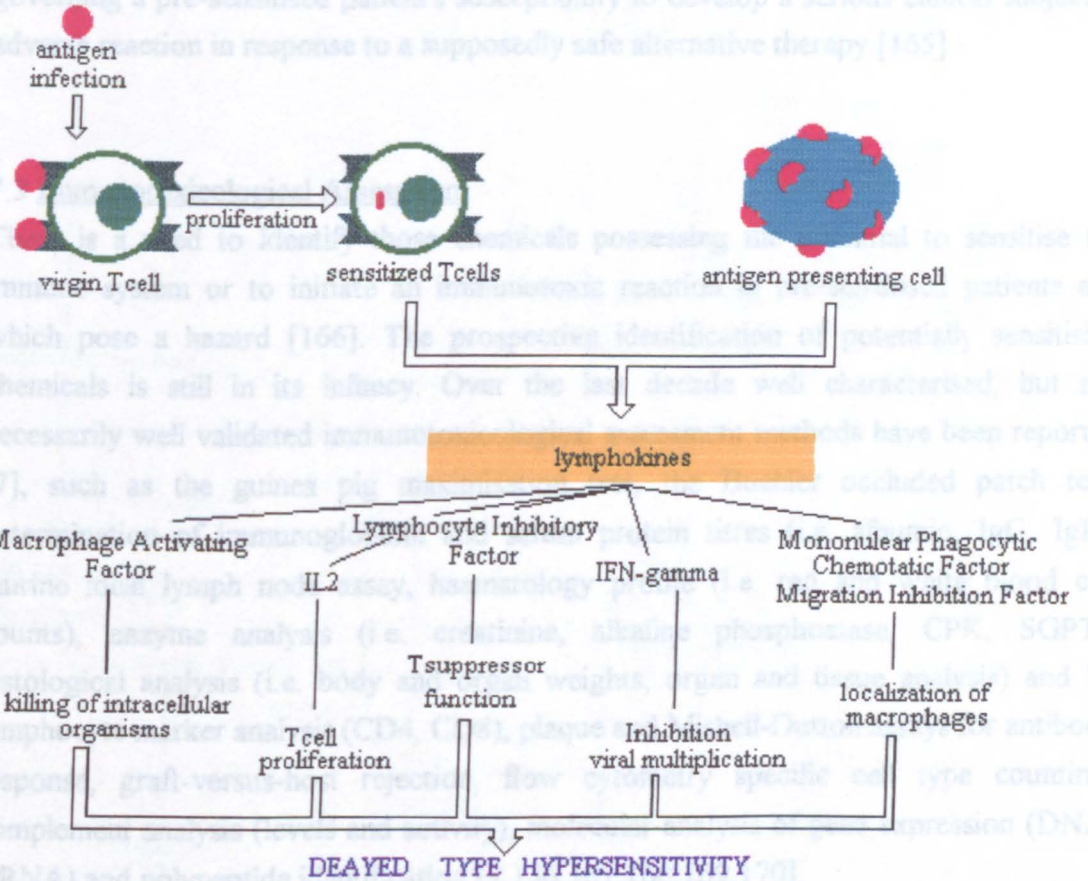
7.2312 Delayed Hypersensitivity Reactions

Delayed or Contact hypersensitivity reactions, are cell-mediated immune responses, in which epidermal sensitisation results in dermatitis. The process occurs in two stages (figure 7.2):

Primary or Induction Phase in which a small sensitising antigenic agent, such as simple chemicals, metal elements, rubber, poison ivy, passes through the skin tissue where it interacts and is processed by the proteins of the epidermal Langerhan cells. The Langerhan cells are the dendritic cells of the epidermis and as such play a vital role in cutaneous immune responses [164]. The Langerhan cell-allergen complex migrates from the skin through the draining afferent lymphatic's to the regional lymph nodes, undergoing both phenotypic and functional changes during migration, which renders it highly immunogenic and transfers it into a potent antigen-presenting cell [164]. In the regional lymph nodes an immune response is initiated which results in an increase in the population of allergen sensitised T lymphocytes and which with the assistance of sensitised T_{helper} cells, release a range of mediators causing the initiation of a mild immune response in the lymphoid organs but not in the tissues.

The Elicitation or Challenge Phase. A subsequent exposure to the same, a closely related or structurally similar allergen causes the provocation of an accelerated and more aggressive immune response at the site(s) of exposure [161]. During this secondary exposure a variety of cell types are capable of presenting the allergen to the circulating primed or reactive memory T-lymphocytes and hence initiate a local immune response [134] in the skin tissue with the classical symptoms of redness, oedema, irritation and in severe case's tissue damage about the site of exposure may result.

Figure 7.2 Diagrammatic Representation of the Cellular Events of Delayed Hypersensitivity



7.24 Cross-Reactivity

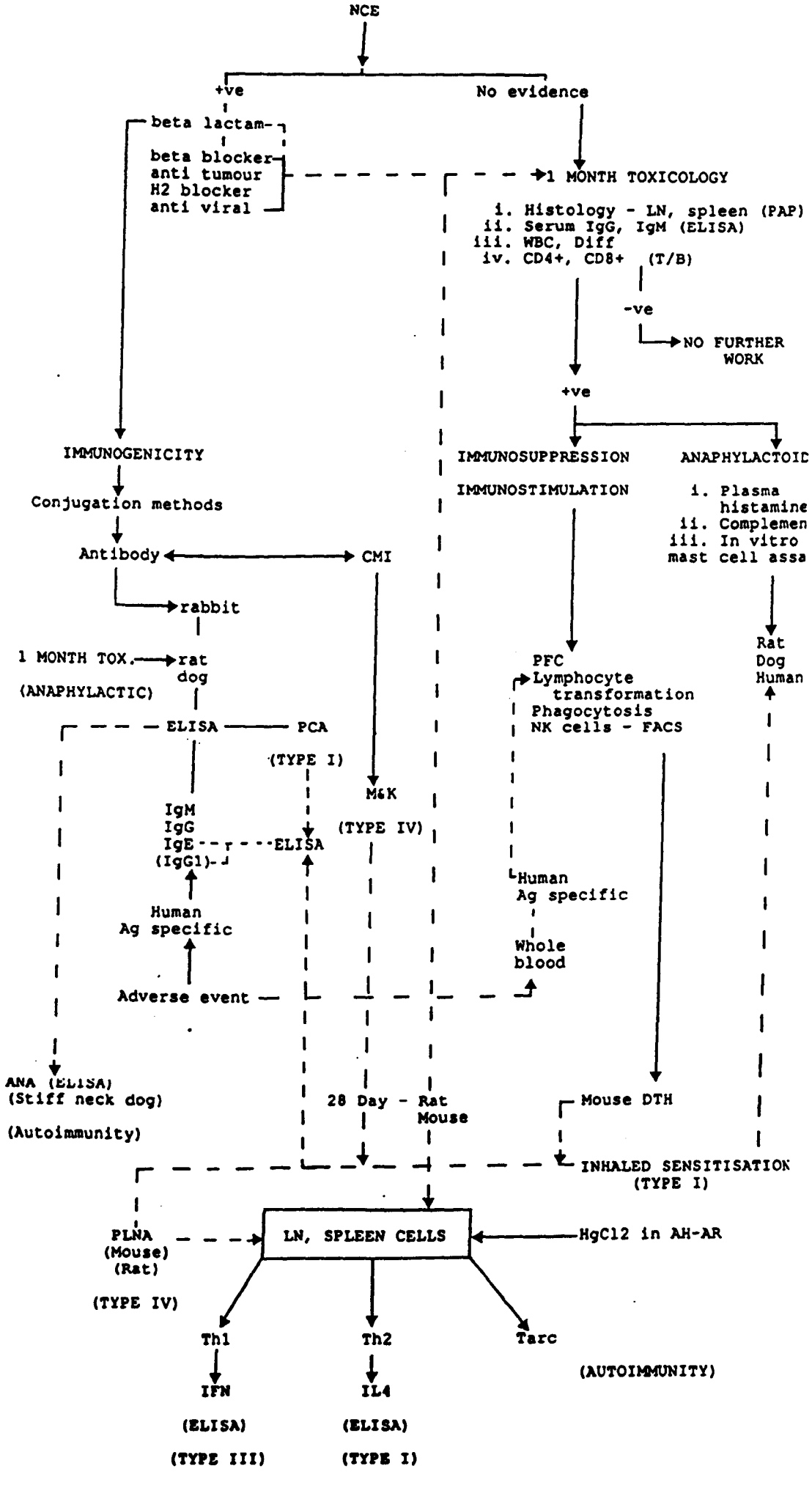
The recognition of an allergen by an antibody raised *in vivo* to a different, yet similar chemical antigen, may lead to the phenomenon of cross-reactivity, in which the new antigen is recognised by the immune components as the sensitising allergen and the normal process's of Immediate or Delayed immune activation, as previously explained, are initiated leading to the development of unexpected immunotoxic reactions. Cross-reactivity between new therapeutic compounds and antibodies is a major factor governing a pre-sensitised patient's susceptibility to develop a serious clinical subjective adverse reaction in response to a supposedly safe alternative therapy [165].

7.3 Immunotoxicological Assessment

There is a need to identify those chemicals possessing the potential to sensitise the immune system or to initiate an immunotoxic reaction in pre-sensitised patients and which pose a hazard [166]. The prospective identification of potentially sensitising chemicals is still in its infancy. Over the last decade well characterised, but not necessarily well validated immunotoxicological assessment methods have been reported [7], such as the guinea pig maximisation test, the Buehler occluded patch test, determination of immunoglobulin and serum protein titres (i.e. albumin, IgG, IgE), murine local lymph node assay, haematology profile (i.e. red and white blood cell counts), enzyme analysis (i.e. creatinine, alkaline phosphoatase, CPK, SGPT), histological analysis (i.e. body and organ weights, organ and tissue analysis) and T-lymphocyte marker analysis (CD4, CD8), plaque and Mishell-Dutton assays for antibody response, graft-versus-host rejection, flow cytometry specific cell type counting, complement analysis (levels and activity), molecular analysis of gene expression (DNA, mRNA) and polypeptide identification [3,156,167,168,169,170].

As no single test can completely asses the immune function and immunotoxicity of a xenobiotic[168], therefore Vos [123], Luster et al [4], DeWeck [2] and Hadden [153] have each proposed a tiered approach for immunotoxicity assessment, as outlined in figure 7.3. The first tier includes the assessment of non-functional parameters, such as the quantitation of immune cells, cell viability, serum immunoglobulins, organ weights, histopathological assessment, Cell-mediated immune response assay and plaque forming assay. Subsequent tiers include functional tests, immune competence and host resistance studies, all of which are chosen on the basis of the results obtained in the first tier of testing [3,7,151].

Figure 7.3 Outline of a Tiered Panel of Immunotoxicological Tests [171]



In this study the aim is to evaluate both immunoglobulin levels (10.0) and cytokine secretion profiles (11.0) following antibiotic simulation, and therefore the assays of interest and which are discussed in detail herein are the enzyme linked immuno-sorbent assays (7.31) and the murine local lymph node assay (7.32).

7.31 Immunoassays

Antibodies have long been used as diagnostic reagents, but it is only in the last 30 years that their use in the assessment of the immunological system has been fully realised and acted upon.

The basis of any immunoassay is the formation of an antibody-antigen complex, followed by estimation of the bound verses free components by use of labelled reaction components [172]. Immunoassays were first introduced in 1960 with an assay for thyroxin, which was based on the strategy of saturation analysis, in which limited amounts of antibody are reacted with excess of labelled antigen. Mile & Hales introduced the use of labelled antibodies in the immunometric type assay. Here excess antibody is added to standard concentrations of antigen. The bound and free components are then separated the bound antibody estimated. No matter how low the concentration of antigen if sufficient antibody is used, some level of complex formation will result over a given time and thus be detected [173].

Recent advances in antibody technology have led to the production of highly specific, multi-epitope monoclonal antibodies in very pure forms, which have enabled the use of two reagent systems in immunoassays, thus increasing assay specificity and sensitivity whilst reducing interference. This coupled with the fact that at almost every step of the general assay procedure it is possible to select from a wide range of reagents and techniques, have given an enormous methodological flexibility to immunoassays and have enabled the development of a range of procedures enabling the assessment of extensive immunological components [174,175,176].

7.311 Enzyme Immuno Assays

Enzyme Immuno Assays (EIAs) were developed in the 1960's to enable identification and localisation of antigens in histological preparations [174]. Observations that it was possible to immobilise either the antibody or the antigen onto a solid phase led to the modification of the strategy to enable the quantitative determinations of immuno reactants in test-tubes, using enzyme rather than radio labelled reactants. EIAs are based on two important biological phenomena [174,175,177,178]; the extraordinary

discriminatory power of antibody for a specific antigen and the high catalytic power and specificity of enzymes. EIAs lend themselves to a wide range of analytical applications, including the quantitation of immunoglobulins and drugs [175,178].

EIAs can be classified into two main types:

Heterogeneous assays that involve an enzyme label as a means of detection and in which it is necessary to separate bound and free antigen using solid phase systems [179].

Homogenous assays that do not require the separation of the two antigenic phases because the process of binding the antigen to the antibody on the solid phase acts to modify the activity of the enzyme label in such a way as to enable the detection of the bound antigen in the presence of the unbound antigen.

7.3111 Enzyme Linked ImmunoSorbent Assay (ELISA)

ELISAs are Heterogeneous EIAs, which are adaptable and diverse group immunoassays in current usage. The first ELISA techniques were described in the early 1970s by two groups of workers - Engvall & Perlmann [176] and Schuurs & Vann Weemann [175].

The assays were developed to ease the process by which bound antigen is separated from free antigen and also to introduce labels for detection other than the radio-labels.

The basic principles of an ELISA are the reaction of the immunoreactants and the subsequent detection of the reaction using a suitable enzyme label.

ELISAs have numerous advantages they have increased sensitivity, detectability and specificity, generate highly reproducible results, give objective evaluation, are highly versatile (variety of enzymes available for the detection of various antibodies), use monoclonal antibodies to their full advantage, use small concentrations of sample and reagents, have increased reagent shelf life, have lower operating costs (reagents, equipment), have no need of specialist training, reagents, storage and disposal procedures or equipment and have reduced health risk.

There are two basic types of ELISA, Competitive & Non-Competitive assays.

7.31111 Competitive ELISAs

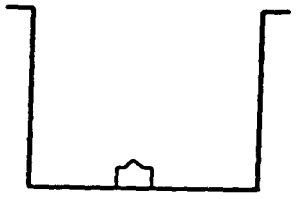
The general method for a Competitive ELISA (figure 10.7) involves the binding of the antibody to the walls of a plastic well and the subsequent addition of the test and labelled antigens, which compete with each other to bind with available bound antibody. After a suitable reaction time the reaction well is washed to remove any unbound reagents. The amount of enzyme-antigen-antibody complex bound is measured by monitoring an enzymic reaction, following the addition of a suitable substrate, under controlled conditions. In this method a second assay is run concurrently, in which no test sample in added, only the labelled antigen. By comparing the amount of labelled antigen detected in each assay type it is possible to determine the amount of test antigen bound and so evaluate the concentration of test antigen in a given sample.

7.31112 Non-Competitive ELISA

The double sandwich assay is the most useful and most commonly used assay of this type. The procedure is (figure 7.4) the binding of antibody to the walls of a plastic well, washing to remove excess antibody, addition of antigen that binds to the bound antibody, washing to remove excess antigen, addition of a second antibody conjugated to an enzyme label, is added which bind to the antigen, washing, addition of a suitable enzyme substrate determination of the concentration of product formed.

Figure 7.4 Diagrammatic Representation of a Non-Competitive ELISA

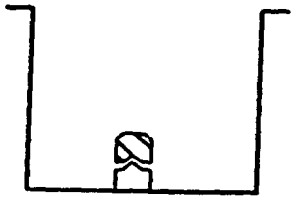
1. Plate coated with antigen



Benzyl Penicillin-Cytochrome C

WASH

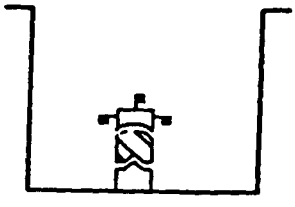
2. Specific antibody binds to antigen (antiserum stage)



Test serum (Penicillin positive rabbit, control rabbit, ampicillin positive rabbit).

WASH

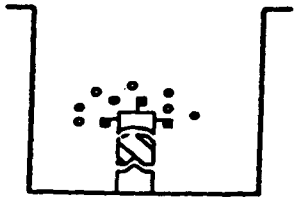
3. Enzyme labelled specific antibody binds to antibody



Goat anti rabbit IgG2a peroxidase

WASH

4. Enzyme substrate added



TMB substrate, peroxidase converts substrate O to product ●.

7.32 Animal Models

The sensitising potential of chemicals is currently assessed using a variety of animal models, such as the Guinea Pig maximisation test [180], the Buehler Occluded Patch test [181], Mouse Ear swelling test and the Mouse Local Lymph Node assay [182], all of which employ a biphasic protocol of an Induction phase, in which the respective animal is sensitised and an Elicitation phase, where the animal is challenged with a test chemical [183]. For the guinea pig tests the sensitising potential is determined by evaluation of the frequency of challenged induced erythematous skin reactions by visual assessment. For the Mouse Ear Swelling test (MEST) sensitising potential is determined from changes in ear thickness following challenge. For the Mouse Local lymph Node Assay sensitising potential is evaluated by quantitation of radioactive material or of specific cytokines following culturing of the draining lymph node lymphocytes.

Until recently the species of choice for the routine predictive toxicological assessment has been the guinea pig [160], however, the guinea pig does not have a well characterised IgE antibody [183] and so provides no provision for the determination of immediate hypersensitivity. The mouse, by virtue of its extensive use in experimental studies for the advancement of our knowledge of the cellular and molecular process involved in an immune response [160], has a well-characterised IgE immune response, which also, has the advantage of being induced and regulated in a manner similar to that seen in man [184], thus the mouse as an animal model lends itself to use for determination of sensitising potential and of Immediate hypersensitivity. Therefore recent attention has focused on the mouse as an alternative model, for routine determination of immunotoxicity, with the mouse Local Lymph Node assay (mLLNA) being at the forefront of current development.

7.321 Lymph Node Assay

The murine local lymph node assay (mLLNA) was first developed at the ICI Central Toxicology Laboratory and is based on the understanding that a chemical causes both skin sensitisation and lymphocyte proliferation, in the lymph nodes draining the site of exposure, during the Induction phase of hypersensitivity reactions [185]. The mLLNA can be used with the incorporation of radio-labelled thymidine to assess the proliferative responses in the lymph nodes or can be used to asses the differential cytokine secretion patterns exhibited by the T_{helper} lymphocytes following chemical challenge.

An outline mLLNA is: 25µl of test chemical, in a suitable vehicle, is applied to the dorsum of each ear of each mouse in the test group.(a control group are treated with vehicle only) on three consecutive days. Single sex mice of either the strain BALB/c or CBA/ca, at between 6-10 weeks of age are used. Five days after the first topical exposure the mice are sacrificed and the draining (auricular) lymph nodes are excised and pooled. (If assessment of sensitivity is by radio-active determination of lymphocyte proliferation then radio-active thymidine is injected into the mouse at day 5 prior to sacrifice). Single cell suspensions of the lymph nodes, from each test group, are prepared. The pooled Lymph Node Cells (LNCs) are pelleted by centrifugation, washed, resuspended and cultured in a suitable culture medium, with the test material. After a suitable incubation time has elapsed the relevant end-point is quantitated and sensitising potential determined [148,182,185]. The mLLNA, therefore provides an alternative predictive test for the identification and possible quantitation of the sensitising potential of topically administered chemical agents.

Evaluation and validation of the mLLNA by the pharmaceutical industry is still on going. It is hoped, by the developers, that the assay, may in time prove to be relatively rapid, robust, cost effective and reliable, and will be adopted as a routine assay within all immunotoxicology laboratories and by the respective authorities.

8.0 Proposed Aim of the Study

The current general schemes of immunotoxicological testing, performed within the pharmaceutical industries, are complex, time and resource consuming.

Ultimately, it is hoped to develop QSARs that reliably model β-lactam immunotoxicity and which may be used to predict the immunotoxic potential and the direction of triggering of the immune system by 'new' antibiotics of the β-lactam class. Use of these QSARs in the early stages of drug development and immunotoxicity studies will provide a means of better safety assessment, as well as highlighting those compounds that will have a high immunotoxic or cross-reactive potential. Thus indicating the need to test these suspect compounds more extensively or to eliminate them completely from further development, so saving time and resources without a reduction in drug safety.

It was the aim of this study is to use QSAR and immunological techniques to develop a means for the mechanistic assessment of β-lactam antibiotic immunotoxicity.

The works concentrated on the penicillins and cephalosporins, as a range of compounds and vast amounts of data regarding structures and extensive clinical trials were available.

The starting point of the study was to use the dedicated techniques of QSAR to identify any structures within the β-lactams which may be responsible for activation of the immune system, and thus the development of an allergic reaction and to develop statistically valid QSARs that will enable the prediction of a new β-lactam antibiotics' potential allergenicity to be made.

The second aim of the study was to develop an ELISA technique which would provide a means of generating quantitative data with regards a compound's potential to cross-react with anti-benzylpenicillin serum antibodies in pre-sensitised animal models, and again use the techniques of QSAR to determine the molecular properties of importance and to produce statistically significant models which will enable valid predictions of potential cross-reactivity for new compounds to be made.

Finally, using the knowledge of the differential patterns of cytokine secretion released in response to the differential stimulation of the immune system, it was hoped to develop an *in vitro* protocol for the quantitative analysis of cytokine production, particularly interleukin-2 and interleukin-4 following specific antibiotic simulation. This cytokine analysis may thus provide an indication as to the direction of triggering of the immune system induced by specific β-lactam antibiotics, as well as a further insight into the phenomenon of cross-reactivity.

9.0 Prediction of the Frequency of β-Lactam Induced Adverse Reactions

9.1 Introduction

Since their first clinical use penicillin and cephalosporin antibiotics have become amongst the most commonly prescribed drugs in the world [186,187]. However, despite their vast antibacterial spectrum of activity and wide therapeutic index the β-lactam antibiotics are also responsible for initiating a vast array of adverse responses, some of which may be allergic reactions [188]. Initially these adverse reactions were attributed to impurities in the crude preparations. However, subsequent studies have shown that samples free of any contamination still elicit adverse responses and so suggest that it is the compounds themselves which are responsible for eliciting the problem reactions [187].

An adverse response (AR) is defined as 'any response to medication which is noxious and unintended and that occurs at doses used in therapy' [189] and can be classified into two types; **Type A** (augmented) reactions, which are pharmacologically predictable from the known activity of the drug and **Type B** (bizarre) reactions, which are unpredictable, rare, not dose related and may be clinically serious [190].

An allergic reaction can be defined as 'the adverse, tissue-damaging and sometimes fatal consequences of the stimulation of specific immune responses to an exogenous antigen' [7]. In toxicology it is allergic reactions resulting from immune responses to chemicals and drugs which are of the greatest relevance. The allergy may take a variety of forms, including immediate and delayed hypersensitivity reactions, as well as various types of comparatively ill-defined reactions which may resemble other reaction types.

Padlan [191] hypothesised that a compound's intrinsic immunogenicity, i.e. its ability to initiate an immunological response, arises from an overall structural property exclusive to that compound. As penicillin and cephalosporin antibiotics are structural analogues it should be possible to relate their immunotoxic potential to a common structural feature(s). It is therefore the aim of this work to explore such possibilities using quantitative structure-activity relationship (QSAR) studies.

9.2 Generation of a Data-Base Pertaining to the Frequencies of β-Lactam Antibiotic Induced Adverse Reactions

9.21 Method

In order to determine the frequency of allergic reactions initiated in man, in response to the therapeutic use of a specific β-lactam antibiotic, it was decided to review all relevant published clinical trial literature. The review was performed using 'Medline', cited references, relevant pharmaceutical publications such as 'Merck Index' [192], Martindale's Pharmacopoeia [193], Therapeutic Drugs [194], the US Pharmacopoeia [195], the Encyclopaedia of Antibiotics [196], and Antibiotic and Chemotherapy [197], as well as via communication with the manufacturing companies and specialists in the field.

A review of the literature showed there to be no uniformity in the protocols adopted for the recording of adverse reaction frequencies. It was decided therefore, in order to attain a complete picture of the scope and frequencies of adverse reactions exhibited in response to specific β-lactam therapy, that the data reviewed should encompass world-wide clinical trials of all types (comparative, retrospective, double-blind, etc.), using both healthy and clinically ill (suffering a range of disease states), volunteer and hospitalised subjects of both sexes, ranging in age from new-born to elderly, with a study duration of between 4 and 50 days.

It is very difficult in some cases to differentiate between drug allergies and idiosyncratic reactions, and thus a level of confusion between what is an adverse response or an allergic reaction has arisen, with the term 'allergic reaction' often being applied in an uncritical fashion to all adverse reaction resulting from drug therapy, whether it be a recognised side-effect, a toxic effect or an immunologically mediated response [26]. It is for this reason, therefore, that in this study it was decided to determine the frequency of all adverse responses initiated by specific β-lactam antibiotics as well as the frequency of observed 'true' allergic reactions. In the context of 'true' allergic reactions to the β-lactam antibiotics it is those reactions which are immunologically mediated which are of the most significance. β-lactam allergies may be classified on the basis of the immune mechanism involved; neutralisation, cytotoxic, immune complex, granulomatus, immediate or delayed type hypersensitivity [32].

Anaphylaxis is the most serious allergic reaction of the β-lactam antibiotics to the individual, as it may have fatal consequences. But it occurs rarely and is not usually reported in clinical trial data; the frequency of anaphylaxis was therefore omitted from this study.

The majority of allergic reactions commonly manifest themselves clinically as cutaneous lesions, with urticaria being indicative of IgE-mediated immediate hypersensitivity reactions and maculopapular or morbilliform rashes as well as pruritis and erythematous exanthemas being indicative of non-IgE mediated responses. It is appreciated that determination of the frequency of urticaria and therefore IgE mediated reactions would be very beneficial, as these reactions play a significant part in patient morbidity and mortality [198]. This was not possible for every compound investigated, as in the majority of clinical trials it is the presence of 'rash' that is recorded and not the specific types of cutaneous lesion [199]. The frequency of all cutaneous rashes was determined and assumed to be indicative of the frequency, of all allergic (immunologically mediated) reactions.

Although drug-induced disruption of the lower gastro-intestinal tract is not immunologically mediated and is not therefore an allergic reaction, it is a significant side-effect exhibited in response to β-lactam therapy, and as the literature reviewed specifically reported its frequency, it was decided to include it in the data compilation and subsequent modelling.

All the above detailed adverse responses investigated are of the sub-type A. No responses of sub-type B were investigated, as their occurrence is rare and may or may not be as a result of the specific antibiotic.

The data gleaned from the literature reviewed for each of the penicillins and cephalosporins were mathematically manipulated in accordance with equation 9.1, in order to generate values of percentage frequency of adverse / allergic reaction which were comparable. This was not possible in all cases as the number of patients involved in the trial and / or the number of patients eliciting a specific response were not always given in all the literature. In cases where only one reference is cited percentage frequencies quoted therein were used. In those cases where more than one reference is cited but not all the references provided the required patient numbers, average frequency values for each specific response were calculated only from those studies giving patient numbers.

$$\% \text{ frequency} = \frac{\Sigma \text{ Number of patients in trials eliciting reaction}}{\Sigma \text{ Total number of patients in trials}} \times 100 \qquad (9.1)$$

9.22 Results & Discussion

Initially the literature was reviewed in order to glean information for as many penicillins and cephalosporins as possible, complete data sets were obtained for 70 β-lactam antibiotics (28 penicillins and 42 cephalosporins), spanning numerous years and areas of development. It was felt that this was a suitably sized data base for the project and particularly for QSAR analysis. Appendix 1 lists the structures of the 70 β-lactams reviewed. Some compounds (penicillins N, BT, O, S, amylpenicillin, clometocillin, diphenicillin, fenbenicllin and hydroxybenicillin) were eliminated from the study on the grounds that they were found to be severely toxic or of an insufficient antibacterial potency during development and so were not tested in the clinical situation. For other compounds (hetacillin, BLP-1645, phenethicillin, sulbenicillin, cefazaflur, cephanone, ceftioflur, ceftezole and cefepimizole) complete clinical data sets were unavailable, but information on these compound structures were collected so that they could be used as 'test' compounds for the developed QSARs.

In total, data from over 600 clinical trials, pertaining to the therapeutic use, efficacy and safety of 70 specific β-lactam antibiotics were combined to produce a data base of reaction frequencies which it was felt, reflected a comprehensive therapeutic picture.

The recalculated percentage frequency values of all ARs, cutaneous rash and GI disruption for each of the 28 penicillins and 42 cephalosporins investigated are given in tables 9.1 and 9.2 and presented graphically in figures 9.1 and 9.2 respectively. The data are presented more specifically with regard to the type of antibiotic (penicillin or cephalosporin) and mode of administration (oral or parenteral) in figures 9.3, 9.4 ,9.5 and 9.6.

Examination of the data shows the extent of AR and GI frequencies to be quite similar for both the penicillins (2.69% to 37.00 % AR and 0.42% to 13.33% GI) and the cephalosporins (1.10% to 34.18% AR and 0.00% to 16.46% GI).

However, for cutaneous rash, and therefore allergic reactions, the frequencies observed for the penicillins (0.00% to 10.88%) are considerably lower than those observed for the cephalosporins (0.00% to 22.00%), showing a trend equivalent to that proposed by Norrby [200].

These relationships are maintained when the compounds are sub-classified on the basis of their mode of administration, with values for oral penicillins and cephalosporins ranging respectively, from 2.69% to 23.21 % and 1.88% to 34.18% for AR, from 0.00% to 8.77% and 0.00% to 18.81% for cutaneous rash and from 0.53% to 12.59% and 0.23% to 16.46% for GI disruption, and levels for parenteral penicillins and cephalosporins ranging respectively, from 3.45% to 37.00 % and

1.10% to 29.84% for AR, from 1.10% to 10.88% and 0.00% to 22.00% for cutaneous rash and from 0.42% to 13.33% and 0.00% to 8.30% for GI disruption.

It can also be seen from these respective values that it appears that the parenteral antibiotics are slightly more allergenic than are the orals, this relationship being in agreement with the world survey of Sullivan et al [201], who proposed that the differing safety levels are due in part to the lower doses given orally and to different absorption levels.

At first glance it is somewhat surprising that the parenteral antibiotics cause any GI disruption, let alone at such high levels as given here, as the nature of their administration (intravenously or intramuscularly) prevents absorption in the GI tract which is necessary if a direct effect is to be had on the gut flora, so causing GI disruption. However, parenteral administration is systemic and thus exposes the whole of the gut secretory cells to the antibiotic via its blood supply; thence high levels of the antibiotic may pass into the gut along its entire length, hence affecting the flora. Also, parenteral antibiotics undergo biliary excretion [200], which has more profound effects on both the aerobic and anaerobic faecal flora than do renally cleared antibiotics, so inducing significant levels of GI-disruption. In some instances biliary excreted antibiotics may cause an increased secretion of biliary salts into the GI tract, where their increased concentration, rather than the antibiotic [202] affects the flora.

The age, sex, state of health, and state of renal capabilities of each individual coupled with the presence of any additional medications will also have a bearing on the level of GI-disruption incurred as a result of antibiotic therapy.

Comparison of the data obtained for each individual β-lactam showed there to be a wide variation in the frequencies of adverse responses reported in different studies by different investigators in different laboratories worldwide. This occurrence is disturbing and indicates a lack of uniformity in the methods used for the evaluation of adverse effects. It is likewise suprising that reported frequencies vary quite significantly from country to country [186,200]. Many factors, such as diet and genetic varaition, may account for this latter variation. It is also important to recognise that each of the respective β-lactam antibiotics was administered therapeutically over a range of different dosages. These facts suggest that the data generated in this study, being obtained by reconciling the results of numerous trials performed in a variety of centres, undoubtedly contain substantial errors. These errors may impair the derivation of significant QSARs and mean that any model derived will not be definitive and thus will provide only an indication as to the molecular properties and biological mechanisms which lie behind the induction of an adverse response.

Table 9.1 Frequencies of Adverse Responses Due to 28 Penicillin Antibiotics

Penicillin Generic Name	Percentage Incidence of Adverse Effect & Number of Persons in Combined Trials (xx)			References
	AR*	Rash*	GI*	
Amdinocillin	6.53 (337)	4.01 (299)	0.89 (337)	203,204
Amdinocillin pivoxil	6.00 (NG)	1.00 (NG)	3.33 (NG)	205
Amoxycillin	15.70 (5235)	2.92 (5235)	2.55 (4121)	206-212
Ampicillin	16.91 (1534)	4.72 (7902)	12.59 (3446)	206-208,210,212-226
Apalcillin	9.96 (241)	5.83 (241)	0.42(241)	227-242
Azidocillin	23.21 (NG)	3.75 (NG)	5.63 (NG)	243
Azlocillin	17.51 (1262)	2.93 (1262)	1.74 (1262)	244-247
Bacampicillin	8.93 (918)	2.71 (1476)	1.56(1476)	208,210,212,248,249
Benzylpenicillin	6.70 (1553)	2.70 (1553)	2.50 (1201)	250,251
Carbenicillin	18.57 (168)	6.56 (183)	0.78 (128)	224,244,251-257
Cardinacillin	13.00 (NG)	0.80 (NG)	5.00 (NG)	258-259
Carfecillin	5.05 (NG)	2.560 (NG)	0.53 (NG)	260,261
Ciclacillin	10.03 (1286)	1.74 (2763)	4.65 (3719)	211,213,225,226,262-265
Cloxacillin	37.00 (79)	1.50 (NG)	2.50 (NG)	207,265-268
Dicloxacillin	6.92 (NG)	0.80 (NG)	3.70 (NG)	269,270
Epicillin	7.44 (215)	0.93 (215)	6.05 (215)	271-273
Flucloxacillin	36.67 (30)	3.33 (30)	13.33 (30)	267,274
Lenampicillin	3.89 (1261)	0.79 (1261)	1.27 (1261)	275-298
Methicillin	16.38 (254)	10.88 (193)	12.20 (40)	248,251,267,299-301
Mezlocillin	7.88 (1369)	2.73 (1369)	1.98 (1369)	245-247,251,252,302-304
Nafcillin	12.28 (57)	8.77 (57)	6.11 (229)	248,299-301,305,306
Oxacillin	20.20 (129)	2.50 (80)	2.00 (50)	251,266,300,307,308
Phenoxymethylpenicillin	2.69 (1749)	2.10 (1749)	2.53 (1749)	309-312
Piperacillin	5.77 (2528)	1.27 (2043)	2.49 (2528)	207,244,247,253,254,309, 313-317
Pivampicillin	11.22 (508)	3.21 (2929)	1.74 (2929)	206,208,210,212,249,318-321
Talampicillin	20.69 (55)	2.18 (1282)	1.49 (1282)	208,210,212,214,216,217,322, 323
Temocillin	3.45 (232)	2.50 (40)	0.52(192)	324-327
Ticarcillin	13.50 (NG)	1.10 (NG)	1.70 (NG)	248,251,328

* **KEY**: See end of Table 9.2

Figure 9.1 Relative Frequencies of Adverse Reactions Exhibited by the General Population in Response to Therapy with 28 Penicillin Antibiotics

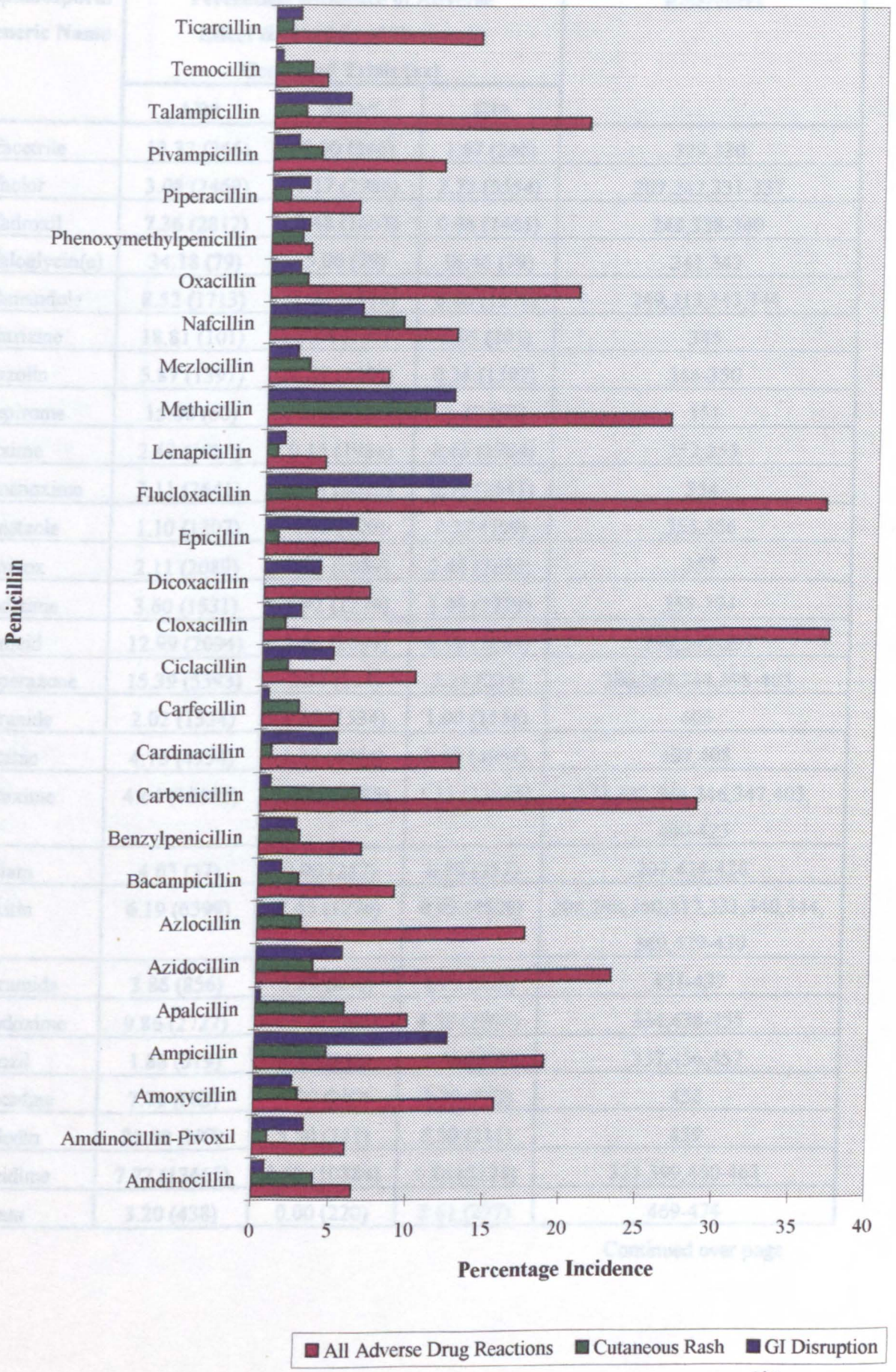


Table 9.2 Frequencies of Adverse Responses Due to 42 Cephalosporins

Cephalosporin Generic Name	Percentage Incidence of Adverse Effect & Number of Persons in Combined Trials (xx)			References
	AR*	Rash*	GI*	
Cefacetrile	13.33 (266)	0.00 (266)	1.67 (266)	329,330
Cefaclor	3.08 (2469)	1.17 (2986)	2.72 (5554)	207,242,331-337
Cefadroxil	7.36 (2812)	0.48 (1461)	0.48 (1461)	248,338-340
Cefaloglycin(e)	34.18 (79)	5.06 (79)	16.46 (79)	341,342
Cefamandole	8.52 (1713)	1.70 (1278)	0.80 (1278)	249,313,343,344
Cefatrizine	18.81 (101)	5.94 (101)	4.95 (101)	345
Cefazolin	5.87 (1397)	1.57 (1397)	0.36 (1397)	346-350
Cefepirome	15.00 (60)	5.00 (60)	3.33 (60)	351
Cefixime	2.42 (1984)	0.15 (1984)	0.45 (1984)	352,353
Cefmenoxime	3.11 (2641)	0.84 (4015)	0.76 (2641)	354
Cefmetzole	1.10 (1207)	0.58 (700)	0.27 (700)	355,356
Cefminox	2.11 (2089)	1.15 (2089)	0.48 (2089)	357
Cefodizime	3.60 (1531)	0.92 (1279)	1.40 (1279)	358-394
Cefonicid	12.99 (2094)	1.96 (2094)	2.10 (2094)	250,395-397
Cefoperazone	15.39 (5593)	2.54 (134)	3.21 (235)	206,269,344,398-405
Ceforanide	2.02 (1534)	1.65 (1534)	1.00 (1534)	406
Cefotetan	4.76 (4954)	1.29 (4954)	2.68 (4954)	407,408
Cefotaxime	4.64 (13585)	1.82 (13585)	1.11 (13585)	331,332,344,346,347,403, 409-423
Cefotiam	4.03 (37)	0.00 (257)	0.39 (257)	207,424-428
Cefoxitin	6.19 (6399)	2.45 (1276)	0.93 (4508)	206,248,250,317,331,340,344, 349,429-430
Cefpiramide	3.86 (856)	1.29 (856)	1.05 (856)	431-437
Cefpodoxime	9.86 (2727)	0.51 (2343)	4.28 (2594)	334,438-455
Cefprozil	1.88 (319)	0.90 (319)	1.45 (298)	337,456,457
Cefroxadine	7.70 (NG)	1.30 (NG)	2.20 (NG)	458
Cefsulodin	22.00 (NG)	5.50 (111)	8.30 (111)	459
Ceftazidime	7.77 (13465)	2.60 (10384)	1.86 (5223)	331,399,460-468
Cefteram	3.20 (438)	0.00 (220)	3.61 (277)	469-474

Continued over page

Table 9.2 Continued

Cephalosporin Generic Name	Percentage Incidence of Biological Effect & the Number of Persons in Combined Trials (xx)			References
	AR	Rash	GI	
Ceftibuten	8.40 (119)	0.00 (92)	5.40 (92)	241,332,333,475
Ceftizoxime	10.87 (2943)	0.92 (2943)	0.78 (2943)	331,343,344,399
Ceftriaxone	5.41 (28260)	1.24 (29334)	2.54 (24354)	331,450,476-479
Cefuroxime	3.70 (1908)	0.83 (1939)	0.72 (1939)	207,248,309,480,481
Cefuroxime-axetil	8.96 (1283)	1.16 (1283	3.58 (1283)	309,482-487
Cefuzonam	7.82 (2316)	3.26 (928)	0.23 (928)	358,425,485-502
Cephalexin	15.00 (NG)	1.00 (NG)	0.33 (NG)	309,503
Cephaloridine	19.00 (NG)	1.00 (NG)	0.00 (NG)	504,505
Cephalothin	10.00 (290)	2.00 (501)	0.70 (290)	266,307,348,398,504,506,507
Cephamycin	7.50 (NG)	0.00 (NG)	2.50 (NG)	258,331,508
Cephapirin	29.84 (124)	0.00 (124)	0.80 (124)	509
Cephradine	7.36 (516)	4.19 (430)	2.45 (490)	274,510,511
Flomoxef	3.12 (1058)	1.61 (1058)	0.76 (1058)	512-540
Latamoxef	10.59 (10674)	2.40 (18308)	2.36 (14810)	250,331,344,399,405
T-2588	3.56 (1152)	0.35 (1152)	1.22 (1152)	541-572

* KEY:

AR = all adverse reactions associated with drug therapy

Rash = skin rash, pruritus, eruption or urticaria associated with drug therapy

GI = GI disruption, primarily diahorrea associated with drug therapy

NG = no data pertaining to patient numbers.

Figure 9.2 Relative Frequencies of Adverse Reactions Exhibited by the General Population in Response to Therapy with 42 Cephalosporin Antibiotics

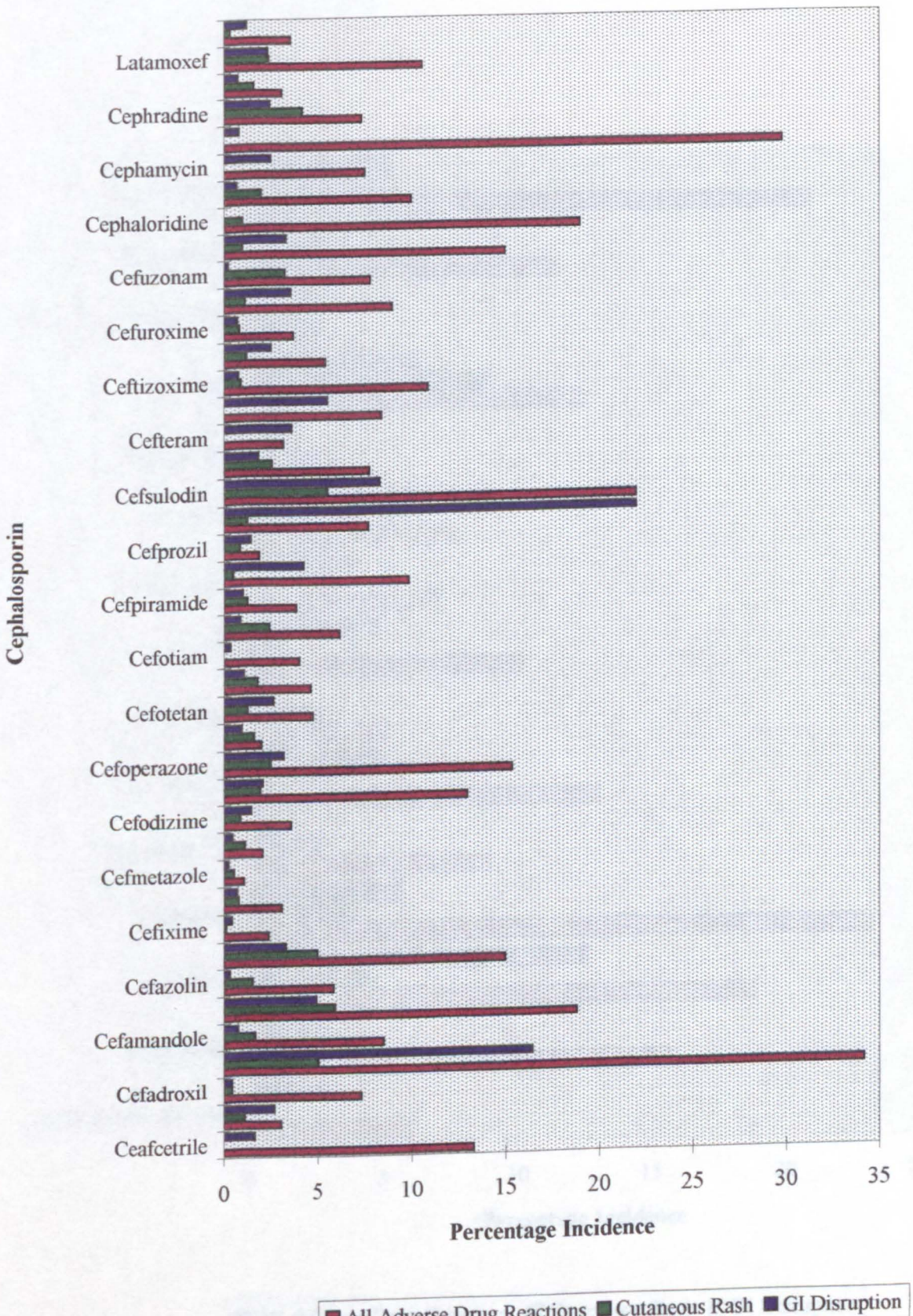


Figure 9.3 Relative Frequencies of Adverse Reactions Exhibited by the General Population in Response to Therapy with 15 Oral Penicillin Antibiotics

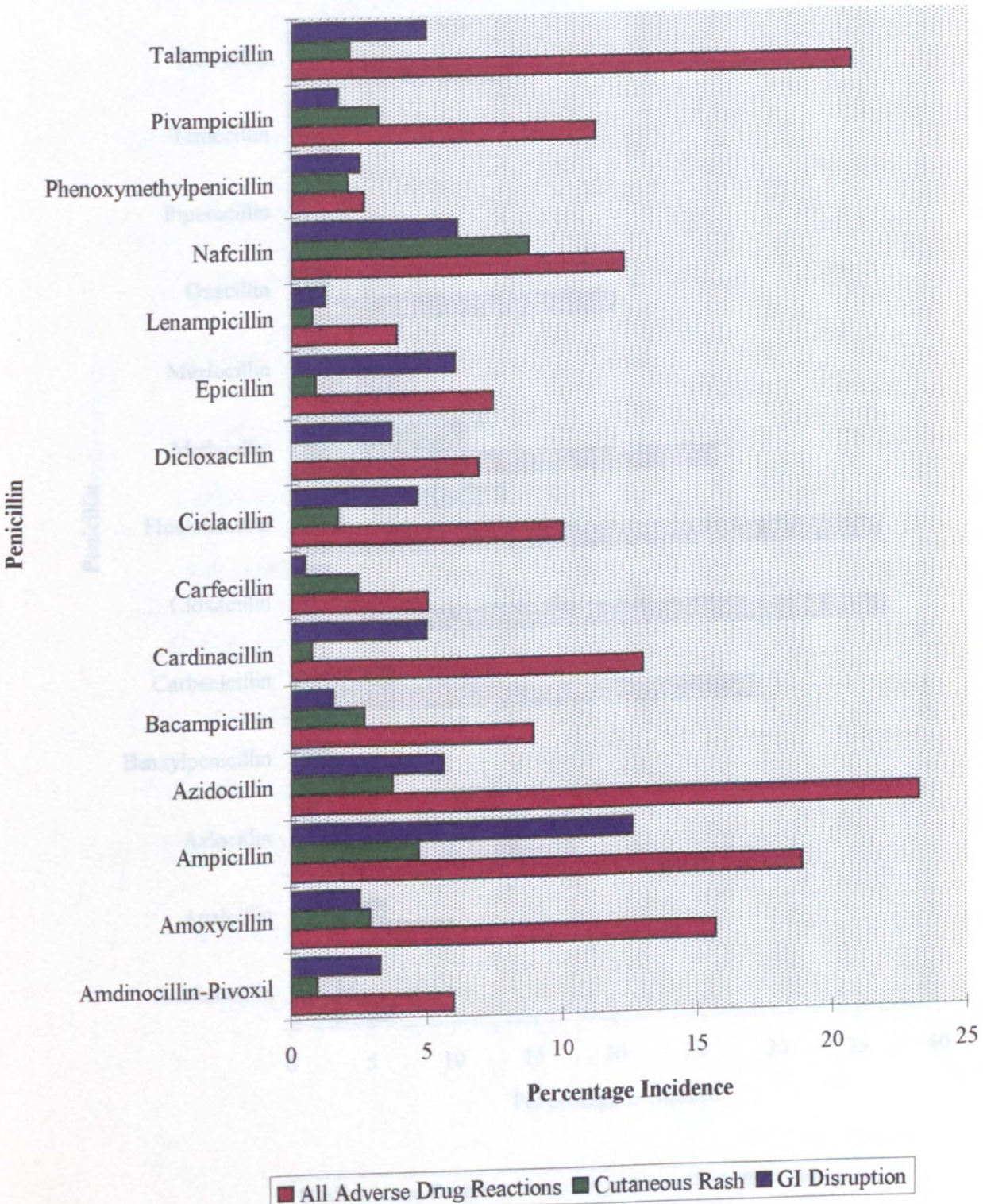


Figure 9.4 Relative Frequencies of Adverse Reactions Exhibited by the General Population in Response to Therapy with 13 Parenteral Penicillin Antibiotics

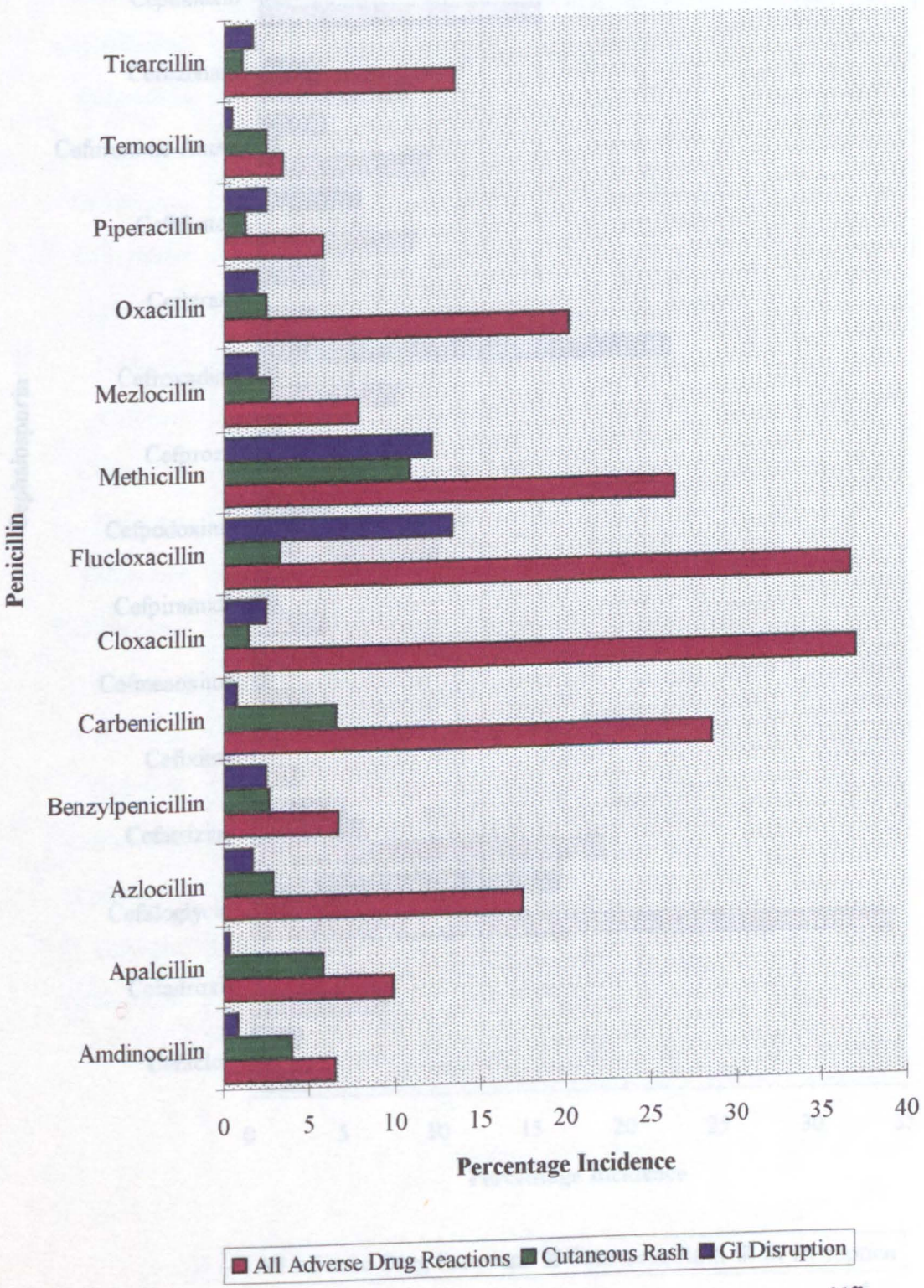


Figure 9.5 Relative Frequencies of Adverse Reactions Exhibited by the General Population in Response to Therapy with 17 Oral Cephalosporin Antibiotics

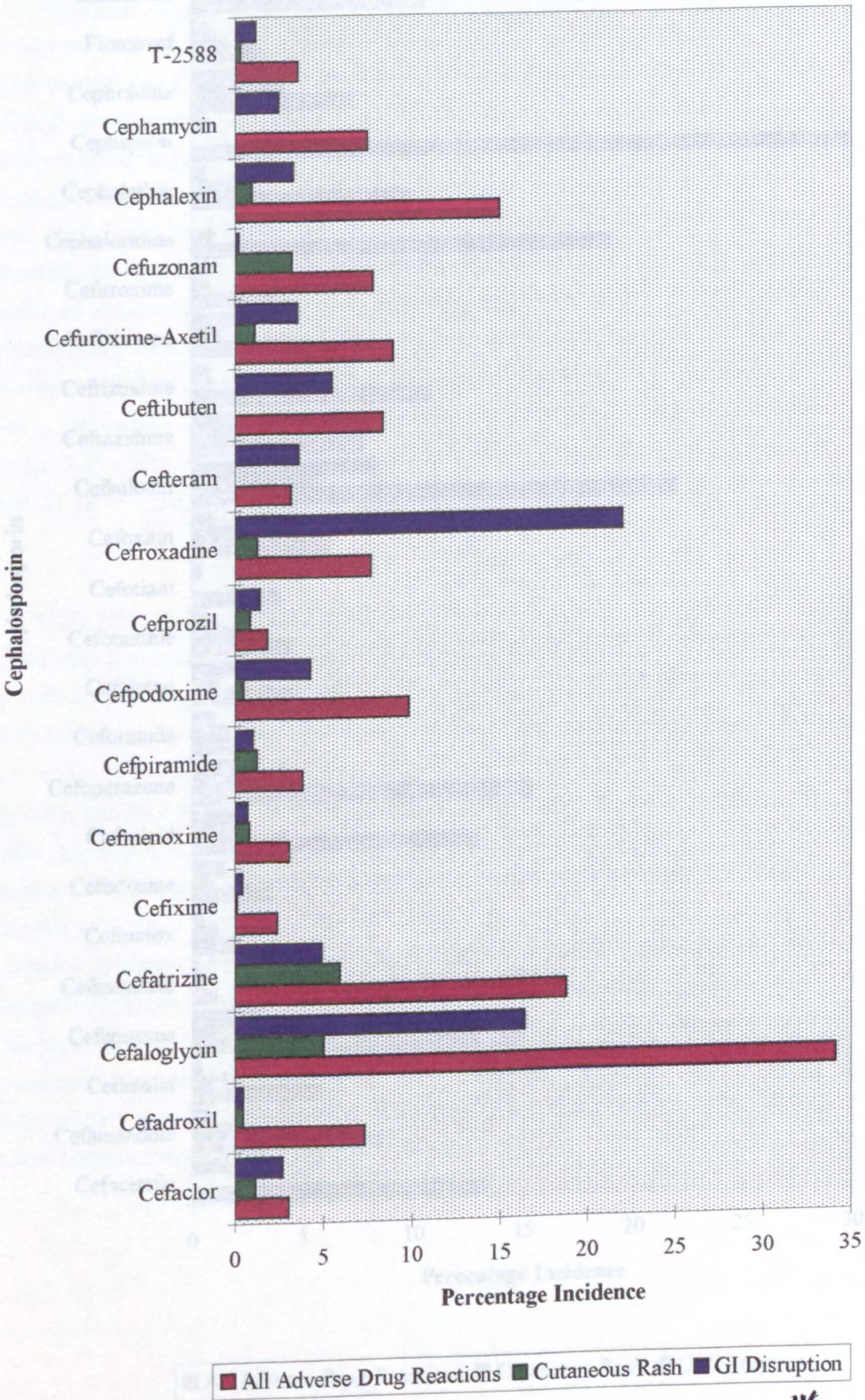
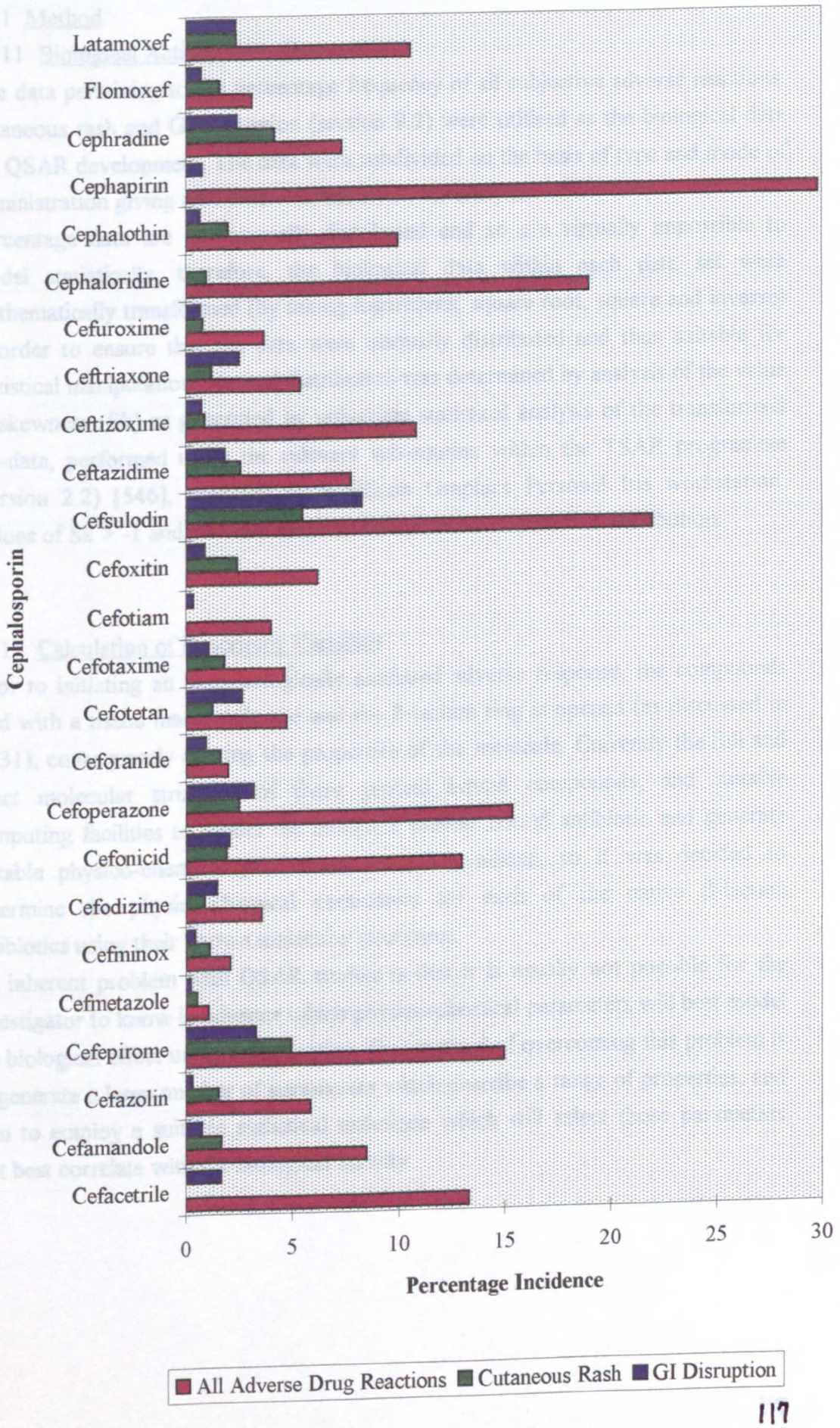


Figure 9.6 Relative Frequencies of Adverse Reactions Exhibited by the General Population in Response to Therapy with 25 Parenteral Cephalosporin Antibiotics



9.3 Development of QSAR Models of β-Lactam Allergenicity

9.31 Method

9.311 Biological Activity Data Generation

The data pertaining to the percentage frequency of all subjective adverse reactions, cutaneous rash and GI disruption (section 9.2) were utilised as the biological data for QSAR development. The data were subdivided on the basis of type and mode of administration giving nine data sets, table 9.3, for analysis.

Percentage data are not normally distributed and so are virtually impossible to model statistically, therefore, the biological data within each data set were mathematically transformed (by taking logarithms, square root, square and inverse) in order to ensure that the data were normally distributed and thus suitable for statistical manipulation. Normal distribution was determined by analysis of the value of skewness (Sk) as generated by univariate statistical analysis of the transformed bio-data, performed using the relevant sub-routine within the TSAR programme (version 2.2) [546], operated on a Silicon Graphics Personal Iris workstation. Values of $Sk > -1$ and <1 were taken to be indicative of a normal distribution.

9.312 Calculation of Descriptor Variables

Prior to initiating an immunologically mediated adverse response, the compounds bind with a tissue macromolecule and the β-lactam ring is opened (as discussed in 7.231), consequently altering the properties of the molecule. Currently the full and exact molecular structure of these protein bound compounds, and suitable computing facilities to model the complete protein bound antibiotic and generate suitable physico-chemical parameters are not available, so it was decided to determine the physico-chemical parameters for each of the native β-lactam antibiotics using their known molecular structures.

An inherent problem with QSAR studies is that it is usually not possible for the investigator to know in advance which physico-chemical parameters will best model the biological effect under investigation. One method of overcoming this problem is to generate a large number of parameters which describe a range of properties, and then to employ a suitable statistical technique which will select those parameters that best correlate with the biological activity.

Table 9.3 Data Sets for QSAR Analysis

Data Set #	Compounds	Biological Data	# Compounds in Data Set
1	All β-Lactams	All ARs	70
		Cutaneous Rash	70
		GI Disruption	70
2	All Oral β-Lactams	All ARs	32
		Cutaneous Rash	32
		GI Disruption	32
3	All Parenteral β-Lactams	All ARs	38
		Cutaneous Rash	38
		GI Disruption	38
4	All Penicillins	All ARs	28
		Cutaneous Rash	28
		GI Disruption	28
5	Oral Penicillins	All ARs	15
		Cutaneous Rash	15
		GI Disruption	15
6	Parenteral Penicillins	All ARs	13
		Cutaneous Rash	13
		GI Disruption	13
7	All Cephalosporins	All ARs	42
		Cutaneous Rash	42
		GI Disruption	42
8	Oral Cephalosporins	All ARs	17
		Cutaneous Rash	17
		GI Disruption	17
9	Parenteral Cephalosporins	All ARs	25
		Cutaneous Rash	25
		GI Disruption	25

The structures of the 70 β-lactams were entered as SMILES (Simplified Molecular Input Line Entry System) codes into the Cobra programme (version 3.0) [573], run on a Silicon Graphics Personal Iris workstation. SMILES is a simple chemical notation system designed for modern chemical information processing. SMILES encodes the molecule in the form of a simple hydrogen-suppressed string. Each atom is represented by its atomic symbol. Lower case notation implies aromaticity, with branches shown in parentheses. Cyclic structure are represented by breaking a bond within the ring structure and designating the ring opening bond with a digit immediately following the atomic symbol and the same digit at the ring closure bond. Other symbols used include = representing a double bond and # representing a triple bond. The molecule can be written from any starting point, as long as the connectivity of the molecule is maintained. Therefore, many different but equally valid codes for the same molecule may exist.

Several conformations relative to each of the SMILES codes were generated, the conformation labelled C1_1_1.pdb was assumed to be the conformation of the lowest energy and was the conformation used in further analysis. Appendix 2 details the SMILES codes used in this study for each of the 70 β-lactam antibiotics used in the model generation process. The conformations were imported into and pictorially displayed in the Pimms programme (version 1.45) [574], where they were checked for correct conformational arrangement and were energy minimised, in the COSMIC sub-routine [575], using the MIN02 quasi Newton-Raphson method, until the root mean square of the energy was reduced to its lowest possible point, this being between 50 and 70 Kcal/mole for the β-lactams. The energy minimised conformations were fitted to the β-lactam nucleus of the benzylpenicillin structure, thus ensuring all structures were in the same orientation, and thus would produce comparable parameters from shape, topological and vectorial analysis.

The structures were imported into the TSAR program [576] (version 2.0) and various relevant physico-chemical and structural parameters generated therein. Further parameters were generated using programs run interactivley on a Western Systems 486 PC, supported by a VAX Mainframe:

Medchem (version 3.54) [576] was used to generate values of the n-octanol/water partition coefficient (CLogP) and molar refractivity (MR).

MOPAC (version 2.0) [577] was used to calculate partial atomic charges for the compounds using the MNDO routine, with the keywords 1scf, am1, mmok, polar, enpart and nointer being employed. Whole molecule parameters of HOMO (highest occupied energy level) and LUMO (lowest unoccupied energy level) energies, dipole moment, total electronic energy of the minimised molecule, and the polarizability were also evaluated.

Indicator values of hydrogen bonding were calculated manually in accordance to the method of Yang et al as outlined in 4.32235, using published fragment values [80]. The respective parameter values for each antibiotic were entered manually into a TSAR database.
Appendix 3 lists the 84 descriptor variables generated and used in the model generation procedure.

In order to reduce the possibility of chance correlations, as may occur when large numbers of physico-chemical (desciptor) variables are utilised, each of the parameters was correlated against the others using the PCA analysis routine, available within TSAR. If correlations were produced in which the multiple correlation coefficient (R^2) > 0.8, one or more of the cross-correlated parameters were removed from the database prior to statistical analysis, so that no cross-correlated parameters were used in the model generation procedure (the correlation matrices for each model (biological data and descriptor variables) generated are presented in appendix 4). The selection criteria used for the selection of a descriptor variable to remain in the data set was based on the variables utility, that is the ability to interpret its significance biologically.
(TSAR, Cobra and Pimms are all available within the CHEST package from Oxford Molecular Ltd., Oxford,UK.).

9.313 Data Analysis

Initially for the larger data sets (all β-lactams, all penicillins and all cephalosporins) each repective class of biological data, were correlated using stepwise regression analysis (available within TSAR) against a selected majority of the physico-chemical and structural descriptors (some of the descriptors related specifically to the cephalosporins, i.e. R_2 side chain properties, and so were not relevant for use in the generation of models pertaining to all β-lactams or penicillins). Those descriptors of most significance in each case were noted. Each class of biological data within each of the three data sets was then regressed against selected combinations of those descriptors showing the most significance using the regression routine in TSAR.
For the smaller data sets (orals and parenterals) each class of biological data was regressed directly against combinations of those parameters showing significance for the larger data sets and if necessary with additional parameters thought to be of relevance.

9.314 Determination of models' significance

For each data set the resultant models' statistical significance was determined by examination of the values of the correlation coefficient (r), and the standard error of the estimate (s) and of the validity plots of predicted biological values against actual biological values and residual values against predicted biological values. These two plots were also used in conjunction to determine if any compound was behaving as an 'outlier'. An 'outlier' can be defined as any compound which following statistical analysis lies a significant distance away form the resultant regression line and other compounds used in the model generation. That it to say the biological activity predicted by the model varies significantly from the actual biological activity, i.e./ the residual variance is significantly greater for this compound than for other compounds used in the model generation.

If a compound was seen to be acting as an outlier and a valid reason could be found to explain this behaviour, the regression analysis was repeated without that compound and the significance of the new model examined.

The models' significance with regards their predictive powers was evaluated via the 'leave-one-out' cross-validation procedure within the TSAR regression analysis and determined from the value of the cross-validated regression coefficient ($r(CV)^2$). Wold [115] has proposed that values of $r\ (CV)^2$ greater than 0.6 indicate that the model is predictively valid, but Clementi [46] has suggested a lower cut-off value of 0.1 as being indicative of predictive ability.

9.315 Data Randomisation

As the initial stepwise regression parameter selection procedure comprised a large number of descriptor variables, it is possible that any given equation developed may be the result of a chance correlation with the biological data. In order to ensure this was not so, once a significant model was developed the biological data were randomised (the descriptor variables being left as they were) some twenty times and re-input into the data base. Regression analysis against the previously highlighted descriptors was repeated and the value of the resultant 'new' regession coefficient determined. If the value of r was >0.6 for any of the randomised models this would indicate that the original model could have been a result of a chance correlation and therefore was not valid. However, if the value of r for each of the randomised models was significantly below that of the original model it could be concluded that the original model was not derived by chance and that it is a true reflection of the physico-chemical properties which control that specific biological response. Thus it can be used to predict the frequency of the given biological response for new, untested or hypothesised compounds and used to aid investigations into the biological systems responsible for initiating a specific response.

9.32 Results and Discussions

9.321 Assessment of the Biological data

The percentage frequency values of all ARs, cutaneous rash and GI disruption for the β-lactams analysed are given in tables 9.1 and 9.2.

Transformation and univariate analysis of the respective biological data sets (as outlined in table 9.3) generated data with a range of skewness values and distribution, as given in tables 9.4, 9.5 and 9.6 for each biological response respectively. Examination of each respective transformation for each related data set (all ARs, cutaneous rash and GI disruption) showed that in all cases one transformation generated normally distributed data for all the related data sets, i.e. Log_{10} transformation of cutaneous rash due to all β-lactams. In order to ensure continuity between related data sets and the resultant models it was decided for each data set (all ARs, cutaneous rash GI disruption) to adopt that transformation which resulted in vaid values of skewness for all the repective sub-data sets (all β-lactams, all oral β-lactams, all parenteral β-lactams, all penicillins, oral penicillins, parenteral penicillins, all cephalosporins, oral cephalosporins and pareanteral cephalosporins).

Table 9.4 Univariate Skewness Values Generated Following Various Mathematical Transformations of the AR Biological Data

Data set	Skewness value (Sk) for the respective transformation of:				
	%I	Log_{10} %I	$\sqrt{}$%I	%I^2	$1/_{\%I}$
All β-lactams	1.478	-0.153	0.681	2.812.	2.326
All Oral β-lactams	1.355	-0.041	0.619	2.869	1.131
All parenteral β-lactams	1.418	-0.241	0.656	2.518	2.591
All penicillins	1.264	-0.063	0.604	2.316	1.362
All oral penicillins	0.386	-0.359	0.047	0.923	1.311
All parenteral penicillins	0.996	0.088	0.592	1.465	1.053
All cephalosporins	1.621	-0.034	0.798	2.994	1.950
All oral cephalosporins	1.912	0.336	1.120	2.902	0.735
All parenteral cephalosporins	1.206	-0.207	0.506	2.427	2.138

%I represents the percentage incidence.

In this instance it was the Log_{10} transformation which generated normally distributed data for each of the sub-data sets. Therefore, Log_{10} data were used in the generation of the QSAR models pertaining to the frequency of all ARs.

Table 9.5 Univariate Skewness Values Generated Following Various Mathematical Transformations of the Cutaneous Rash Data

Data set	Skewness value (Sk) for the respective transformation of:				
	%I	Log_{10} %I	$\sqrt{}$%I	%I^2	$1/_{\%I}$
All β-lactams	1.821	-1.873	0.337	3.978	2.892
All Oral β-lactams	1.010	-1.628	0.088	2.046	2.650
All parenteral β-lactams	1.731	-2.105	0.383	3.124	3.000
All penicillins	1.648	0.122	0.893	2.729	0.908
All oral penicillins	0.363	-0.253	0.017	1.165	0.580
All parenteral penicillins	0.950	0.140	0.578	1.511	0.728
All cephalosporins	1.323	-1.404	0.152	2.234	1.965
All oral cephalosporins	1.554	-0.890	0.663	2.078	1.544
All parenteral cephalosporins	1.113	-1.807	-0.230	2.140	2.199

%I represents the percentage incidence.

In this instance it was the square root ($\sqrt{}$) transformation which generated normally distributed data for each of the sub-data sets. Therefore the square root data were used in the generation of the QSAR models pertaining to the frequency of cutaneous rash.

Table 9.6 Univariate Skewness Values Generated Following Various Mathematical Transformations of the GI Disruption Data

Data set	Skewness value (Sk) for the respective transformation of:				
	%I	Log_{10} %I	$\sqrt{}$%I	%I^2	$1/_{\%I}$
All β-lactams	2.647	-1.212	1.216	4.424	7.950
All Oral β-lactams	2.215	-0.412	0.917	3.621	2.608
All parenteral β-lactams	2.498	-1.231	1.421	3.376	5.662
All penicillins	1.539	-0.079	0.840	2.216	1.592
All oral penicillins	1.525	-0.321	0.632	2.674	2.114
All parenteral penicillins	1.366	0.261	0.968	1.652	0.989
All cephalosporins	3.290	-1.296	1.373	5.372	5.988
All oral cephalosporins	2.426	-0.194	1.155	3.312	1.899
All parenteral cephalosporins	2.424	-1.743	0.898	4.028	4.402

%I represents the percentage incidence.

In this instance it was the Log_{10} transformation which generated normally distributed data for the majority of the sub-data sets. Therefore the Log_{10} data were used in the generation of the QSAR models pertaining to the frequency of GI distribution.

9.322 Regression analysis

A total of 84 physico-chemical descriptors (listed in appendix 3) were generated and input into the TSAR data base along with the biological data.

A summation of the final results attained from regression analysis (including a description of the preliminary validation plots) and from cross-validation analysis for each of the 27 models under investigation is given in table 9.7. It can clearly be seen, from the results summation, that statistically significant and predictively valid QSAR models have been generated for the majority of the data sets (models 9.1 to 9.27 inclusive). A summary of the physico-chemical and structural parameters used in each of the final models generated is presented in table 9.8, full parameter descriptions are given along with the full model.

Appendix 4 presents a listing of the relevant data (compound names, biological and physico-chemical parameter values, residual and predictive values, and the subsequent correlation matrices, as well as representations of the two validity plots for each individual model produced.

Table 9.7 Summation of Regression Statistics, Plots and Cross Validation Results

Data Set	Biological Response	n	# params	# outliers	r	r^2	s	plot 1	plot 2	$r\ (CV)^2$
All β-lactams	All ARs (log10)	70	8	3	0.642	0.412	0.267	sk	hetro	0.090
	Rash (√)	70	8	5	0.731	0.534	0.492	lin	homo	0.303
	GI Disrpn. (log10)	70	7	1	0.684	0.468	0.355	sk	hetro	-0.078
Oral β-lactams	All ARs (log10)	32	4	3	0.790	0.624	0.196	lin	homo	0.527
	Rash (√)	32	4	4	0.895	0.800	0.294	lin	homo	0.602
	GI Disrpn. (log10)	32	4	3	0.780	0.609	0.190	sk	homo	0.380
Parenteral β-lactams	All ARs (log10)	38	3	4	0.732	0.535	0.230	lin	homo	0.370
	Rash (√)	38	4	1	0.789	0.622	0.453	lin	homo	0.233
	GI Disrpn. (log10)	38	2	3	0.784	0.615	0.248	lin	hetro	0.539
All Penicillins	All ARs (log10)	28	3	2	0.627	0.393	0.216	sk	homo	0.269
	Rash (√)	28	4	1	0.857	0.734	0.341	lin	homo	0.634
	GI Disrpn. (log10)	28	3	1	0.893	0.798	0.160	lin	homo	0.782
Oral Penicillins	All ARs (log10)	15	2	1	0.786	0.618	0.177	lin	homo	0.475
	Rash (√)	15	1	1	0.907	0.823	0.181	lin	homo	0.765
	GI Disrpn. (log10)	15	2	0	0.841	0.706	0.138	sk	homo	0.499
Parenteral Penicillins	All ARs (log10)	13	2	0	0.872	0.760	0.166	lin	homo	0.585
	Rash (√)	13	2	1	0.843	0.711	0.404	lin	hetro	0.425
	GI Disrpn. (log10)	13	2	1	0.928	0.861	0.174	lin	homo	0.768
All Cephs	All ARs (log10)	42	3	8	0.852	0.725	0.190	lin	homo	0.576
	Rash (√)	42	4	4	0.796	0.633	0.368	lin	homo	0.560
	GI Disrpn. (log10)	42	4	3	0.814	0.663	0.312	sk	homo	0.240
Oral Cephs.	All ARs (log10)	17	1	3	0.859	0.737	0.167	lin	homo	0.382
	Rash (√)	17	2	4	0.950	0.902	0.213	lin	homo	0.844
	GI Disrpn. (log10)	17	2	4	0.840	0.706	0.162	sk	homo	0.376
Parenteral Cephs.	All ARs (log10)	25	3	4	0.885	0.782	0.178	lin	homo	0.648
	Rash (√)	25	3	1	0.834	0.695	0.317	lin	homo	0.564
	GI Disrpn. (log10)	25	4	1	0.902	0.813	0.260	sk	homo	0.565

Key:

sk - data are skewed about the line of best fit, so the error disribution is not normal.

lin - data are linear, so the distribution of error is normal.

hetro - data are heteroskedastic about the zero line, so there is a large scope for variation, the model is not a good fit with regard to the data used in its construction.

homo - data are homoskedastic about the zero line, there is even distribution of variation, the model is a good fit with regard to the data used to construct it.

Table 9.8 Summary of the Descriptor Variables Used in the QSAR Models Generated

Biological Response	Parameters used in the QSAR models for each respective data set								
	All β-lactams	All Oral β-lactams	All Parenteral β-lactams	All Penicillins	Oral Penicillins	Parenteral Penicillins	All Cephalosporins	Oral Cephalosporins	Parenteral Cephalosporins
All ARs (Log10)	Flexibility of R1 # R1 Amino groups R3 Indicator R4 Indicator # Oxygen atoms SS Cefmetazole SS Flucloxacillin SS Cephapirin	CLogP Dipole Vector Z SS Cefmetazole SS Cephapirin	# R1 Amino groups Charge C of β-ring carbonyl group SS Flucloxacillin	R1 Hyrdogen bond donor ability Flexibility of R1 S Lipophilicity BP	CLogP R1 Hydrogen bond donor ability	CLogP Sterimol B2 of whole molecule	Hydrogen bond acceptor ability Total Dipole # Amino groups R1 Sterimol B3 of R2 # R2 N atoms # R2 Ox atoms SS Cefmetazole SS Cephapirin	CLogP SS Cefmetazole SS Cephapirin	# R1 Amino groups Sterimol L of R2 # Oxygen atoms Charge C of β-ring carbonyl group
Cutaneous Rash (√)	Sterimol B2 SS Benzylpenicillin # Carbons in R1 Log H2O Solubility SS Methicillin SS Cefepirome SS Ceftibuten SS Cefatrizine	SS Benzylpen. Log H2O Sol. SS Ceftibuten SS Cefatrizine	Sterimol B2 # Carbons in R1 Log H2O Solubility SS Methicillin	SS Benzylpen. Sterimol B3 Administration ID SS Methicillin	SS Benzylpen.	Sterimol B3 SS Methicillin	Administration ID SS Cefacetrile SS Cefepirome SS Cefatrizine	Log H2O sol. SS Cefatrizine	Dipole Vector Z SS Cefacetrile SS Cefepirome

Continued over page

Table 9.8 Continued

Biological Response	Parameters used in the QSAR models for each respective data set								
	All β-lactams	All Oral β-lactams	All Parenteral β-lactams	All Penicillins	Oral Penicillins	Parenteral Penicillins	All Cephalosporins	Oral Cephalosporins	Parenteral Cephalosporins
GI Disrupn. (Log10)	# R1 Methyl groups # Sulphur atoms Sum of charges on carbonyl oxygens Kappa 3 Ionisation ptl Administration ID SS Cephaloridine	Surface Area Total Dipole R1 Hydrogen acceptor ability # R1 Methyl gps	# R1 Methyl groups SS Cefsulodin	Surface Area # R1 Methyl gps. Administration ID	Surface Area # R1 Methyl gps.	Surface Area # R1 Methyl gps	Dipole vector Z Sum of charges on carbonyl oxygens SS Cephaloridine SS Cephalexin	Total Dipole SS Cephalexin	Dipole Vector Z Sum of charges on carbonyl oxygens SS Cephalexin

Full descriptions of each parameter and the full models follow.

9.3221 QSAR Models of AR Frequencies

9.32211 All β-Lactam Antibiotics

The log_{10}AR data has a skewness value of -0.153 indicating normal distribution and that the data is suitable for modelling. Stepwise regression against 70 physico-chemical and structural parameters highlighted fourteen descriptors which correlated with the transformed biological data. Regression analysis with the eight most significant descriptors yielded model 9.1, which described only 41% of the information contained within the biological data, indicating that the model was not statistically significant. The cross-validated r^2 for the resultant model (0.09) showed that the model was not predictively valid. The lack of a significant correlation between the AR data of all the β-lactams assessed may be due to the fact that the data contained information pertaining to numerous biological responses, which are brought about via numerous biological mechanisms. Also the different antibiotic types (penicillins or cephalosporins) and modes of administration (oral or parenteral) may lead to the responses being brought about via differing biological mechanisms. Each of these different biological mechanisms may be initiated in response to a different physico-chemical property or combination of properties of the antibiotics under invesigation. Therefore the generation of a simple linear model which correlates the complexity and diversity of such an array of biological mechanisms is probably not possible.

Three compounds, azidocillin, cefaloglycin(e) and cefuroxime, appear to be behaving as outliers. Azidocillin was determined to be an outlier on the premise that it was the only β-lactam containing an azido ($N=N^+=N^-$) functional group, the exact properties of which were problematic to model using Cobra and Pimms. Cefaloglycin(e) was believed to be an outlier by virtue of the fact that the biological data pertaining to the frequency of ARs due to this compound were disproportionately high compared with the other antibiotics. It was believed that this may adversely effect the model generation process. Cefuroxime is with the exception of its prodrug cefuroxime-axetil, the only β-lactam in which the R_1 five membered heterocyclic ring substituents contains a strained oxygen component atom. In equivalent compounds the oxygen atom is replaced with a sulphur atom. It was felt that the presence of the oxygen may differentially affect the electron distribution within the molecule, thus making accurate comparisons with other compounds impossible. The additional R_4 substituent on the prodrug may act to rectify the disproportionate electron distribution, so enabling comparisons to be made with this compound,therefore cefuroxime was believed to be an outlier.

Model 9.1

$Log_{10}AR$ due to all β-lactam antibiotics $= -0.06(0.028)FlexR_1 - 0.178(0.083)\#AminoR_1 - 0.405(0.075)R_3I - 0.202(0.045)R_4I + 0.065(0.059)\#Ox - 1.183(0.103)SSCefmet + 0.801(0.767)SSFluclox + 1.166(0.327)SSCephap + 1.178(0.876)$

$n=67$ $r=0.642$ $r^2=0.412$ $s=0.267$ $F=5.007$ probability$=6.13^{-5}$ $r(CV)^2=0.090$

Where:

$FlexR_1$ is the flexibility of the R_1 acyl side chain of the antibiotic, calculated in TSAR and in accordance with equation 4.10.

$\#AminoR_1$ is the number of amino groups within the R_1 side chain

R_3I is an indicator variable of value 1 or 2 pertaining to the absence or presence, of a substituent group at the R_3 position of the β-ring.

R_4I is an indicator variable of values 0, 1 or 2 pertaining to the presence of either no atom, a hydrogen atom or another substituent, respectively, at the R_4 position.

#Ox is the number of oxygen atoms present within the molecule.

SSCefmet is a measure of the shape similarity of the antibiotic to that of cefmetazole, this antibiotic giving the lowest frequency of ARs.

SSFluclox is a measure of the similarity in shape of the antibiotic to flucloxacillin, this penicillin giving the highest frequency of ARs.

SSCephap is a measure of the similarity in shape of a antibiotic to cephapirin, this cephalosporin antibiotic, in the regression data set, giving the highest frequency of ARs (cephaloglycin results in the highest frequency of ARs of the cephaolsporins but is an outlier and so is not significant with regard to the final model).

Shape similarity is determined through a TSAR/Asp (version 3.01) interface and is calculated in accordance to the method of point counting as proposed by Meyer. Values of similarity obtained range between 0 and 1, with values approaching 1 indicating increased similarity to the chosen lead compound.

9.32212 All Oral β-Lactam Antibiotics

The value of skewness for the $Log_{10}AR$ data for all the oral β-lactams is -0.041 and as such indicates that the data are normally distributed and so suitable for modelling.

Regression analysis of the biological data against combinations of the descriptor parameters highlighted as significant for all β-lactams (9.32211) and other parameters considered to be of relevance to oral compounds, yielded the statistically significant and predictively valid model 9.2, which comprised four descriptors and modelled 62% of the variance contained in the biological data.

Azidocillin and cefaloglycin, both oral antibiotics, were ommitted as outliers for the reasons given in 9.32211. Carfecillin was also isolated as an outlier as it is the only oral β-lactam which possess an R_1 substituent comprising two independent benzene rings. It was felt that the flexibility of the bonds attaching the two rings would make accurate and comparible shape parameter determinations problematic.

Model 9.2

$Log_{10}AR$ due to all oral β-lactams

$$= -0.912(0.031)\frac{CLogP}{10} + 0.214(0.081)\frac{DVz}{100} - 3.316(0.331)SSCefmet + 2.267(0.542)SSCephap + 1.648(0.250)$$

$n=29$ $r=0.790$ $r^2=0.624$ $s=0.196$ $F=9.943$ probability$=3.89^{-5}$ $r(CV)^2=0.527$

Where: CLogP is the logarithm of the octanol-water partition coefficient calculated in Medchem, version 3.54.

DVz is a numerical value pertaining to the dipole moment and therefore electron directing in an arbitary vector Z of the molecule.

SSCefmet is a measure of the shape similarity of the given antibiotic to that of cefmetazole, the antibiotic giving the lowest frequency of ARs.

SSCephap is a measure of the similarity in shape of a given antibiotic to cephapirin, the oral antibiotic in the regression data set giving the highest frequency of ARs (cephaloglycin results is the highest frequency of ARs of the oral cephaolsporins, but is an outlier and so not significant with regard to this final model)

9.32213 All Parenteral β-Lactam Antibiotics

The skewness value for the $\log_{10}$AR data of parenteral β-lactams is -0.241 indicating the data are normally distributed and so suitable for modelling.

Regression analysis of the biological data against combinations of the descriptor parameters highlighted as significant for all β-lactams (9.32211) and other parameters considered to be of relevance to parenteral compounds, yielded a statistically significant and predictively model 9.3, comprising three descriptors which modelled 54% of the information contained within the biological data.

Cefuroxime is an parenteral antibiotic and is determined to be an outlier for the reasons as given in 9.32211. Cefepirome, cefmetazole and cefsulodin were also eliminated from the regression procedure as outliers. Cefepirome because it is the only parenteral compound possessing a fused heterocyclic ring substituent at the R_2 position. It was felt that this substituent, being quite large, may have an adverse bearing on the compound's similarity to flucloxacillin which does not have a substituent group at the R_2 position, thus adversely affecting its ability to enter a binding site and therefore being deleterious to the fitting of this compound's frequency data to the regression model. Cefmetazole is believed in this instance to be a justifiable outlier on the basis of the fact that it the only β-lactam which contains an R_1 straight chain substituent which terminates in a carbon triple bonded to a nitrogen atom. It was felt that the electron distribution and charge about this nitrogen atom would not be effectively modelled, thus affecting the accuracy of some of the descriptors. Cefsulodin was removed as an outlier due to the fact that it comprises a charged nitrogen containing heterocyclic ring in the R_2 substituent. It was not possible to generate relevant parameters which related the effects of this charge.

Model 9.3

$Log_{10}AR$ due to all parenteral β-lactams $= -0.312(0.037)\#AminoR_1 - 1.296(0.029)ChargeβC^*=O + 1.516(0.112)SSFluclox + 3.703(0.152)$

$n=34$ $r=0.732$ $r^2=0.535$ $s=0.230$ $F=11.52$ probability$=2.52^{-5}$ $r(CV)^2=0.370$

Where: $\#AminoR_1$ is the number of amino groups found in the R_1 acyl side chain.

$ChargeβC^*=O$ is the charge on the carbonyl carbon of the β-lactam ring multiplied by a factor of 10 and as determined using Pimms.

SSFluclox is a measure of the similarity in shape of the given antibiotic to flucloxacillin, this penicillin antibiotic giving the highest frequency of ARs.

The descriptor variables included in model 9.3 suggests that as with the oral β-lactams (model 9.2), it is some type of shape dependent binding process which governs both the initiation and extent of stimulation of the biological mechanism(s) responsible for bringing about the development of adverse responses to parenterally adminstered β-lactam antibiotics.

9.32214 All Penicillin Antibiotics

The $Log_{10}AR$ data pertaining to the frequency of ARs due to all penicillin antibiotics has a skewness value of -0.063, indicating it to be normally distributed an so suitable for modelling.

Stepwise regression of the $Log_{10}AR$ data against 70 chosen descriptors highlighted three parameters which correlated with the biological data. Regression analysis of the $Log_{10}AR$ data with respect to the three highlighted descriptor variables yieled model 9.4, which modelled only 39% of the variance present in the biological data.

Two compounds, azidocillin and flucloxacillin were eliminated from the data set as outliers. Azidocillin was assumed an outlier for the reasons given in 9.32211. Flucloxacillin was regarded as an outlier as it produces a significantly higher frequency of ARs compared with the related compounds oxacillin and dicloxacillin. Flucloxacillin differs as it is the only penicillin containing a fluorine group in its structure and it is believed that it is the presence of this fluorine atom which by some means causes an increase in the frequency of ARs induced.

Model 9.4

$Log_{10}AR$ due to all penicillins $= 0.208(0.029)HBDR_1 - 0.073(0.020)FlexR_1 + 0.412(0.115)SBPLipo + 1.009(0.181)$

$n=26$ $r=0.627$ $r^2=0.393$ $s=0.293$ $F=7.744$ probability=0.009 $r(CV)^2=0.269$

Where: $HBDR_1$ is the hydrogen bond donor ability of the R_1 acyl side chain as evaluated by the method of Yang et al [80].

$FlexR_1$ is a measure of the the flexibility of the R1 acyl side chains of the antibiotic, calculated in TSAR and in accordance with equation 4.10.

SBPLipo is a measure of the similarty in liphophilicity of the given penicillin antibiotic to benzylpenicillin determined through a TSAR. Values of between 0 and 1 were obtained, with values approaching 1 indicating increased liphophilic similarity to benzylpenicillin.(chosen because it is believed that the majority of the population are sensitised to this penicillin rather than others, as a result of its wide and extended use).

Examination of the model statistics shows that there is still some 60% of the biological variance not accounted for by the descriptors presented in the model.

The lack of statistical significance is probably because the data represent both orally and parenterally administered penicillins, which possibly initiate ARs via different biological mechanisms, the activation of which is dependent on different physico-chemical properties. The integral properties of the oral and parenteral penicillins which govern the processes of absorption, transportation, distribution and excretion, by virtue of the nature of the compound itself, will vary significantly and it may be that these properties are related to the AR initiation process. If so, it will be impossible to fit a simple linear model to these complex and diverse systems.

9.32215 Oral Penicillin Antibiotics

Data relating to the $Log_{10}AR$ frequency due to oral penicillin antibiotics are normally distributed, with a skewness value of -0.359 and thus are suitable for modelling.

Regression analysis of the $Log_{10}AR$ data with respect to the three descriptors highlighted in 9.32214 and other parameters believed to be of relevance yielded the statistically significant and predictively vaild model 9.5, which modelled 62% of the infomation contained within the biological data.

Azidocillin was determined to be behaving as an outlier an outlier for the same reasons given in 9.32211.

Model 9.5

$Log_{10}AR$ due to oral penicillins $= -0.81(0.19)\frac{CLogP}{10} + 0.203(0.058)HBDR_1 + 0.686(0.042)$

n=14 r=0.786 r^2=0.618 s=0.177 F=8.901 probability=0.004 $r(CV)^2$=0.475

9.32216 Parenteral Penicillin Antibiotics

The $Log_{10}AR$ data for the parenteral penicillins are normally distributed (Sk= 0.088) and as such is suitable for modelling.

Regression analysis was performed using combinations of the three descriptors highlighted in 9.32214 and with other parameters which could be of relevance. Model 9.6 uses two descriptors to model 76% of the variance contained within the biological data and is both statistically significant and predictively valid.

No compounds were found to be behaving as outliers in this data set.

Model 9.6

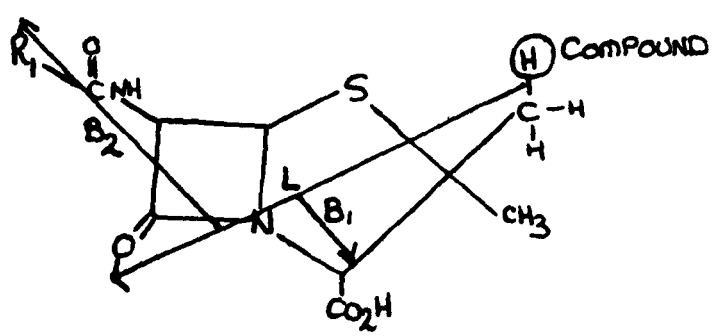
$$Log_{10}AR \text{ due to parenteral penicillins} = 1.207(0.478)\frac{CLogP}{10} - 2.872(1.038)\frac{B2}{10} + 2.115(0.483)$$

n=13 r=0.872 r^2=0.760 s=0.166 F=15.84 probability=4.3^{-4} $r(CV)^2$=0.585

Where: B_2 is the B_2 Sterimol width parameter for the penicillin calculated in TSAR. Although it is not possible to evaluate Sterimol parameters for whole molecules, it is possible within TSAR to measure values of a substituent from a common bond. In this instance the common bond chosen was one of the carbon-hydrogen bonds within the first methyl group attached to the thiazolidine ring. Thus the hydrogen atom became the "compound" and the remaining structure became the substituent and so an indicator of whole molecular shape was evaluated and used in the model, as illustrated in figure 9.6a. If another bond were to be used then the generated Sterimol parameter would differ from those in the above model, and may not be valid.

The presence of the Sterimol parameter in the model is significant as it may be related to the flexibility of the R_1 acyl side chain, as indicated in figure 9.6a. The larger the R1 substituent the larger B2, and if the rotational nature of the substituent bonds allows the more flexible R1 becomes.

FIGURE 9.6a DIAGRAM OF THE POSSIBLE DERIVATION OF THE B2 STERIMOL PARAMETER



9.32217 All Cephalosporin Antibiotics

The $Log_{10}AR$ data for all the cephalosporins are normally distributed (Sk = -0.034) and as such are suitable for modelling. Stepwise regression of the $Log_{10}AR$ data against 81 chosen physico-chemical parameters highlighted numerous parameters which correlated with the bio-data. Regression anlaysis against combinations of the eight most significant descriptors yielded model 9.7, which described 73% of the variance contained within the data.

Three compounds cephaloglycin, cefsulodin and cefuroxime were indicated as outliers, the reasons being the same as those detailed in 9.32211 and 9.32213.

Model 9.7

$$Log_{10}AR = 5.267(1.095)^{HA/100} - 3.35(1.820)TD/10 - 2.146(0.762)\#AminoR_1 + 2.04(0.190)B_3R_2/10 - 2.05(0.320)\#NR_2/10 - 7.900(0.800)\#OxR_2/100 - 2.059(0.223)SSCefmet + 1.094(0.378)SSCephap + 1.227(0.266)$$

due to all cephalosporins

$n=39$ $r=0.852$ $r^2=0.725$ $s=0.190$ $F=9.905$ probability$=2.58^{-7}$ $r(CV)^2=0.576$

Where: HA is the ability of the whole cephalosporin molecule to accept hydrogen bonding, as determined by the method of Yang et al [80].

TD is a measure of the total dipole moment of the cephalosporin, an indicatotion of the electron directing occurring within the entire molecule.

B_3R_2 is the Sterimol B_3 width parameter of the R_2 substituent.

$\#NR_2$ is the number of nitrogen atoms contained within the R_2 substituent.

$\#OxR_2$ is the number of oxygen atoms present within the R_2 substituent.

The resultant model 9.7 is significant not only statistically but also because some of the parameters contained within the model are equivalent to those highlighted as being of significance in the induction of ARs by all β-lactam antibiotics, model 9.1. The model suggests that more the cephalosporin antibiotic resembles cefmetazole and the less it resembles cephapirin the less likelty it is to be able to 'fit' the receptor site(s) and initiate binding.

It is interesting to note that with the cephalosporins the R_2 substituent has profound effects on the induction of ARs, both through its shape and its electronic properties.

9.32218 Oral Cephalosporin Antibiotics

The data petaining to the Log_{10} of the frequency ARs induced in response to therapy with oral cephalosporin antinbiotics has a skewnes of 0.336, which suggests normal distribution and thus that the data are suitable for modelling.

Cephaloglycin was determined in 9.32211 to be an outlier and was therefore eliminated from the model generation procedure.

Regression analysis was therefore carried out for 16 of the 17 cephalosporins, using combinations of the eight descriptor variables proposed as being significant in 9.32217 and other physico-chemical parameters believed to be of relevance to oral cephalosporins. The resultant model 9.8 effectively modelled 74% of the biological variance and was both statistically significant and predictively valid.

Model 9.8

$$\text{Log}_{10}\text{AR due to oral cephalosporins} = -9.995(2.500)\frac{\text{CLogP}}{100} - 4.617(0.817)\text{SSCefmet} + 3.657(2.083)\text{SSCephap} + 1.564(1.217)$$

n= 6 r=0.859 r^2=0.737 s=0.167 F=11.23 probability=4.04^{-4} $r(CV)^2$=0.382

The model proposes that the two descriptor variables (SScefmet and SS cephap) and CLogP are of significance in controlling the induction of ARs by oral cephalosporins. The resultant model therefore supports the theory of AR induction as previously discussed in 9.32217, that some means of shape dependent receptor recognition and binding are responsible for governing the extent and duration of a specific AR response.

9.32219 Parenteral Cephalosporin Antibiotics

The skewness value of $Log_{10}AR$ frequency due to parenteral cephalosporins is -0.271 and indicates normal distribution and that the data are suitable for modelling. Three compounds were removed prior to regression analysis, cefuroxime, cefepirome and cefsulodin, due to their previously being identified as being outliers (9.32211, 9.32213).

Regression analysis of the transformed biological data against combinations of those descriptors believed to be of relevance yielded model 9.9, which was both statistically significant and predictively valid and which modelled 78% of the information contained within the biological data.

Model 9.9

$Log_{10}AR$ due to parenteral cephalosporins $= -3.572(0.652)\#AminoR_1/10 - 8.678(2.113)LR_2/100 + 1.055(0.4141)\#Ox/10 - 2.859(0.706)ChargeβC^*=O + 9.302(1.970)$

$n=39$ $r=0.852$ $r^2=0.725$ $s=0.190$ $F=9.905$ probability$=2.58^{-7}$ $r(CV)^2=0.576$

Where: LR_2 is the Sterimol measurement of the length of the R_2 substituent. In this instance the sterimol parameters were evaluated along the bond attaching the R_2 substistuent to the core nucleus of the cephalsoporin, towards the terminal atom of the R_2 substituent.

#Ox is the number of oxygen atoms present in the entire cephaolosporin.

The model has further significance as it contains the parameters $\#AminoR_1$ and #Ox which were highlighted as being significant in the earlier related model 9.7, and ChargeβC*=O which was highlighted as being significant in the induction of ARs by all parenteral β-lactams (model 9.3) and a Sterimol shape parameter which is related to the R_2 substituent shown to be of significance in model 9.7.

Overview of All Adverse Reactions QSARs

The data pertaining to the frequency of all ARs relate to the development of one or more multiple effects, which are initiated through multiple reaction mechanisms. It has not therefore, been possible to produce models which 'fit' exactly to each reaction mechanism. However, it has been possible to some extent to produce linear regression equations relating to the frequency of responses initiated to the molecular properties of the antibiotics (9.1 to 9.9 inclusive).

Of the nine QSARs developed seven present some level of statistical and predictive significance, whereas the two remaining (all β-lactams and all penicillins)are not statistically significant.

Examination of the descriptor variables present in the nine developed models (table 9.8 page 129) has enabled the development of a generalised picture pertaining to the molecular properties responsible for initiating such adverse responses to be drawn.
In each instance the derived models comprise a shape, hydrophobicity, electronic and / or R1 substituent parameter.

The presence of one or more equivalent descriptor types in each of the individual models indicates a pattern of information to be emerging regarding the physico-chemical requirements for the induction of a AR and hence gives credibility to each of the nine models.

The hydrophobicity parameter, in most cases CLogP, by virtue of its differing sign between oral and parenteral sub-groups (negative for orals and positive for parenterals), probably accounts for the different absorption requirements of the different modes of administration.

The presence of the molecular shape and / or electronic parameters suggests that the majority of the adverse reaction types assessed in the data may be induced by the binding of the antibiotic to a specific receptor. The shape of the antibiotic being that which governs whether the antibiotic has the capability to enter and 'fit' with the receptor site, and the electronic property being that which controls the extent of binding and thus initiation of the response.

The presence of parameters relating specifically to the R1 acyl side chain substituent of the antibiotics, provides some support to previous hypotheses [579,580] that the R1 substituent is predominant in controlling the inductions of ARs.

However, due to the complexity and diversity of the biological mechanisms involved in giving rise to all the AR responses, it was not possible, using these data, to generate models which provided information as to the location or nature of the receptor sites, as these could be numerous. Further analysis into the frequencies of specific responses, i.e. cutaneous rash, may lead to the generation of regression models which can be used more specifically in determining the exact biological mechanisms giving rise to them.

However, it must be remembered that the biological data used must, by virtue of their nature, contain substantial error, and it may be that the correlations obtained represent the limit of useful information that can be extracted.

9.3222 QSAR Models of Cutaneous Rash Frequency

9.32221 All β-Lactam Antibiotics

The square root transformation of the biological data pertaining to the frequency of cutaneous rash for all the 70 β-lactam antibiotics under investigation presented a skewness value of 0.337, therefore showing them to be normally distributed and so suitable for modelling.

Stepwise regression against 70 descriptor variables indicated twelve which were related to the cutaneous rash data. Regression analysis against combinations of the eight most significant descriptors produced the statistically siginificant and predictively valid regression model 9.10, which modelled only 53% of the variance contained with the biological data. This is probably due to the fact that the data contains information relating to both oral and parenterally adminstered β-lactam antibiotics, which possess differing physico-chemical and structural properties that may cause them to act via different mechanisims. Also, the data relate to both penicillin and cephalosporin antibiotics which may induce cutaneous rash via different biological mechanisms.

Four compounds (epicillin, cephaloglycin, cefuzonam and cefroxadine) were isolated as acting as outliers. Epicillin and cefroxadine were believed to be acting as an outliers because they are the only compounds to contain a heterocyclic rather than a benzene ring within the R_1 acyl side chain. This structural variation confers differing properties on the structures, affecting overall molecular shape, and the flexibility of the R_1 side chain, as well as affecting the electron distribution within the molecules. Cephaloglycin was removed as an outlier on the basis of the reasononing given in 9.32211. Cefuzonam was belived to be an outlier because in the R_2 side chain the nitrogen atom of other equivalent substituents is replaced by a sulphur atom, which will confer different electronic properties on the compound, so restricting its statistical comparision.

Model 9.10

$\sqrt{}$Rash due to all β-lactam antibiotcs $= 1.236(0.835)B_2 + 1.092(0.942)SSBP + 0.453(0.127)\#CR_1 - 1.843(0.528)LogH_20sol + 5.138(2.314)SSMeth + 2.624(1.000)SSCefepir - 3.118(0.844)SSCeftib + 1.816(0.512)SSCefatriz - 5.321(1.336)$

$n=66$ $r=0.731$ $r^2=0.534$ $s=0.492$ $F=8.159$ probability$=1.452^{-7}$ $r(CV)^2=0.303$

Where:

SSBp is the shape similarity of the antibiotic to benzylpenicillin, the supposed sensitising β-lacatm, as determined in TSAR.

$\#CR_1$ is the number of carbon atoms in the R_1 acyl side chain.

$LogH_20sol$. is a measure of the antibiotics water solubility, calculated using the MicroQSAR programme PC Version [581].

SSMeth. is a measure of the shape similarity of the antibiotic to the parenteral penicillin methicillin, the antibiotic responsible for inducing the greatest frequency of cutaneous rash.

SSCefepir. is a measure of the shape similarity of the antibiotic to the parenteral cephalosporin cefepirome, the parenteral cephalosporin responsible for inducing the highest frequency of cutaneous rash.

SSCeftibut. is a measure of the shape similarity of the antibiotic to the oral cephalosporin ceftibuten, the oral cephalosporin responsible for inducing the lowest frequency of cutaneous rash.

SSCefatriz. is a measure of the shape similarity of the antibiotic to the orall cephalosporin cefatrizine, the oral β-lactam responsible for inducing the highest requency of cutaneous rash.

9.32222 All Oral β-Lactam Antibiotics

The transformed biological data have a skewness value of 0.088, thus indicating that they are normally distributed and therefore suitable for regression analysis.

Four compounds (epicillin, cephaloglycin, cefuzonam and cefroxadine) were previously (Model 9.10) isolated as acting as outliers and were removed from the data set prior to analysis, for the reasons as outlined in 9.32221. Regression analysis with combination of the those parameters highlighted in model 9.10 which were believed to be of relevance yielded model 9.11 which was both statistically significant and predictively valid and which modelled 80% of the variance contained within the biological data.

Model 9.11

$\sqrt{}$ Rash due to oral β-lactam antibiotcs = 2.025(1.088)SSBp - 1.412(0.283)Log$H_2$0sol - 2.053(0.419)SSCeftib + 2.868(2.602)SSCefatriz - 1.128(1.130)

n=28 r=0.895 r^2=0.800 s=0.294 F=23.06 probability=2.245^{-8} $r(CV)^2$=0.602

The derived model suggests that for oral β-lactam antibiotics there is a positive realtionship between the incidence of rash and the similarity in shape of the oral antibiotic with benzylpenicillin and cefatrizine, and a negative realtionship with the similarity in shape to ceftibuten and water solubility.

The less the oral antibiotic ressembles benzylpenicillin (the major sensitising agent) and cefatrizine (that oral antibiotic giving rise to the greatest frequency of cuatneous rash) and the more it resembles ceftibuten (that oral antibiotic inducing the least amount of cutaneous rash) the more likely is the antibiotic not to be recognised by the antibody and bind in the shape specific receptor sites and therefore, the lower will be the resultant frequency of cutaneous eruptions induced. The data set contains information pertaining to both penicillins and cephalosporins and it may be that the specific antibody involved in the recognition of these subtly different antigenic types, this may be reflected in the need for three shape parameters in regression model 9.11.

9.32223 All Parenteral β-Lactam Antibiotics

The skewness value of the square root transformed data within this set was 0.383, indicating them to be normally distributed as so suitable for regression analysis.

No compound was found to be behaving as an outlier and so regression analysis and model generation were carried out using each of the 38 parenteral β-lactams.

Regression analysis was performed using combinations of those descriptor variables highlighted as being relevant in 9.32221 (model 9.10) and produced the regression model 9.12 which was marginally statistically significant, describing only 56% of the biological variance, and only slightly predictively valid. The lack of statistical significance and predicability may be due to an inability to produce a linear model which describes differences in the biological reaction mechanisms adopted by the penicillins and cephalosporins. Alternatively it may be that is was not possible to model the different biological mechanisms involved in giving rise to different reaction responses, i.e/ different types of cutaneous rash.

The model is however, useful in that it provides some support to the previously derived related, yet more generalised model of cutaneous rash (9.10), in that the descriptors utilised are equivalent to some of those signified therein, thus indicating similar mechanisms to be operating, as would be expected.

Model 9.12

$\sqrt{}$ Rash due to parenteral β-lactam antibiotcs

$$= 2.005(1.317)\frac{B_2}{10} + 1.051(0.218)\frac{\#CR_1}{10} - 1.647(0.927)LogH_20sol + 5.883(1.098)SSMeth - 4.607(0.842)$$

$n=38$ $r=0.748$ $r^2=0.559$ $s=0.490$ $F=10.46$ probability=9.03^{-6} $r(CV)^2=0.233$

9.32224 All Penicillin Antibiotics

The square root transformation of the biological data pertaining to the frequency of cutaneous rash exhibited following therapy with 28 penicillin antibiotics gave a skewness value of 0.893, indicating them to be normally distributed and so suitable for modelling. Regression analysis of the data was performed against combinations of those physico-chemical parameters found previously to be of significance in the modelling of cutaneous rash (models 9.10, 9.11 and 9.12), plus others which were believed to be of relevance. The statistically significant and predictively valid regression model 9.13, which described 73% of the variance contained within the biological data was generated.

Ticarcillin was isolated as being an outlier on the basis that it is given at a therapeutic dose (20g/day) which far exceeds that of the other penicillins (averaging between 2 and 5g/.day). Therefore, the frequency of rash reported in the clinical trials may be dose dependent and not a true or comparable frequency. Appendix 5 details the therapeutic dose levels of each of the 70 β-lactams.

Model 9.13

$$\sqrt{\text{Rash due to all penicillin antibiotics}} = 2.54(0.19)\text{SSBP} + 3.64(0.48)\frac{B_3}{10} + 6.36(1.21)\frac{\text{Admin}}{10} + 5.34(0.83)\text{SSmeth} - 9.15(4.08)$$

$n=27$ $r=0.857$ $r^2=0.734$ $s=0.341$ $F=15.18$ probability$=1.601^{-6}$ $r(CV)^2=0.634$

Where: B_3 is the Stermiol B_3 width measurement of the whole molecule.

In this instance the parameter is a measure of the width of the whole molecule as measured from the carbonyl bond of the β-lactam ring.

Admin is an indicator variable pertaining to the mode of administration of the penicillin, where 3 indicates oral and 4 parenteral.

The regression model generated (9.13) yet again highlights the importance of the shape of the antibiotic itself in controlling the extent of cutaneous rash induced. In this instance there is a negative relationship between the incidence of rash and the similarity in shape of the penicillin to benzylpenicillin (the primary sensitiser) or methicillin (that penicillin which induces thee greatest frequency of rash).

This model also suggests that the mode of administration (oral or parenteral) of the penicillin is of significance when coupled to the shape of that penicillin, in governing the frequency of rash induction. This would be expected on the basis of the effects which the differential absorption, transportation and distribution requirements of the oral and parenteral penicillins would have on rash induction by the respective compounds.

Separate examination of the different administration modes may therefore yield more significant and more biologically significant and explainable regression models.

9.32225 Oral Penicillin Antibiotics

Univariate statistical analysis showed the square root tranformed data to have a skewness value of 0.017, indicating that they are normally distributed and suitable for statistical modelling.

Epicillin was removed from the data set prior to regression analysis, as it was believed to be an outlier for the reasons previously stated in 9.32221.

Regression analysis of the biological data of the remaining 27 penicillins against combinations of the the physico-chemical parameters signified in 9.32224 (model 9.13) as being of importance, yielded the regression model 9.14 which consisted of one descriptor and which was both statistically significant, modelling some 82% of the variance presented in the biological data, and predictively valid.

Model 9.14

$\sqrt{}$Rash due to oral penicillins = 3.824(0.263)SSBP - 1.754(0.182)

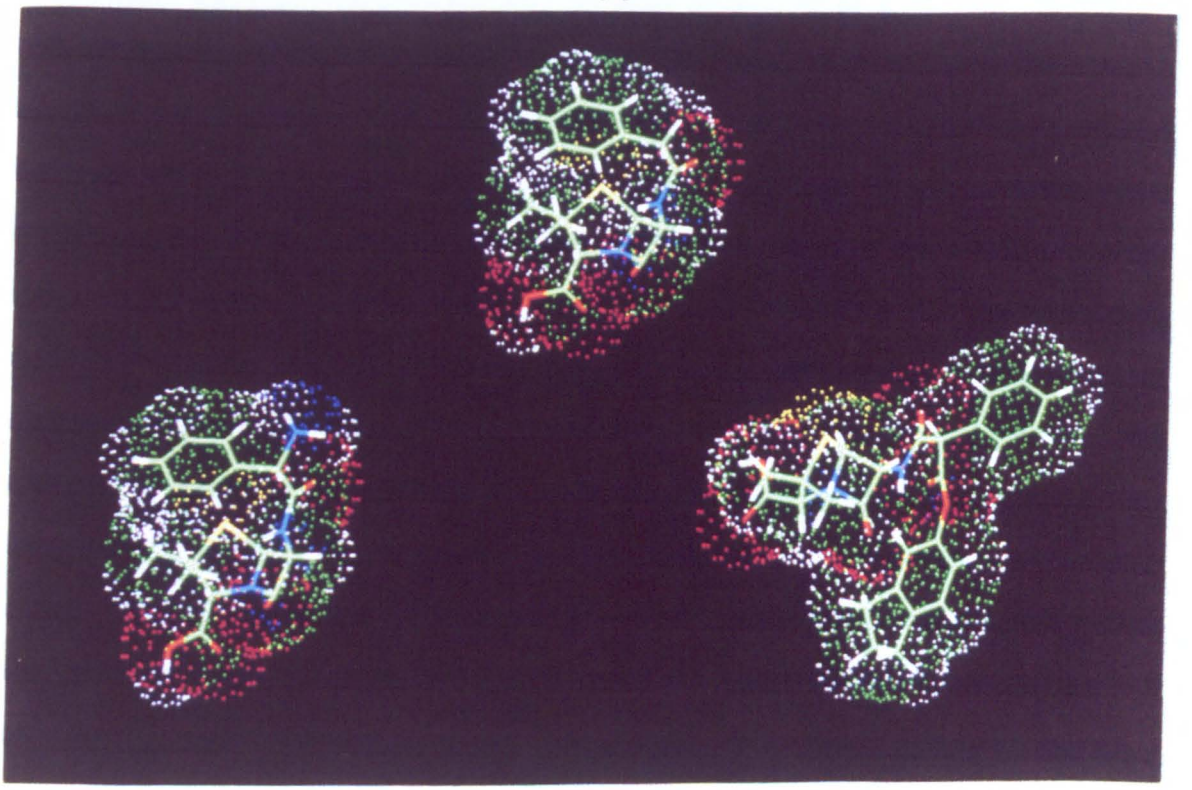
n=14 r=0.907 r^2=0.823 s=0.181 F=55.73 probability=4.728^{-6} $r(CV)^2$=0.765

The model suggests that the frequency of cutaneous rash as exhibited in response to therapy with oral penicillins is directly related to the similarity in shape of the pencillin to benzylpenicillin. The more the penicillin resembles benzylpenicillin the greater the frequency of cutaneous rash it will induce. This relationship is displayed pictorially in figure 9.7. (Note: the molecules presented are in the same oreintation and so the shapes presented are directly comparable).

The model is also biologically significant as it can be related back to the biological situation *in vivo*, since the majority of the population are believed to be already sensitised to benzylpenicillin (BP) as a result of environmental exposure [1] and thus possess antibodies against benzylpenicillin. A second administration of benzylpenicillin or a structurally similar compound will lead to the initiation of a hypersensitivity reaction, as discussed in 7.231. If another oral penicillin is administered, its shape similarity to the sensitising benzylpenicillin will govern the extent of allergic reaction initiated. An oral penicillin which is very similar in shape to benzylpenicillin will be able to occupy the same epitope on the antibody and so will be fully recognised by the anti-benzylpenicillin serum antibodies; a full allergic response will result, giving high levels of cutaneous rash. Penicillins which bear less similarity in shape to benzylpenicillin will be recognised by or fit with only part of the antibody epitope, thus only a fractional allergic response will result, giving lower incidence of cutaneous rash. Penicillins with only slight similarity in shape to benzylpenicillin will not be recognised by the antibodies or fit with any part of the epitope and so no reaction and hence no rash will result from their use.

Figure 9.7 Representation of the Relationship Between the Frequency of Cutaneous Rash Induced by Oral Penicillins and Their Shape Similarity to Benzylpenicillin

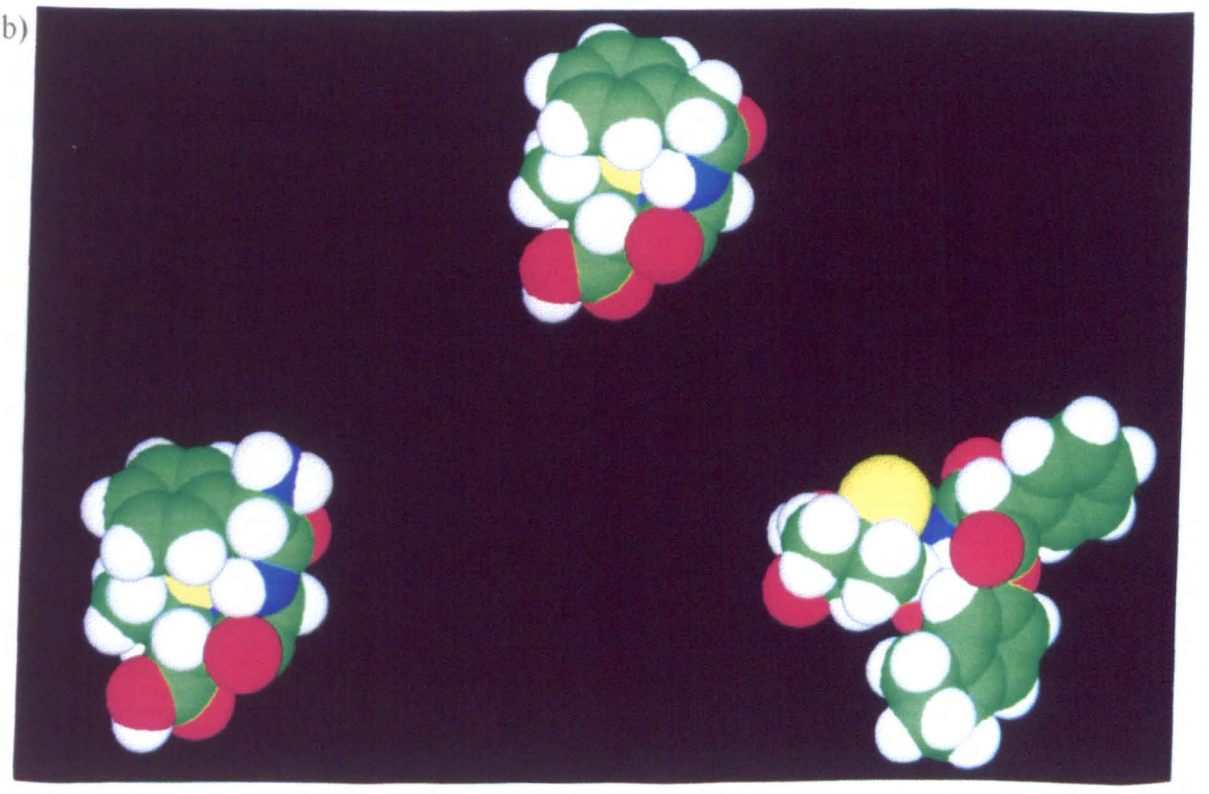
a)



Ampicillin
Shape similarity = 0.991
Frequency of rash = 16.91%

Cardinacillin
Shape similarity = 0.674
Frequency of rash = 0.80%

b)



9.32226 Parenteral Penicillin Antibiotics

The skewness value of 0.578 obtained for the parenteral pencillin cutaneous rash frequency data showed them to be normally distributed and so suitable for analysis.

Ticarcillin was removed from the data set prior to analysis as an outlier, the reasons being the same as those outlined in 9.32221.

Regression analysis of the transformed biological data against combinations of those descriptor variables highlighted as significant in 9.32224 (model 9.13) produced the statistically significant and predictively valid model 9.15, which modelled 71% of the infomation contained within the biological data.

Model 9.15

$\sqrt{\text{Rash due to parenteral penicillins}} = 4.76(0.88)\frac{B_3}{10} + 5.90(2.06)\text{SSMeth} - 5.86(1.72)$

$n=12$ $r=0.843$ $r^2=0.711$ $s=0.404$ $F=11.09$ probability $=0.002$ $r(CV)^2=0.425$

The model clearly suggests that there is a positive relationship between the frequency of cutaneous rash initiated by a parenteral penicillin and the shape of the molecule itself. In this instance it is the similarity in shape of the parenteral penicillin to methicillin (that parenteral penicillin giving rise to the greatest frequency of rash) coupled with a Sterimol width measurement which are the significant controlling factors. The more the parenteral penicillin resembles methicillin and the greater the B_3 width the higher will be the resultant fequency of cutaneous rash induced. The need for two shape descriptors may relate to the nature of the receptor site, in that one parameter alone is unable to fully represent all aspects of the receptor. The nature of the Sterimol parameters prevent identification of the exact region of the molecule modelled by each respective parameter and thus restricts the ability hypothesize as to the receptor requirements.

9.32227 All Cephalosporin Antibiotics

The square root transformed data pertaining to the frequency of cutaneous rash induced by cephalosporin therapy produces data which are normally distributed, having a skewnes value of 0.152, and thus which are conducive to regression analysis.

Two cephalosporins (cephaloglycin and cefuzonam) were eliminated from the data set prior to analysis on the grounds that they behaved as outliers. Cephaloglycin and cefuzonam were for of the reasons given in 9.32221. Two further compounds ceftibuten and cephamycins were found during analysis to be behaving as outliers. Ceftibuten was believed to be acting as an outlier because it is administered at a much lower therapeutic dose (0.4g/day) than other cephalosporins (average dose 4g/day), as a result of which its plasma concentration at its half-life is also much lower (6μg/ml) compared with the other cephalosporin antibiotics (average plasma concentration at $t_{1/2}$ being 120μg/ml), detailed in appendix 5. This fact may account for the relatively lower frequency of rash. Should ceftibuten be given at equivalent doses the frequency may increase considerably. Cephamycin is essentially a group of four cephalosporin like β-lactams, and it is not clear from the literature whether the frequency data presented for cutaneous rash, are for one or a combination of these cephamycins. This uncertainty regarding the accuracy of the clinical data is the reason for the omission of cephamycin as an outlier.

Regression analysis against combinations of those descriptors indicated in 9.32221 (model 9.10) as being of significance, and against others which were thought may be relevant, yielded the statistically significant and predictively valid regression model 9.16, which explained 63% of the variance presented in the biological data.

Model 9.16

$$\text{/ Rash due to all cephalosporins} = 2.08(0.06)\frac{\text{Admin}}{10} - 4.16(0.35)\text{SSCefacet} + 3.34(0.68)\text{SSCefep} + 3.75(0.71)\text{SSCefatriz} - 1.51(0.98)$$

$n=38$ $r=0.796$ $r^2=0.633$ $s=0.368$ $F=14.25$ probability $=3.949^{-7}$ $r(CV)^2=0.560$

Where: SScefacet is a measure of the similairity in shape of the antibiotic to the parenteral cephalosporin cefacetrile, which is the cephalosporin (in the data set) giving rise to the lowest frequency of cutaneous rash. Ceftibuten actually presents the lowest frequency but as it was excluded as an outlier its inclusion as a descriptor was felt to be unsuitable.

The model indicates that the more the cepahlosporin resembles both cefepirome and cefatrizine and the less it resembles cefacetrile, in shape, the greater will be the frequency of cutaneous eruption which will result. The model also proposes that, when coupled to the shape of the cephalosporin, the type of administration (oral or parenteral) is significant in governing the overall extent of rash induced. This factor probably relates to the different adsorption, transportation and distribution requirements of the oral and parenteral cephalosporins prior to their reacting with the specific receptor site.

The relatively low correlation coefficient is probably related to the fact that the cutaneous rash data are a combination of the frequencies presented by both oral and parenteral cephalosporins, the reaction mechanism(s) or physico-chemical properties responsible for which may vary slightly, thus limiting the ability to 'fit' a better model to them. Analysis of the individual administration types should enable the production of more statistically significant and biologically explainable models.

9.32228 Oral Cephalosporin Antibiotics

The skewness value attained from univariate analysis of the square-root transformed biological data, pertaining to the frequency of cutaneous rash due to oral cephalosporins (Sk=0.663) showed the data to be normally distributed, and hence suitable for further statistical analysis.

Three compounds, cephaloglycin, cefuzonam and cefroxadine, were believed to be outliers and so were removed from the data set prior to regression analysis for the reasons given in 9.32221. During analysis cefuroxime-axetil was found to be behaving as an outlier. The reasoning for this was that it is a pro-drug of the parenteral cephalosporin cefuroxime and as such has an increased plasma half-life ($t_{1/2}$) of some six hours, which far exceeds that of any other oral cephalosporin (other oral cephs. $t_{1/2}$ = one hour, detailed in appendix 5). This prolonged time of the compound being in circulation may lead to an increased initiation of the immunological reaction mechanisms, than would be the case if $t_{1/2}$ were equivalent to other cephalosporins. It was therefore assumed that the reported frequencies of cutaneous rash for cefuroxime-axetil could be erroneous and not suitable for comparative statistical analysis.

Regression analysis against combinations of those parameters previously suggested as being relevant, enabled the generation of the stastistically significant and predictively valid model 9.17, which describes 90% of the biological variance.

Model 9.17

$$/\ \text{Rash due to oral cephalosporins} = -4.469(0.537)\frac{\text{LogH}_2\text{0sol}}{10} + 4.583(0.805)\text{SSCefatriz} - 2.600(0.547)$$

n=13 r=0.950 r^2=0.902 s=0.213 F=46.21 probability =2.304^{-6} $r(CV)^2$=0.844

The model suggests that cutaneous rash due to oral cephalosporins is a combination of a positive relationship with shape similarity to cefatrizine, and a negative relationship with water solubility, which may relate partitioning or to bioavailability, e.g. low solubility generally means a low rate of dissolution. Hence there ia a tendency for less polar compounds to have a greater rash inducing effect.

9.32229 Parenteral Cephalosporin Antibiotics

A skewness value of -0.230, for the square-root transformed rash data, suggests normal distribution and indicates that the data are suitable for statistical modelling.

Regression analysis produced model 9.18 which was statistically significant and preidictively valid, and which described 70% of the biological variance contained within the data.

Cephapirin was eliminated from the data set as an outlier, on the grounds that it was the only parenteral cephalosporin which possessed a nitrogen atom in a R_1 pyridine ring substituent, thus adversely effect the properties of cephapirin, so rendering it unsuitable for inclusion in the model generation process.

Model 9.18

/ Rash due to parenteral cephalosporins

$$= -7.224(1.800)\frac{DVz}{100} - 3.190(0.634)SSCefacet + 2.614(1.002)SSCefep + 1.653(1.076)$$

n=24 r=0.834 r^2=0.695 s=0.317 F=15.21 probability =1.136^{-5} $r(CV)^2$=0.564

The model proposes that the more the compound resembles cefacetrile, and the less it resembles cefepirome in its structural shape, the more unlikely it is to be able to interact with the receptor and initiate a response. The negative relationship with dipole moment suggests that a repulsion effect may be in existence. The greater the magnitude of the dipole Z vector the more the compound is repulsed at the receptor surface and thus the initiation of the cutaneous reaction mechanism is restricted.

Overview of Cutaneous Rash QSARs

Nine models (9.10 to 9.18 inclusive) pertaining to the incidence of cutaneous rash have been developed. Five of these models show significant statistical and predictive validity, two show partial statistical significance and twoare of limited statistical significance.

However, examination of the descriptor variable in each respective model (table 9.8 page 129), indicates a high degree of consistency in the parameters present. The main constituent parameter types are ones which relate to the molecular shape and the hydrophobicity of the antibiotic.

The presence of a shape descriptor is of significance in that it indicates that cutaneous rash is possibly induced as a consequence of some form of shape specific receptor binding. Such a reaction mechanism supports the hypothesis that cutaneous rash is initiated via the specific binding of the antibiotic with a pre-existing circulating antibody, as would be expected if, as assumed, the responses are mediated through the immediate or delayed hypersensitivity immunological mechanisms.

As with the QSARs pertaining to all ARs (page 143/4) the hydrophobicity term, when present, may possibly reflect differences in the absorption characteristics of the antibiotics.

9.3223 QSAR Models of GI Disruption Frequency

9.32231 All β-Lactam Antibiotics

The Log_{10} transformed GI disruption data, for all 70 of the β-lactams, although producing the best skewness value of the four transformations tested gave a Sk value of -1.212, indicating that it lay marginally outside the boundaries of normal distribution and that statistical analysis may not yield models of such high levels of statistical significance.

Sepwise regression against 70 descriptors hightlighted a number which were related to the biological data. The final model was derived, by regression analysis with respect to a combination of the eight most significant descriptor parameters. This yielded model 9.19 which described only 47% of the information contained within the biological data, indicating that the model is not statistically significant. The cross-validated r^2 for the resultant model (-0.078) showed that the model was not predictively valid. The lack of a significant correlation between the GI disruption data of all the β-lactams assesed may be due to the fact that the data contain information pertaining to numerous biological responses, which are brought about via numerous biological mechanisms. Also, the different antibiotic types (penicillins or cephalosporins) and modes of administration (oral or parenteral) may lead to the responses being brought about via differing mechanisms. This latter effect is particularly significant as it has previously been reported [189,200,202] that compounds administered by different routes are excreted via differing routes, which differentially affect the faecal flora, thus initiating GI disruption to varying extents. Each of the differing biological mechanisms may be initiated in response to a different physico-chemical property or combination of properties of the antibiotics under invesigation, therefore the generation of a simple linear model which correlates the complexity and diversity of such an array of biological mechanisms may not be possible.

Two compounds (flucloxacillin and cephaloglycin) were identified as acting as outliers. Flucloxacillin was thought to be an outlier on the grounds that the fluorine group present in its structure may adversely affect the flora in such a way as to cause superinfection and therefore increased activity, or possibly by direct irritation of the gut mucosa, resulting in a higher than expected frequency of disruption. Cefaloglycin(e) was believed to be an outlier by virtue of the fact that the frequency of GI disruption due to this compound is disproportionately high, at 16.5% compared with the other cephalosporins.

Model 9.19

$Log_{10}GI$ due to = 0.311(0.018)#CH_3R_1 - 0.113(0.091)#S
all β-lactam antibiotics - 0.383(0.119)ΣCcarbonylOxs - 0.128(0.079)K_3
+ 0.128(0.199)Ionptl - 0.195(0.071)Admin
-3.133(2.109)SSCepdalor + 2.085(0.707)

n=68 r=0.684 r^2=0.468 s=0.355 F=7.535 probability =1.12^{-6} $r(CV)^2$=-0.078

Where: #CH_3R_1 is the number of methyl groups in the R_1acyl side chain.
#S is the number of sulphur atoms in the entire molecule.
ΣCcarbonylOxs is the sum of the charges on the carbonyl oxygens within the whole molecule, as determined in Pimms.
K_3 is the Kier and Hall Kappa 3 topological indices, as calculated in TSAR.
Ionptl. is a measure of the antibiotic's ionisation potential, generated by the MOPAC 6.0 software.
Admin is an indicator variable pertaining to the mode of administration of the β-lactam, where 3 indicates oral and 4 parenteral administration.
SSCepdalor is a measure of the shape similarity of the antibiotic to cephaloridine, a parenteral β-lactam which does not induce GI disruption.

9.32232 Oral β-Lactam Antibiotics

The skewness value of -0.412 for the oral β-lactam Log_{10} transformed GI frequency data indicates that this sub-data set is normally distributed and so should be amenable to statistical modelling. Regression analysis of the Log_{10} biological data against combinations of those descriptors suggested in 9.32231 as being significant and others believed to be relevant, yielded regression model 9.20, which was statistically significancant and predictively valid and which described 61% of the biological variance. Three oral antibiotics (cephaloglycine, cefuzonam and cefadroxil) were determined as being outliers. Cephaloglycine for the reason outlined in 9.32231. Cefuzonam was believed to be an outlier because it was felt the additional sulphur atom in the molecule (in the R_2 side chain the nitrogen atom of other equivalent substituents is replaced by a sulphur atom) may either react with the flora iself inducing a response or it may alter the electron distribution in the molecule which in turn affects the floral response. Cefadroxil was classed as an outlier because of its high plasma half life (12 hours) which would give the compound an extended period to interact. Details of comparative $t_{1/2}$ levels given in appendix 5.

Model 9.20

$$\text{Log}_{10}\text{GI of oral } \beta\text{-lactams} = -0.316(0.060)\frac{SA}{100} - 0.511(0.134)\frac{TD}{10} - 0.959(0.561)\frac{R_1HA}{10} + 0.359(0.083)\#CH_3R_1 + 1.899(0.173)$$

n=29 r=0.780 r^2=0.609 s=0.190 F=9.351 probability =6.256^{-5} $r(CV)^2$=0.380

Where: SA is a measure of the external surface area of the molecule.

TD is the total dipole moment and is a measure of electron directing.

R_1HA is the hydrogen bond accepting ability of the R_1 acyl side chain, determined using the method of Yang et al [80].

$\#CH_3R_1$ is the number of methyl groups in the R_1acyl side chain.

In model 9.19 the shape parameter was that of shape similarity to cephaloridine, but this β-lactam is a parenteral antibiotic and so its properties were not felt to be relevant to purely oral compounds. In model 9.20, however, shape is still highlighted as being significant by virtue of the presence of the measurement of the antibiotics external surface area (SA). The importance of binding interactions is indicated by the presence in the model of the electronic related parameter total dipole, and the hydrogen bonding parameter. The number of methyl groups in the R_1 acyl side chain may be an indicator of the extent of branching and therefore of the shape or flexibility of the substituent, which thus affects receptor recognition, or may reflect some hydrophobic or bonding factor.

9.32233 Parenteral β-Lactam Antibiotics

A skewness value of -1.231 indicates that the Log_{10} transformed data lie outside the boundaries of normal distribution and so may not be compatible with statistical analysis and hence it may not be possible to generate a fully significant model. Regression analysis of the transformed data with combinations of those descriptors indicated previously (9.32231) and others believed to be of relevance, yielded the statistically significant and predictively valid regression model 9.21 which described 62% of the variance shown in the biological data.

Three parenteral β-lactams were determined to be acting as outliers. Flucloxacillin on the grounds that the presence of the fluorine group in the R_1 side chain was itself adversely affecting the flora and yet its effect was not parametrised as flucloxacillin is the only β-lactam to include a fluorine atom. Cephaloridine, although highlighed in 9.3231 as being a significant compound, was in this instance felt to be inflicting untoward bias on the model generation procedure as it causes no disruption to the lower GI flora, the only parenteral antibiotic not to do so. Cefamandole is given therapeutically at doses in excess of 12 grams per day, a level which far exceeds the doses of other parenteral β-lactams, as detailed in appendix 5. The levels of GI disruption reported may therefore be a function of this increased dosage and so it was deemed safer to remove it as an outlier.

Model 9.21

$$Log_{10}GI \text{ due to all parenteral } \beta\text{-lactams} = 4.934(0.535)\frac{\#CH_3R_1}{10} + 2.711(0.187)SSCefsul - 1.948(0.109)$$

$n=35$ $r=0.784$ $r^2=0.615$ $s=0.248$ $F=25.55$ probability $=1.686^{-7}$ $r(CV)^2=0.539$

Where: SSCefsul. is a measure of the shape similarity of the parenteral β–lactam with the parenteral cephalsporin antibiotic cefsulodin ,which is responsible for inducing the highest frequency of GI disruption of the compound in the data set. (Flucloxacillin is responsible for inducing the greatest disruption but is an outlier and so was not felt to be a relevant parameter).

The model indicates that the more the shape of a compound resembles that of cefsulodin coupled with the more methyl groups it possesses in its R_1 acyl side chain, the greater will be the resultant level of GI disruption it induces.

The fact that it has been possible to produce this valid model with data with slightly worse than a normal distribution casts some doubt over its true worth. However, the distribution may merely be a reflection of the errors present in the data used, and it may be that the use of more accurate data would lead to the generation of a model of yet greater validity.

9.32234 All Penicillin Antibiotics

The Log_{10} transformed GI disrpution data pertaining to all penicillin antibiotics were shown through univariate statistical analysis to be normally distributed (Sk=-0.079) and so are suitable for regression analysis.

Flucloxacillin was removed from the data set as being an outlier for the reasons oultined in 9.32233.

Regression anlaysis was carried out with combinations of those parameters indicated as significant in 9.343231. Analysis yielded the statistically significant and predictively valid regression model 9.22, which modelled 80% of the information contained witin the biological data.

Model 9.22

$$Log_{10}GI \text{ due to all penicillins} = -0.416(0.043)\frac{SA}{100} + 0.532(0.043)\frac{\#CH_3R_1}{10} - 0.415(0.030)Admin + 3.047(0.242)$$

n=27 r=0.893 r^2=0.798 s=0.160 F=30.34 probability =1.97^{-8} $r(CV)^2$=0.782

The model indicates a positive realtionship with the size and extent of branching of the R_1 acyl side chain is of significance. It may be that in this instance the number of methyl groups represents the extent of branching occurring in the side chain and thus, is a crude measure of shape. This is perhaps why a further size indicator, in this instance surface area, is also required.

The model suggests that the smaller the surface area of the penicillin the greater the frequency of GI disruption it will induce.

Examination of the different administration modes may yield more significant and more biologically explainable regression models.

9.32235 Oral Penicillin Antibiotics

The Log_{10} transformed GI disrpution data pertaining to the oral penicillin antibiotics were shown to be normally distributed (Sk = -0.321) and so suitable for regression analysis. Regression analysis was carried out with combinations of those parameters indicated as significant in 9.32234 and yielded the statistically significant and predictively valid regression model 9.23, which modelled 70% of the variance contained witin the biological data.

It was not felt that any of the oral penicillins were behaving as outliers.

Model 9.23

$$Log_{10}GI \text{ due to oral penicillins} = -0.471(0.109)\frac{SA}{100} + 0.308(1.275)\frac{\#CH_3R_1}{10} - 2.005(0.351)$$

n=15 r=0.841 r^2=0.706 s=0.138 F=14.44 probability =3.951^{-4} $r(CV)^2$=0.499

The model indicates a positive realtionship with the size and extent of branching of the R_1 acyl side chain are of significance and a negative realtionship with the surface area.

9.32236 Parenteral Penicillin Antibiotics

The Log_{10} transformed GI disruption data pertaining to the parenteral penicillin antibiotics were shown through univariate statistical analysis to be normally distributed (Sk = 0.261) and were so suitable for regression analysis.

Flucloxacillin was removed from the data set as being an outlier for the reasons oultined in 9.32233.

Regression anlaysis was carried out with combinations of those parameter indicated as being significant in 9.32234 and yielded the statistically significant and predictively valid regression model 9.24, which modelled 86% of the biological variance.

Model 9.24

$$Log_{10}GI \text{ due to parenteral penicillins} = -0.356(0.031)\frac{SA}{100} + 0.570(0.031)\frac{\#CH_3R_1}{10} + 1.158(0.171)$$

$n=12$ $r=0.928$ $r^2=0.861$ $s=0.174$ $F=27.80$ probability$=4.998^{-5}$ $r(CV)^2=0.768$

The model indicates that for penicillin antibiotics the size and extent of branching of the R_1 acyl side chain are of significance. The smaller the surface area of the penicillin the greater the frequency of GI disruption.

9.32237 All Cephalosporin Antibiotics

The Log_{10} transformed data for all cephalosporins are not normally distributed (Sk= -1.296) and so the production of a highly significant statistical correlation with descriptor variables is dubious. The lack of normal distribution is most likely to be a legacy of the errors presented in the clinical data used in the model generation procedure.

Regression analysis of the data with combinations of the descriptors highlighted in 9.32231 and others believed to be relevant, produced the statistically significant and predictively valid regression model 9.25, which described 66% of the biological variance.

Two compounds were eliminated as outliers. Cephaloglycin was removed for the reasons stated in 9.32231, and cefepirome because the predicted frequency of GI disruption is some ten times lower than the reported frequency. This discrepancy may be a result of the positively charged fused heterocyclic ring structure which forms part of the R_2 substituent. Parameterising the effects of this positive charge on the rest of the molecular properties was not possible.

Model 9.25

Log_{10}GI due to all cephalosporins = -0.302(0.670)DVz/10 +0.810(0.106)ΣCcarbonylOxs -3.886(2.004)SSCephalor - 2.829(0.567)SSCephalex - 0.465(1.102)

n=40 r=0.814 r^2=0.663 s=0.312 F=16.71 probability =5.549^{-8} $r(CV)^2$=0.240

Where: DVz is is a measure of the electron distribution within the molecule in the arbitary Z vector, as evaluated in Pimms.

SSCephalex. is a measure of the shape similarity to cephalexin, that oral cephaolsporin which presents the lowest frequency of GI disruption.

The statistical significance of model 9.25 is not particularly high; this may be due in part to the lack of normal distribution displayed by the data and because the data set contains information pertaining to both oral and parenteral cephalosporins, which may depend on different properties in order to elicit their response.

9.32238 Oral Cephalosporin Antibiotics

The Log_{10} transformed data displayed a normal distribution (Sk = -0.194) and so were deemed suitable for statistical analysis. Regression analysis of the biological data with those descriptors previously indicated as being of significance (9.32231/2/7) enabled the generation of the regression model 9.26 which showed both statistical significance and predictive validity and which modelled 71% of the variance present within the biological data.

Four compounds (cephaloglycin, cefuzonam, cefixime and cefadroxil) were removed on the grounds of being outliers, cephaloglycin on the basis of the reason stated in 9.32231, cefuzonam and cefadroxil for the reasons stated in 9.32232. Cefixime was determined to be an outlier as it is administered at a dose of 0.4g/day, which is markedly less than for any other oral cephalosporin. Dosage levels are detailed in appendix 5. It was felt that such low dosing would result in lower levels of GI disruption than if cefixime were given at a dose equivalent to other oral cephalosporins and therefore that the reported frequency was not comparable with those of other oral cephalosporins.

Model 9.26

$$Log_{10}GI \text{ due to oral cephalosporins} = -8.77(2.79)\frac{TD}{100} - 1.57(0.70)SSCephalex - 0.191(0.490)$$

n=13 r=0.840 r^2=0.706 s=0.162 F=12.02 probability =0.001 $r(CV)^2$=0.376

The model suggests the more an oral cephalosporin resembles cephalexin, in shape, and the lower its total dipole charge, the less will be the extent of GI disruption induced by it.

9.32239 Parenteral Cephalosporin Antibiotics

The data presented were not normally distributed, having a skewness value of -1.743, and so the production of a highly significant statistical correlation with physico-chemical data is dubious. The lack of normal distribution is most likely to be a legacy of the errors presented in the clinical data used in the model generation procedure.

Cefepirome was removed from the data set as an outlier for the reasons given in 9.32237.

Regression analysis of the $\log_{10}$ transformed bio-data with combinations of those parameters previously highlighted as being sigificant (9.32231/3/7) enabled the prodcution of the statistically significant and predictively valid regression model 9.27, which describes 81% of the infomation contained in the biological data.

The model proposes, as with 9.32237, that shape dependent and electronic interactions are important in controlling the frequency of GI disruption induced by a parenteral cephalosporin.

Model 9.27

$\log_{10}$GI due to parenteral cephs. $= -0.342(0.096)DVz/10 - 0.809(0.181)\Sigma CcarbonylOxs - 3.957(1.192)SSCephalor - 2.816(1.362)SSCephalex - 0.501(0.474)$

$n=24$ $r=0.902$ $r^2=0.813$ $s=0.260$ $F=20.63$ probability $=2.460^{-7}$ $r(CV)^2=0.565$

Overview of GI Disruption QSARs

Nine models pertaining to GI disruption (9.19 to 9.17 inclusive) have been produced. Five of these models display statistical and predictive validity, three show signs of partial significance and one (all β-lactams) shows limited statistical significance.

On closer examination of the descriptor variables presented in each model (table 9.8 page 130) it can clearly be seen that six of the models contain a specific parameter, namely the number of methyl groups in the R1 substituent. This parameter may reflect a number of molecular properties, such as branching, size, shape, flexibility or hydrophobicity. The remaining three models although not specifically containing this #CH_3R1 parameter contain related shape and electronic parameters.

A second descriptor common to most models is that of total surface area, which probably reflects the size of the antibiotic.

The exact biological mechanism(s) of action which give rise to GI disruption are unclear, and so the precise mode of action and the significance of these parameters cannot be determined. It can therefore, only be hypothesised that these parameters are connected with the process of 'fitting' into and binding with a specific receptor site, thus initiating the response.

The nature of the receptor site(s) is not known; it may be a receptor which controls the growth and / or replication of the flora. Alternatively, the antibiotic may bind in the colon in some way as to impair vitamin K2 synthesis. In either case the binding of the antibiotic may act to stimulate the growth of faecal flora, thus leading to an overpopulation or super-infection of bacteria, which results in GI disruption, a phenomenon seen with *Enterococcus faecalis* and *Clostridium difficile* [202]. Or the initiation of GI disruption may be due to direct irritation of the intestinal mucosa by the antibiotic itself [202].

The presence of an indicator as to the route of administration, in the larger models, is significant in that it is well documented [200,202] that the excretion profiles associated with the different routes of administration have some bearing on the levels of GI disruption induced, as previously discussed in section 9.22, page 108.

9.323 Data Randomisation

A problem with using large numbers of descriptors in step-wise regression is that there is a significant risk of chance correlations occurring, according to Topliss and Edwards [46,104]. In order to investigate whether this had happened in this work, the biological data used in each of the 27 models generated were randomised some 20 times and the regression analysis repeated, as discussed in methods section 9.315. Examination of the resultant regression coefficient values for each randomised model, presented in table 9.9, clearly shows that for each of the randomised models the value of r was significantly less than that attained for the original model, and in most cases was approaching zero. This indicates that the randomised models bear no statistical significance and do not model any aspect of the biological information.

It can be assumed therefore, from these randomised results that all the original models produced were not generated by chance, but that the descriptors used reflect the actual physico-chemical and structural properties responsible for initiating and controlling each specific biological response.

Table 9.9 Regression Coeffiecient Values Attained from Randomised Data Analysis

Model	r val ues																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
9.1	0.47	0.17	0.38	0.00	0.21	0.26	0.08	0.29	0.16	0.17	0.38	0.16	0.19	0.02	0.05	0.09	0.33	0.41	0.07	0.15
9.2	0.36	0.21	0.26	0.24	0.19	0.19	0.45	0.02	0.37	0.03	0.23	0.23	0.21	0.13	0.25	0.09	0.14	0.27	0.10	0.13
9.3	0.29	0.25	0.14	0.18	0.14	0.13	0.26	0.29	0.41	0.35	0.21	0.17	0.27	0.16	0.32	0.13	0.01	0.32	0.31	0.03
9.4	0.30	0.02	0.03	0.02	0.39	0.16	0.05	0.08	0.17	0.18	0.10	0.08	0.21	0.00	0.16	0.09	0.21	0.19	0.11	0.13
9.5	0.12	0.00	0.00	0.00	0.05	0.19	0.16	0.13	0.00	0.16	0.00	0.00	0.31	0.01	0.08	0.23	0.16	0.09	0.13	0.14
9.6	0.01	0.31	0.15	0.12	0.16	0.03	0.10	0.16	0.00	0.16	0.06	0.28	0.18	0.13	0.33	0.17	0.13	0.09	0.00	0.00
9.7	0.26	0.19	0.32	0.25	0.12	0.04	0.15	0.00	0.16	0.29	0.00	0.32	0.19	0.23	0.06	0.02	0.21	0.01	0.23	0.09
9.8	0.00	0.21	0.36	0.16	0.13	0.22	0.01	0.10	0.14	0.05	0.08	0.16	0.15	0.18	0.00	0.21	0.19	0.17	0.19	0.11
9.9	0.00	0.00	0.00	0.00	0.00	0.11	0.02	0.21	0.00	0.16	0.31	0.12	0.01	0.15	0.09	0.18	0.18	0.21	0.29	0.31
9.10	0.35	0.00	0.00	0.16	0.21	0.17	0.00	0.03	0.29	0.19	0.00	0.32	0.21	0.01	0.32	0.09	0.23	0.17	0.15	0.21
9.11	0.29	0.00	0.12	0.16	0.26	0.21	0.11	0.00	0.10	0.18	0.16	0.00	0.11	0.01	0.24	0.19	0.08	0.31	0.15	0.16
9.12	0.00	0.23	0.16	0.11	0.16	0.03	0.19	0.16	0.00	0.12	0.13	0.00	0.12	0.00	0.26	0.17	0.00	0.19	0.12	0.02
9.13	0.15	0.12	0.00	0.21	0.36	0.00	0.05	0.00	0.24	0.13	0.01	0.00	0.00	0.14	0.14	0.13	0.23	0.21	0.12	0.15
9.14	0.23	0.21	0.31	0.12	0.13	0.00	0.05	0.00	0.34	0.00	0.16	0.17	0.26	0.17	0.14	0.18	0.08	0.15	0.10	0.15
9.15	0.00	0.16	0.00	0.05	0.00	0.08	0.22	0.12	0.00	0.12	0.00	0.13	0.19	0.12	0.01	0.17	0.21	0.21	0.23	0.19
9.16	0.21	0.12	0.00	0.19	0.03	0.00	0.08	0.09	0.00	0.27	0.04	0.07	0.13	0.00	0.00	0.21	0.00	0.01	0.02	0.05

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Table 9.9 continued

Model	r val ues																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
9.17	0.32	0.15	0.10	0.21	0.16	0.29	0.13	0.09	0.14	0.08	0.01	0.16	0.08	0.11	0.01	0.00	0.07	0.09	0.00	0.07
9.18	0.14	0.07	0.10	0.21	0.07	0.06	0.12	0.16	0.07	0.31	0.08	0.16	0.02	0.05	0.12	0.08	0.31	0.02	0.01	0.17
9.19	0.30	0.29	0.05	0.06	0.11	0.01	0.04	0.26	0.06	0.09	0.26	0.06	0.07	0.17	0.16	0.18	0.03	0.08	0.12	0.16
9.20	0.12	0.07	0.21	0.16	0.14	0.09	0.03	0.14	0.12	0.13	0.05	0.12	0.31	0.09	0.10	0.15	0.08	0.10	0.01	0.02
9.21	0.26	0.06	0.13	0.08	0.09	0.00	0.13	0.15	0.17	0.07	0.02	0.08	0.15	0.03	0.05	0.17	0.10	0.03	0.12	0.16
9.22	0.08	0.17	0.05	0.09	0.17	0.16	0.11	0.02	0.25	0.08	0.02	0.10	0.09	0.14	0.08	0.13	0.01	0.19	0.14	0.09
9.23	0.34	0.10	0.00	0.11	0.06	0.27	0.12	0.08	0.06	0.16	0.29	0.11	0.12	0.08	0.13	0.06	0.09	0.15	0.02	0.05
9.24	0.16	0.06	0.08	0.05	0.16	0.09	0.09	0.01	0.07	0.07	0.00	0.10	0.15	0.08	0.02	0.10	0.09	0.03	0.16	0.12
9.25	0.19	0.07	0.15	0.05	0.03	0.17	0.07	0.00	0.19	0.00	0.09	0.08	0.09	0.07	0.01	0.01	0.12	0.08	0.21	0.06
9.26	0.07	0.21	0.10	0.17	0.04	0.07	0.09	0.32	0.16	0.06	0.01	0.18	0.17	0.11	0.06	0.23	0.05	0.11	0.08	0.13
9.27	0.01	0.01	0.19	0.12	0.09	0.00	0.10	0.18	0.17	0.00	0.07	0.20	0.00	0.13	0.07	0.11	0.16	0.08	0.10	0.09

9.4 Prediction of the Frequencies of ARs for Untested Compounds

9.41 Method

During the data generation procedure the chemical structures of nine β-lactam antibiotics (4 penicillins and 5 cephalosporins) were obtained, but little or not biological data was gleaned. These structures were input into the modelling packages and the relevant physco-chemical and structural parameters generated, as in 9.32. The biological activities (frequencies of all ARs, cutaneous rash and GI disruption) for these new compounds were then evaluated using the relevant models, as derived in 9.322.

9.42 Results and Discussion

The structure, relevant descrpitor variable values generated and the predicted adverse reaction frequencies (as both Log_{10} or $\sqrt{}$ and the respective % of the treated population) as evaluated using the relevant QSARs for each the 4 penicillins are given in 9.421 and for the 5 cephalosporins in 9.422.

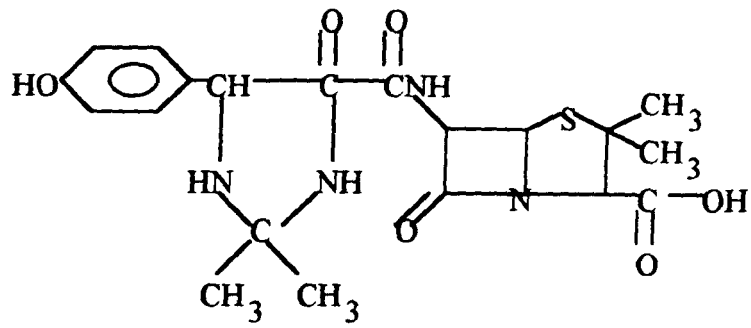
In some cases where the mode of administration of the antibiotic was not known, several models were used to evaluate the several frequency values for the same adverse reaction type. Comparison of these predicted frequencies shows that different values are obtained for each model used. However, on closer inspection it becomes apparent that the values generated by one of the more general models, i.e. all penicillins, and one of the more specialised models, i.e. oral penicillins, are of the same magnitude, thus suggesting the possible mode of administration and potential AR frequency of the antibiotic in question. Comparison of the predicted AR frequencies obtained for these test antibiotics with those of related antibiotics, as generated in 9.2, shows that the predicted AR frequencies fall within the respective reported frequency range, thus giving credence to the predicted values of adverse reaction frequencies evaluated from the developed QSARs.

It is unfortunate that it was not possible to obtain clinical AR frequency data for these nine β-lactam antibiotics, so preventing them constituting a true evaluation data set, as comparison of those actual values against the respective predicted values, as determined herein, would further add to the credibility and validity of the QSARs produced.

9.421 Predicted AR Frequencies of Untested Penicillins

Name: **HETACILLIN**

Mode of administration: Oral



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous Rash		GI Disruption	
	Log10	%	√	%	Log10	%
Oral β-lactams	-0.26	0.55	NC	NC	4.52	error
Oral penicillins	0.85	7.00	0.61	0.37	1.02	10.39

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Descriptor Variables:

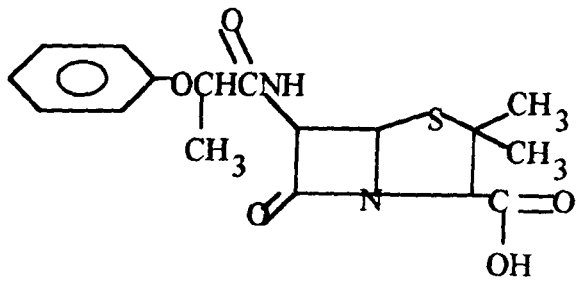
Parameter	Value
Shape similarity to Cefmetazole	0.69
Shape similarity to Flucloxacillin	0.66
Shape similarity to Cephapirin	0.74
Shape similarity to Benzylpenicillin	0.62
Shape similarity to Cefsulodin	0.60
Total diople moment	4.28
Dipole moment in vector Z	-1.66
R1 hydrogen bond donor ability	5

Parameter	Value
CLogP	1.06
Log H_2O solubility	NG
Shape similarity to Ceftibuten	0.65
Shape similarity to Cefatrizine	0.60
# Methyl groups in R1	2
R1 hydrogen bond acceptor ability	3
Surface Area	389.5

NG = It was not possible to generated the respective descriptor value, using the relevant software.

Name: **PHENETHICILLIN**

Mode of administration: Oral



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous Rash		GI Disruption	
	Log10	%	√	%	Log10	%
Oral β-lactams	0.60	3.98	NC	NC	-1.09	0.08
Oral penicillins	2.78	error	0.75	0.54	-3.49	0.00

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Descriptor Variables:

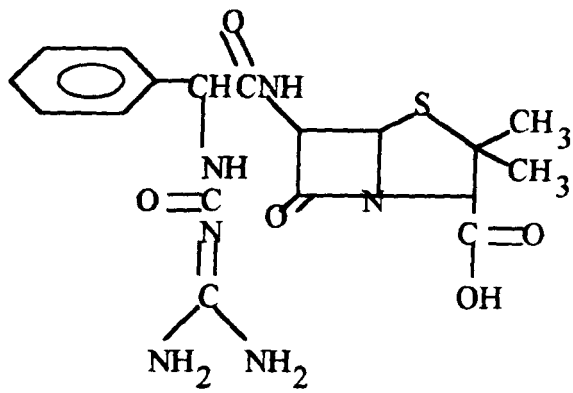
Parameter	Value
Shape similarity to Cefmetazole	0.75
Shape similarity to Flucloxacillin	0.75
Shape similarity to Cephapirin	0.71
Shape similarity to Benzylpenicillin	0.65
Dipole moment in vector Z	2.51
Total Dipole moment	4.18
R1 hydrogen bond acceptor ability	2
R1 hydrogen bond donor ability	11

Parameter	Value
CLogP	1.75
Log H_2O solubility	NG
Shape similarity to Ceftibuten	0.74
Shape similarity to Cefatrizine	0.64
# Methyl groups in R1	1
Shape similarity to Cefsulodin	0.60
Surface Area	321.6

NG = It was not possible to generated the respective descriptor value, using the relevant software.

Name: **BLP-1654**

Mode of administration: unknown



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous	Rash	GI	Disruption
	Log10	%	√	%	Log10	%
All β-lactams	0.03	1.08	NC	NC	NC	NC
Oral β-lactams	0.82	6.53	NC	NC	-0.13	0.74
Parent. β-lactams	5.54	NC	NC	NC	-0.002	1.00
All penicillins	0.278	7.72	oral - 1.76 parent. - 2.40	3.11 5.76	-2.71 -3.72	7.32 9.74
Oral penicillins	0.295	error	1.29	1.66	-2.40	0.0
Parent. penicillins	-10.63	error	2.48	6.15	-0.09	0.81

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

BLP-1654 Descriptor Variables:

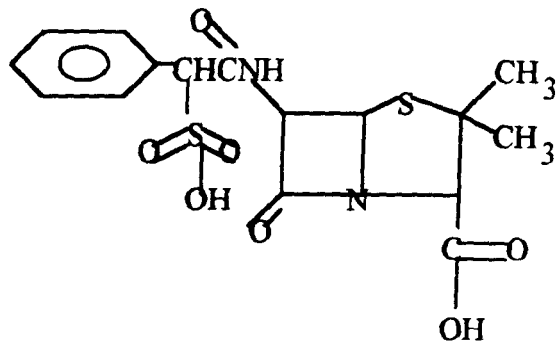
Parameter	Value
Flexibility of R1	10.09
# amino groups in R1	4
R3 indicator variable	1
R4 indicator variable	1
# oxygen atoms in whole molecule	5
Administration ID	3 / 4
Shape similarity to Cefmetazole	0.76
Shape similarity to Flucloxacillin	0.63
Shape similarity to Cephapirin	0.73
Shape similarity to Benzylpenicillin	0.80
Dipole moment in vector Z	1.54
Shape similarity to Cefepirome	0.72
# sulphur atoms in whole molecule	1
Sum charge on carbonyl oxygens	-1.66
Connectivity kappa 3	NG
Ionisation potential	9.06
R1 hydrogen bond donor ability	11

Parameter	Value
Charge on C of β ring carbonyl	0.29
Similarity to Lipophilicity of Bpen.	0.53
Sterimol B2 width from CH_3	4.33
# carbon atoms in R1	10
Sterimol B3 width (β-ring carbonyl)	8.31
Shape similarity Methicillin	0.74
CLogP	-0.33
Log H_2O solubility	NG
Shape similarity to Ceftibuten	0.67
Shape similarity to Cefatrizine	0.75
# Methyl groups in R1	0
Shape similarity to Cephaloridine	0.72
Shape similarity to Cefsulodin	0.72
Total Dipole moment	8.60
R1 hydrogen bond acceptor ability	5
Surface Area	351.3

NG = It was not possible to generated the respective descriptor value, using the relevant software.

Name: **SULBENICILLIN**

Mode of administration: Parenteral



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous Rash		GI Disruption	
	Log10	%	√	%	Log10	%
Parent. β-lactams	6.79	error	NC	NC	-0.15	0.71
Parent. penicillins	1.08	11.91	-1.91	3.64	2.37	error

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Descriptor Variables:

Parameter	Value
Shape similarity to Methicillin	0.79
Shape similarity to Flucloxacillin	0.78
Shape similarity to Cefsulodin	0.66
# amino groups in R1	1
Charge on C of β-ring carbonyl	-1.70
Sterimol B2 width (CH_3)	3.88

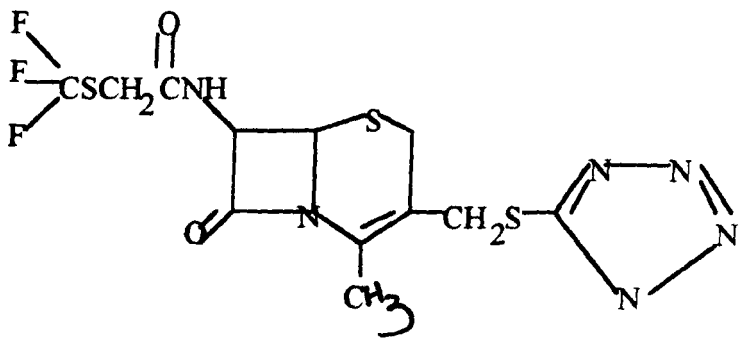
Parameter	Value
CLogP	0.63
Log H_2O solubility	NG
# carbons atoms in R1	8
Sterimol B3 width (β-ring carbonyl)	7.33
Surface Area	340.2
# methyl groups in R1	0

NG = It was not possible to generated the respective descriptor value, using the relevant software.

9.422 Predicted AR Frequencies of Untested Cephalosporins

Name: **CEFAZAFLUR**

Mode of administration: Unknown



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous	Rash	GI	Disruption
	Log10	%	√	%	Log10	%
All β-lactams	1.27	18.41	NC	NC	NC	NC
Oral β-lactams	0.78	5.97	NC	NC	NC	NC
Parent. β-lactams	4.14	error	NC	NC	-0.11	0.78
All cephalosporins	-3.92	0.00	oral - 1.11 parent. - 1.32	1.23 1.74	-2.15	0.01
Oral cephalosporins	0.98	9.48	NC	NC	0.26	1.81
Parent. Cephalosporins	7.47	error	1.72	2.97	-0.30	0.50

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Cefazaflur :Descriptor Variables

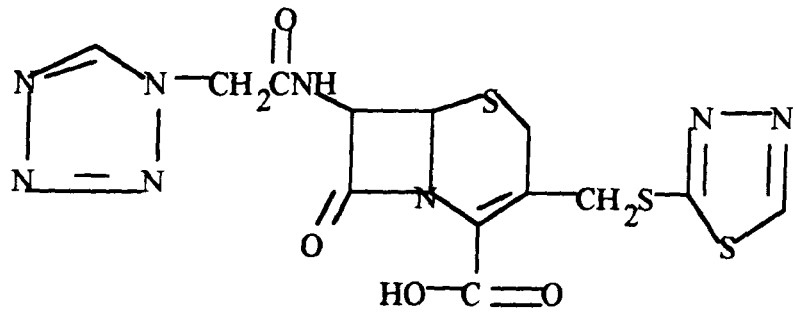
Parameter	Value
Flexibility of R1	7.02
# amino groups in R1	1
R3 indictor variable	1
R4 indicator variable	2
# oxygen atoms in whole molecule	4
Shape similarity to Cefmetazole	0.70
Shape similarity to Flucloxacillin	0.74
Shape similarity to Cephapirin	0.72
CLogP	0.07
Dipole moment in vector Z	-6.12
Charge on C of β-ring carbonyl	0.29
Hydrogen bond acceptor ability	7
Total dipole moment	6.84
Sterimol B3 width of R2	1.93
# nitrogen atoms in R2	4
# oxygen atoms in R2	0
Sterimol L of R2	7.48
Sterimol B2 width (CH_3)	5.21
Shape similarity to Benzylpenicillin	0.79

Parameter	Value
# carbon atoms in R1	3
Log H_2O solubility	NG
Shape similarity to Methicillin	0.72
Shape similarity to Cefepirome	0.68
Shape similarity to Ceftibuten	0.71
Shape similarity to Cefatrizine	0.67
Administration Indicator variable	3 / 4
Shape similarity to Cefacetrile	0.93
# methyl groups in R1	0
# sulphur atoms in whole molecule	3
Sum charge on carbonyl oxygens	-1.19
Connectivity kappa 3	NG
Ionisation potential	9.36
Shape similarity to Cephaloridine	0.72
Surface area	NG
R1 hydrogen bond acceptor ability	4
Shape similarity to Cefsulodin	0.68
Shape similarity to Cephalexin	0.67

NG = It was not possible to generated the respective descriptor value, using the relevant software.

Name: **CEFTEZOLE**

Mode of administration: Unknown



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous	Rash	GI	Disruption
	Log10	%	√	%	Log10	%
All β-lactams	0.56	3.66	NC	NC	NC	NC
Oral β-lactams	0.84	6.87	NC	NC	0.01	1.03
Parent. β-lactams	0.67	4.70	NC	NC	-0.29	0.52
All cephalosporins	-3.71	error	oral -0.25 parent. -0.04	0.06 0.00	-0.88	7.59
Oral cephalosporins	0.72	5.21	NC	NC	1.02	10.52
Parent. Cephalosporins	0.37	2.32	1.03	1.07	0.28	1.90

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Ceftezole :Descriptor Variables

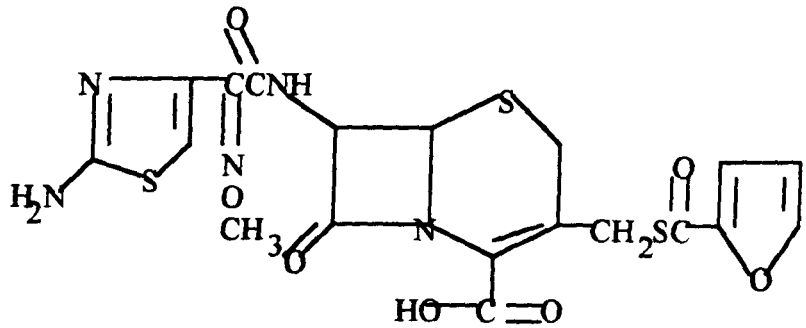
Parameter	Value
Flexibility of R1	3.98
# amino groups in R1	1
R3 indictor variable	1
R4 indicator variable	2
# oxygen atoms in whole molecule	4
Shape similarity to Cefmetazole	0.69
Shape similarity to Flucloxacillin	0.69
Shape similarity to Cephapirin	0.60
CLogP	-1.62
Dipole moment in vector Z	-7.87
Charge on C of β-ring carbonyl	0.29
Hydrogen bond acceptor ability	7
Total dipole moment	7.97
Sterimol B3 width of R2	3.41
# nitrogen atoms in R2	2
# oxygen atoms in R2	0
Sterimol L of R2	8.13
Sterimol B2 width (CH_3)	6.27
Shape similarity to Benzylpenicillin	0.74

Parameter	Value
# carbon atoms in R1	3
Log H_2O solubility	NG
Shape similarity to Methicillin	0.72
Shape similarity to Cefepirome	0.60
Shape similarity to Ceftibuten	0.69
Shape similarity to Cefatrizine	0.60
Administration Indicator variable	3 / 4
Shape similarity to Cefacetrile	0.86
# methyl groups in R1	0
# sulphur atoms in whole molecule	3
Sum charge on carbonyl oxygens	-1.19
Connectivity kappa 3	NG
Ionisation potential	9.11
Shape similarity to Cephaloridine	0.71
Surface area	347.0
R1 hydrogen bond acceptor ability	4
Shape similarity to Cefsulodin	0.61
Shape similarity to Cephalexin	0.78

NG = It was not possible to generated the respective descriptor value, using the relevant software.

Name: **CEFTIOFLUR**

Mode of administration: Unknown



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous	Rash	GI	Disruption
	Log10	%	√	%	Log10	%
All β-lactams	0.59	3.85	NC	NC	NC	NC
Oral β-lactams	0.40	2.51	NC	NC	-0.36	0.44
Parent. β-lactams	1.51	32.43	NC	NC	0.28	1.88
All cephalosporins	-6.66	0.00	oral - 0.28 parent. - 0.49	0.08 0.24	-2.21	0.00
Oral cephalosporins	0.39	2.46	NC	NC	-0.29	0.52
Parent. Cephalosporins	-0.09	0.81	0.93	0.86	-0.42	0.38

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Ceftioflur :Descriptor Variables

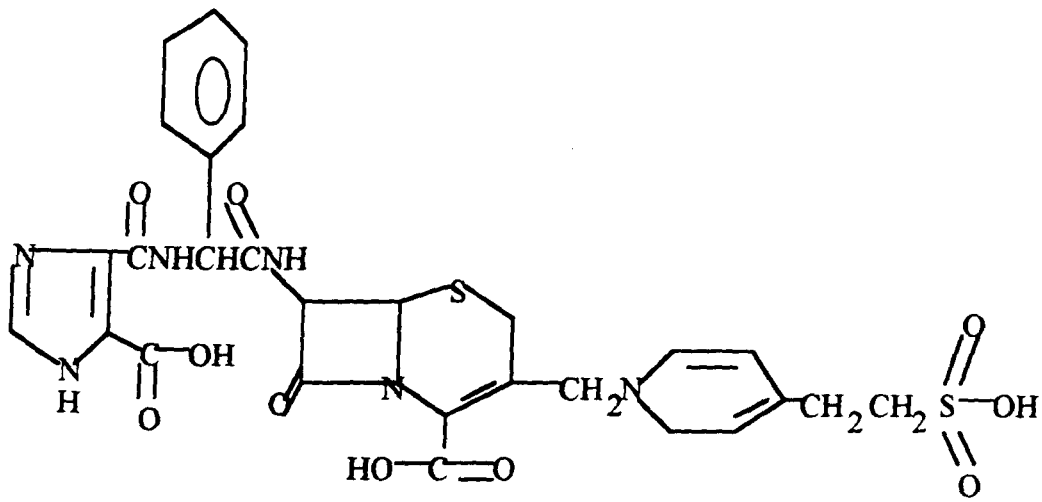
Parameter	Value
Flexibility of R1	7.49
# amino groups in R1	2
R3 indictor variable	1
R4 indicator variable	2
# oxygen atoms in whole molecule	7
Shape similarity to Cefmetazole	0.56
Shape similarity to Flucloxacillin	0.62
Shape similarity to Cephapirin	0.64
CLogP	0.89
Dipole moment in vector Z	-0.21
Charge on C of β-ring carbonyl	0.29
Hydrogen bond acceptor ability	10
Total dipole moment	12.62
Sterimol B3 width of R2	3.59
# nitrogen atoms in R2	0
# oxygen atoms in R2	2
Sterimol L of R2	7.25
Sterimol B2 width (CH_3)	4.93
Shape similarity to Benzylpenicillin	0.72

Parameter	Value
# carbon atoms in R1	5
Log H_2O solubility	NG
Shape similarity to Methicillin	0.75
Shape similarity to Cefepirome	0.66
Shape similarity to Ceftibuten	0.62
Shape similarity to Cefatrizine	0.63
Administration Indicator variable	3 / 4
Shape similarity to Cefacetrile	0.81
# methyl groups in R1	1
# sulphur atoms in whole molecule	3
Sum charge on carbonyl oxygens	-1.16
Connectivity kappa 3	NG
Ionisation potential	8.87
Shape similarity to Cephaloridine	0.69
Surface area	414.4
R1 hydrogen bond acceptor ability	7
Shape similarity to Cefsulodin	0.64
Shape Similarity to Cephalexin	0.64

NG = It was not possible to generated the respective descriptor value, using the relevant software.

Name: **CEFEPIMIZOLE**

Mode of administration: Unknown



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous	Rash	GI	Disruption
	Log10	%	√	%	Log10	%
All β-lactams	0.60	3.97	NC	NC	NC	NC
Oral β-lactams	1.04	10.99	NC	NC	NC	NC
Parent. β-lactams	0.54	3.45	NC	NC	-0.10	0.80
All cephalosporins	-4.75	0.00	oral - 0.35 parent. - 0.55	0.12 0.31	-2.4	0.00
Oral cephalosporins	0.97	9.27	NC	NC	0.14	1.37
Parent. Cephalosporins	1.07	11.78	1.02	1.05	-0.25	1.80

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Cefepimizole Descriptor Variables

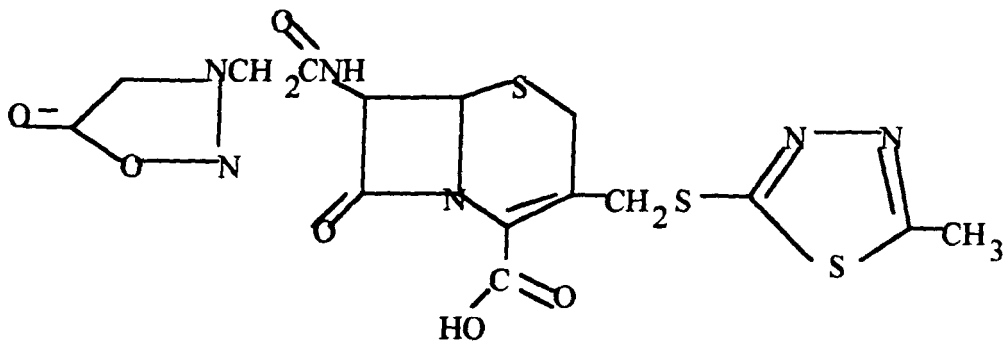
Parameter	Value
Flexibility of R1	9.40
# amino groups in R1	1
R3 indictor variable	1
R4 indicator variable	2
# oxygen atoms in whole molecule	9
Shape similarity to Cefmetazole	0.59
Shape similarity to Flucloxacillin	0.57
Shape similarity to Cephapirin	0.53
CLogP	-1.56
Dipole moment in vector Z	-4.47
Charge on C of β-ring carbonyl	0.29
Hydrogen bond acceptor ability	9
Total dipole moment	7.30
Sterimol B3 width of R2	6.61
# nitrogen atoms in R2	1
# oxygen atoms in R2	3
Sterimol L of R2	6.49
Sterimol B2 width (CH_3)	6.45
Shape similarity to Benzylpenicillin	0.66

Parameter	Value
# carbon atoms in R1	13
Log H_2O solubility	NG
Shape similarity to Methicillin	0.67
Shape similarity to Cefepirome	0.53
Shape similarity to Ceftibuten	0.59
Shape similarity to Cefatrizine	0.67
Administration Indicator variable	3 / 4
Shape similarity to Cefacetrile	0.73
# methyl groups in R1	0
# sulphur atoms in whole molecule	2
Sum charge on carbonyl oxygens	-1.72
Connectivity kappa 3	NG
Ionisation potential	8.78
Shape similarity to Cephaloridine	0.64
Surface area	NG
R1 hydrogen bond acceptor ability	5
Shape similarity to Cefsulodin	0.68
Shape similarity to Cephalexin	0.62

NG = It was not possible to generated the respective descriptor value, using the relevant software.

Name: **CEPHANONE**

Mode of administration: Unknown



Predicted AR Frequencies

Model	Frequency of Adverse Reaction					
	All ARs		Cutaneous	Rash	GI	Disruption
	Log10	%	√	%	Log10	%
All β-lactams	1.10	12.56	NC	NC	NC	NC
Oral β-lactams	NC	NC	NC	NC	NC	NC
Parent. β-lactams	NC	NC	NC	NC	-0.09	0.81
All cephalosporins	NC	NC	oral - 0.56 parent. - 0.43	0.31 0.19	NC	NC
Oral cephalosporins	NC	NC	NC	NC	NC	NC
Parent. Cephalosporins	NC	NC	NC	NC	NC	NC

NC = Due to the lack of one or more descriptor values it was not possible to calculate this frequency, using the respective QSAR model.

Cephanone :Descriptor Variables

Parameter	Value
Flexibility of R1	4.67
# amino groups in R1	1
R3 indictor variable	1
R4 indicator variable	2
# oxygen atoms in whole molecule	6
Shape similarity to Cefmetazole	0.74
Shape similarity to Flucloxacillin	0.73
Shape similarity to Cephapirin	0.59
CLogP	NG
Dipole moment in vector Z	NG
Charge on C of β-ring carbonyl	NG
Hydrogen bond acceptor ability	9
Total dipole moment	NG
Sterimol B3 width of R2	3.03
# nitrogen atoms in R2	2
# oxygen atoms in R2	0
Sterimol L of R2	9.15
Sterimol B2 width (CH_3)	7.30
Shape similarity to Benzylpenicillin	0.80

Parameter	Value
# carbon atoms in R1	3
Log H_2O solubility	0.71
Shape similarity to Methicillin	0.61
Shape similarity to Cefepirome	0.67
Shape similarity to Ceftibuten	0.71
Shape similarity to Cefatrizine	NG
Administration Indicator variable	3 / 4
Shape similarity to Cefacetrile	0.87
# methyl groups in R1	0
# sulphur atoms in whole molecule	3
Sum charge on carbonyl oxygens	NG
Connectivity kappa 3	NG
Ionisation potential	9.27
Shape similarity to Cephaloridine	0.67
Surface area	342.5
R1 hydrogen bond acceptor ability	6
Shape similarity to Cefsulodin	0.68
Shape similarity to Cephalexin	0.68

NG = It was not possible to generated the respective descriptor value, using the relevant software.

9.5 Conclusion

A data base pertaining to the frequencies of adverse responses exhibited by patients following therapy with 70 β-lactam antibiotics has been collated and the data used to develop 27 mathematical models relating the frequencies of specific response types to specific physico-chemical and structural properties of the antibiotics themselves.

The models generated for each specific response vary in statistical validity, but each appears to utilise appropriate and often equivalent descriptors to model that response type (AR, cutaneous rash or GI disruption).

Oral and parenteral antibiotics present models containing somewhat different parameters for the same biological response, probably reflecting the different properties each requires to bring about the response in view of their different absorption, transportation, distribution and elimination needs.

The penicillins and cephalosporins also present models containing somewhat different parameters, suggesting that each antibiotic type initiates a specific biological response via slightly different biological mechanisms which are dependent on slightly different structural properties.

In the majority of instances the model generated includes at least one shape and / or electronic parameter, suggesting that in each reaction type some form of receptor recognition and binding is at the crux of the reaction initiation procedure, a suggestion which is concurrent with theories on immunotoxic reaction mechanisms.

However, the complexity and diversity of the reactions included within each data set precludes receptor identification, typing (immunoglobulin or flora), location and mapping or further interpretations as to the exact biological mechanisms involved.

The models derived provide support to existing theories as to the mechanisms of action of the β-lactam antibiotics, as well as providing a means of predicting the possible frequency of an adverse reaction which a new, untested or hypothetical β-lactam antibiotic may induce if used therapeutically.

Due to the nature of the data used in the model generation procedures the predicted AR frequencies are open to some degree of error and so should be used only as guidelines or in conjunction with other imunogenicity studies.

The ability to generate models pertaining to the frequency of adverse reactions and physico-chemical properties of a given set of compounds bodes well for future research in this field.

10.0 Prediction of Cross-Reactivity between β-lactam Antibiotics and Anti-Benzylpenicillin Serum Antibodies

10.1 Introduction

The phenomenon of cross-reactivity between allergens and antibodies raised *in vivo* to a different previously administered allergen is believed to be a major factor underlying the incidence of immunologically mediated adverse reactions, principally the hypersensitivity type reactions [33,36]. As such it poses a problem when it occurs frequently with phamaceutically administered compounds, such as the β-lactam antibiotics [582]. Cross-reactivity between penicillin and cephalosporin antibiotics is well debated [25,33]. A much cited figure, pertaining to cross-reactivity between penicillins and cephalosporins is 8% and was derived from a retrospective study by Petz [33]. Yet other studies indicate that little if any cross-reactivity occurs between the penicillins and the cephalosporins [25].

A variety of approaches for assessing an antibiotic's cross-reactivity potential have been developed over the years [33,584,585,586,587], e.g. measuring total or individual immunoglobulin titres. However, the complexity of cross-reactivity, and the difficulties associated with such studies, mean that it is difficult to express the extent of cross-reactivity in quantitative terms [165,188,584,588].

The aim of this study: was firstly to develop a quantitative assay for the determination of the cross-reactivity potential of different antibiotics and anti-benzylpenicillin serum antibodies; secondly, as the penicillin and cephalosporin antibiotics are structurally very similar [586], to develop QSARs which correlate the extent of cross-reactivity with specific physico-chemical or structural properties of the antibiotics.

10.2 Development of an Assay for the Quantitative Determination of Cross-Reactivity Between β-Lactam Antibiotics and Anti-Benzylpenicillin Serum Antibodies

Large numbers of studies, in both humans and animals, have previously been performed to investigate the extent to which penicillins and cephalosporins cross-react [188,584,585,586,589]. Previous experimental studies, by Blanca et al [34] and by Petersen & Graham [590], with animal-raised antisera have demonstrated that cross reactions between penicillin and cephalosporin antibiotics are detectable using Enzyme Linked Immuno-Assay (ELISA) techniques.

In this study an existing ELISA for the detection of anti-benzylpenicillin serum antibodies has been modified in such a way as to develop a highly sensitive, reliable, competitive ELISA for the quantitative determination of the level of cross-reactivity occurring between β-lactam antibiotics and anti-benzylpenicillin serum antibodies. The ELISA used involves binding benzylpenicillin, in the form benzylpenicillin cytochrome-C (an antigen), to a solid matrix, the capture of antibodies from a serum sample by the antigen and the subsequent binding of a detectable immunoglobulin marker.

Levine [188] stated that an allergic reaction depends upon the outcome of competition for the allergen (in this instance the β-lactam antibiotic) by several classes of antibodies present in the serum (i.e. within a polyclonal antibody environment). It was felt therefore that the ELISA should be developed using whole serum, which contains polyclonal antibodies. As with the work of Petersen & Graham [590], serum polyclonal antibodies from rabbits sensitised, intradermally and subcutaneously, with protein conjugated benzylpenicillin in the presence of Freund's adjuvant over a period of ninety days (sensitisation's performed on days 0, 21, 56 and 90) were examined, in all experiments, for cross reactivity. It was felt that this polyclonal system would best mimic the clinical situation and enable extrapolation of the results. Rabbit serum was chosen because the rabbit is the species of choice in pharmaceutical immunogenicity studies (in that its immune system is similar to that of humans), and so that the results gleaned from this study could be used in conjunction with those from other immunogenicity studies.

The assay was subsequently used for the quantitative determination of the extent of cross-reactivity occurring between different β-lactam antibiotics and rabbit serum polyclonal anti-benzylpenicillin antibodies.

10.21 Experiment 1: Detection of Serum Anti-benzylpenicillin Antibodies

10.211 Method

Wells of Linbro/Titerk flat bottomed plate (ICN Biomedicals Inc.) were coated by addition of 50µl of 10µg/ml benzylpenicillin cytochrome-C antigen, made in coating buffer (0.05M carbonate bicarbonate buffer pH9.6 (sigma C3041)), and incubated in a Stuart Scientific incubator model S118 for 2 hours at 37°C. Prior to each subsequent step the wells were washed three times with PTS (Phosphate buffered saline pH7.4 (Sigma P3813) + 0.01% Tween 20 (polyoxyethylenesorbitan-monolaurate) (Sigma P1379)), using a a Dynatech MRW automatic plate washer.

The antigen-coated wells were subsequently incubated with;

a - 50µl sensitised serum, positive for polyclonal anti-benzylpenicillin antibodies ($RB\text{-}BP_{101\text{-}105}$) for 1 hour at 37°C at dilution's of 1:200, 1:400, etc. in PTS

b - 50µl anti-rabbit IgG-Horse Radish Peroxidase Conjugate (Sigma A-8275) at 1:1000 dilution in PTS for 1 hour at 37°C

c - 50µl 3,3',5,5' tetramethylbenzidine liquid substrate (TMB) (Sigma T-8540) at room temperature until sufficient colour had developed. The reaction was stopped with the addition of 50µl 0.5M H_2SO_4. Absorbance readings were then taken at 450nm using a BioTek EL900 plate reader.

Controls were established by replacing the anti-benzylpenicillin antibody-positive serum ($RB\text{:}BP_{101\text{-}105}$) with normal, antibody free, rabbit serum (NRS) at dilutions of 1:20,1:40, etc.

Background absorbance levels were determined using equation 10.1

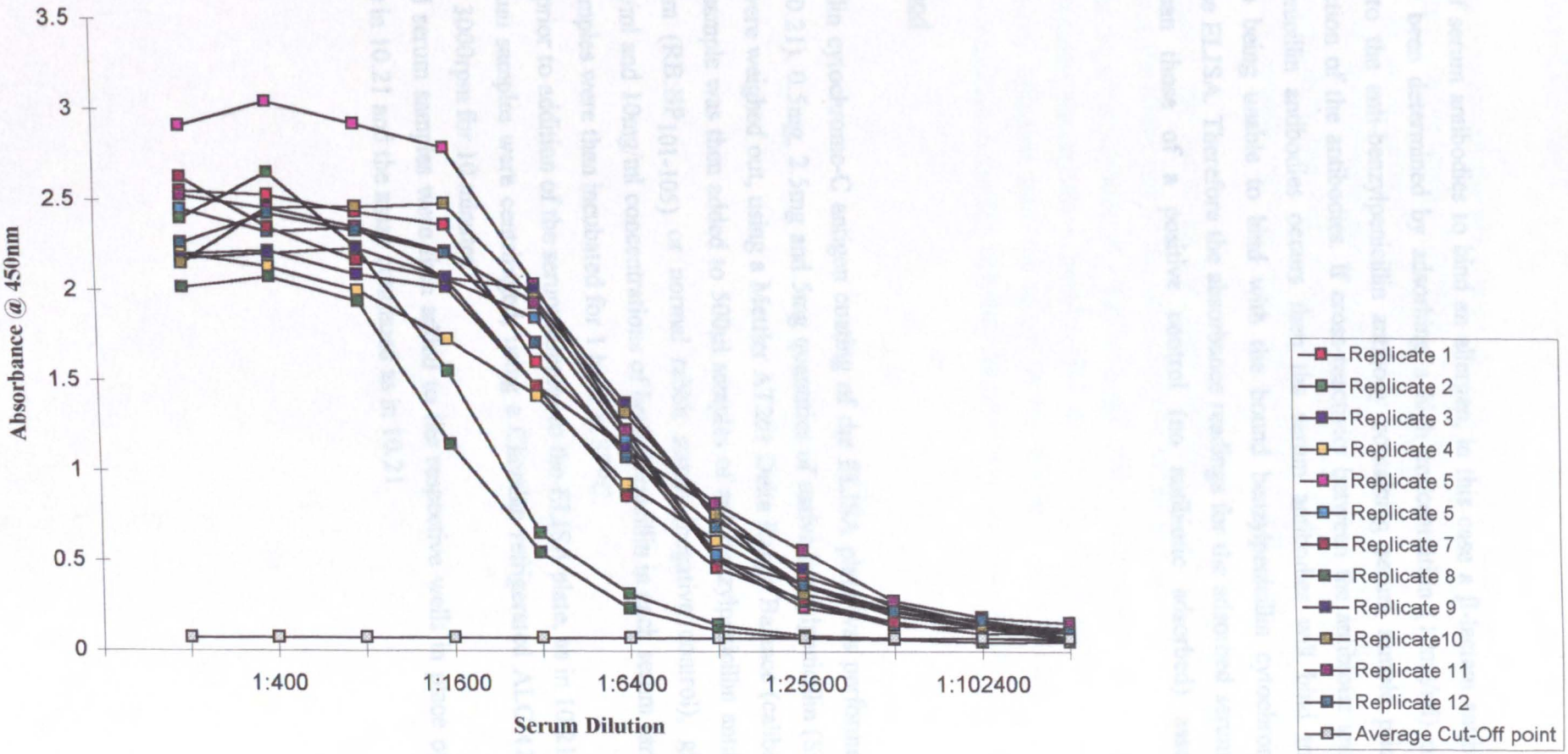
Background = mean absorbance of control at 450nm + 3 standard deviations (10.1)

Twelve replicates of assay, using the same rabbit serum sample, were made in order to determine the reproducibility of the assay.

10.212 Results and Discussion

Graphical representation of the absorbance data, figure 10.1, illustrates that the results obtained for the detection of rabbit serum anti-benzylpenicillin antibodies in each of the twelve replicates and over a range of serum dilutions produces a classical dose-response relationship. It can also be seen that the results obtained for each replicate are very similar with regard to the magnitude of the absorbance level measured, and therefore the amount of antibody present, in each respective serum dilution. It can be concluded therefore that the assay is very reproducible.

Figure 10.1 Reproducibility of Benzylpenicillin Cytochrome-C ELISA forhe Detection of Anti-Benzylpenicillin Antibodies in Rabbit Serum



10.22 Experiment 2: Adsorption of Benzylpenicillin

The ability of serum antibodies to bind an allergen, in this case a β-lactam antibiotic, has normally been determined by adsorbing a high concentration (10mg/ml) of the antibiotic onto the anti-benzylpenicillin antibody containing serum sample prior to ELISA detection of the antibodies. If cross-reactivity between the antibiotic and the anti-benzylpenicillin antibodies occurs then the serum antibodies will bind to the antibiotic, so being unable to bind with the bound benzylpenicillin cytochrome-C allergen in the ELISA. Therefore the absorbance readings for the adsorbed serum will be lower than those of a positive control (no antibiotic adsorbed) assessed concurrently.

10.221 Method

Benzylpenicillin cytochrome-C antigen coating of the ELISA plate was performed as previously (10.21). 0.5mg, 2.5mg and 5mg quantities of native benzylpenicillin (Sigma 48F-0012) were weighed out, using a Mettler AT261 Delta Range Balance (calibrated daily). Each sample was then added to 500µl samples of anti-benzylpenicillin antibody positive serum ($RB:BP_{101-105}$) or normal rabbit serum (negative control), giving 1mg/ml, 5mg/ml and 10mg/ml concentrations of benzylpenicillin in each serum sample. The serum samples were then incubated for 1 hour at 37°C.

Immediately prior to addition of the serum samples to the ELISA plate, as in 10.21, the adsorbed serum samples were centrifuged, using a Chemlab refrigerated ALC 4237R centrifuge, at 3000rpm for 10 minutes.

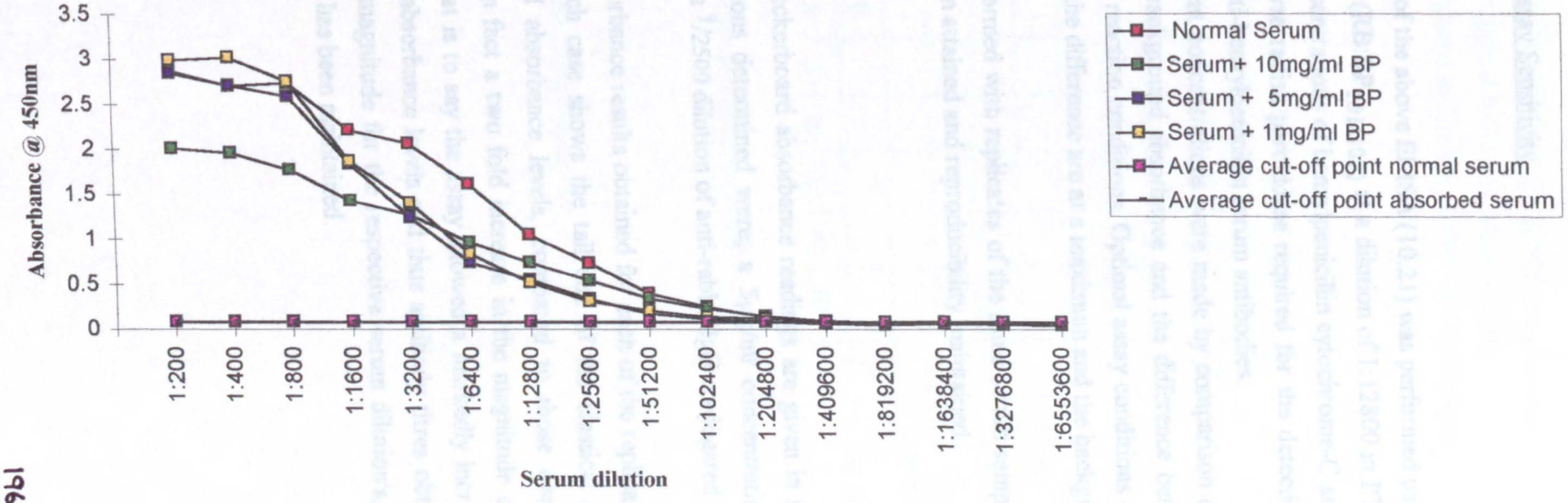
The adsorbed serum samples were then added to the respective wells in place of the serum sample in 10.21 and the assay continued as in 10.21.

10.222 Results and Discussion

Graphical representation of the absorbance data, figure 10.2, illustrates that it is not possible to quantitate the levels of antibody which bind to the native benzylpenicillin (antibiotic) in the serum during the adsorption procedure. Over the range of adsorbed native benzylpenicillin concentrations it is possible only to determine if adsorption, and therefore cross-reactivity, has occurred or not. This is probably due to the lack of assay sensitivity in that the assay does not enable the detection of slight changes in antibody concentration.

It was considered that increasing the assay sensitivity, by optimising the assay conditions about the lower end of the dose-response curve, just prior to its levelling-off, (e.g. about the serum dilution of 1:12800) could make the detection of very slight changes in antibody concentration possible and thus enable the quantitation of the levels of antibody adsorbed from the serum by the allergen, i.e. quantitation of the extent of cross-reactivity occurring between the antibiotic and the anti-benzylpenicillin antibodies.

Figure 10.2 Adsorbance of Three Concentrations of Benzylpenicillin as Detected by BPCC ELISA



10.23 Experiment 3: Increasing Assay Sensitivity

10.231 Method

A checkerboard assay, figure 10.3, of the above ELISA (10.21) was performed using anti-benzylpenicillin positive serum (RB:$BP_{101-105}$) at a dilution of 1:12800 in PTS, in order to determine the optimal concentrations of benzylpenicillin cytochrome-C antigen and anti-rabbit IgG conjugated horse radish peroxidase required for the detection of low concentrations of polyclonal anti-benzylpenicillin serum antibodies.

Determination of the optimal reagent concentrations were made by comparison of the mean test absorbances, the mean background absorbance and the difference between test and background for each set of reaction conditions. Optimal assay conditions occur when both the test absorbance and the difference are at a maximum and the background absorbance is minimal.

The optimised assay was then performed with replicates of the same serum sample, to ensure increased sensitivity had been attained and reproducibility maintained.

10.232 Results and Discussion

Graphical representation of the checkerboard absorbance readings are given in figure 10.4. The optimal reagent conditions determined were; a 5μg/ml concentration of benzylpenicillin cytochrome-C and a $^1/_{2500}$ dilution of anti-rabbit IgG conjugated horse radish peroxidase, in PTS.

Graphical representation of the absorbance results obtained for each of the replicates of the optimised ELISA, 10.5, in each case shows the tail end of the classical dose-response plot at greatly increased absorbance levels, compared to those obtained previously (10.211, figure 10.1). In fact a two fold increase in the magnitude of the absorbance levels was achieved, that is to say the assay showed a markedly increased sensitivity. It is also clear that the absorbance levels and thus antibody titres obtained for each replicate are of a similar magnitude for the respective serum dilution's, thus indicating that assay reproducibility has been maintained.

Figure 10.3 Diagram of the ELISA Plate Layout of a Checkerboard Assay for Determination of Optimal Reagent Concentrations for Detection of Antibodies in 1/12800 Diluted Serum Samples

RB:$BP_{101-105}$ $^{1}/_{12800}$dilution Normal Sera $^{1}/_{10}$ dilution

	1	2	3	4	5	6	7	8	9	10	11	12	
A						B						B	0
B													$^{1}/_{100}$
C													$^{1}/_{500}$ αrHRP
D													$^{1}/_{1000}$
E													$^{1}/_{2500}$
F													$^{1}/_{5000}$
G													$^{1}/_{10000}$
H						B						B	0
	5	10	20	40	100	0	5	10	20	40	100	0	

μg/ml benzylpenicillin cytochrome-C

KEY:

αrHRP - anti-rabbit IgG conjugated horse radish peroxidase
B - control blank well

Figure 10.4 Checkerboard Optimisation of BPCC ELISA Reagent Concentrations for Maximal Detection of Antibodies in 1/12800 Serum Dilution.

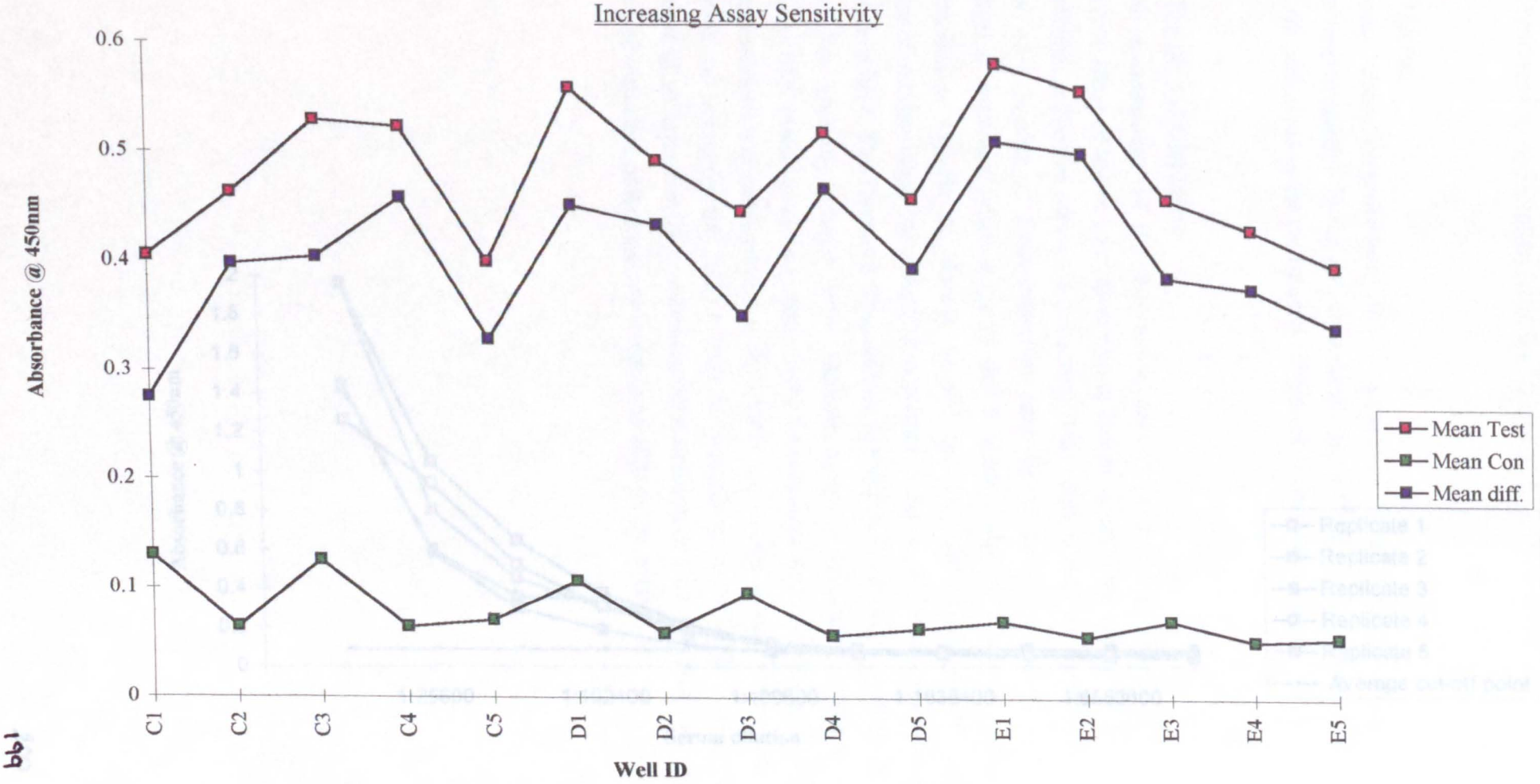
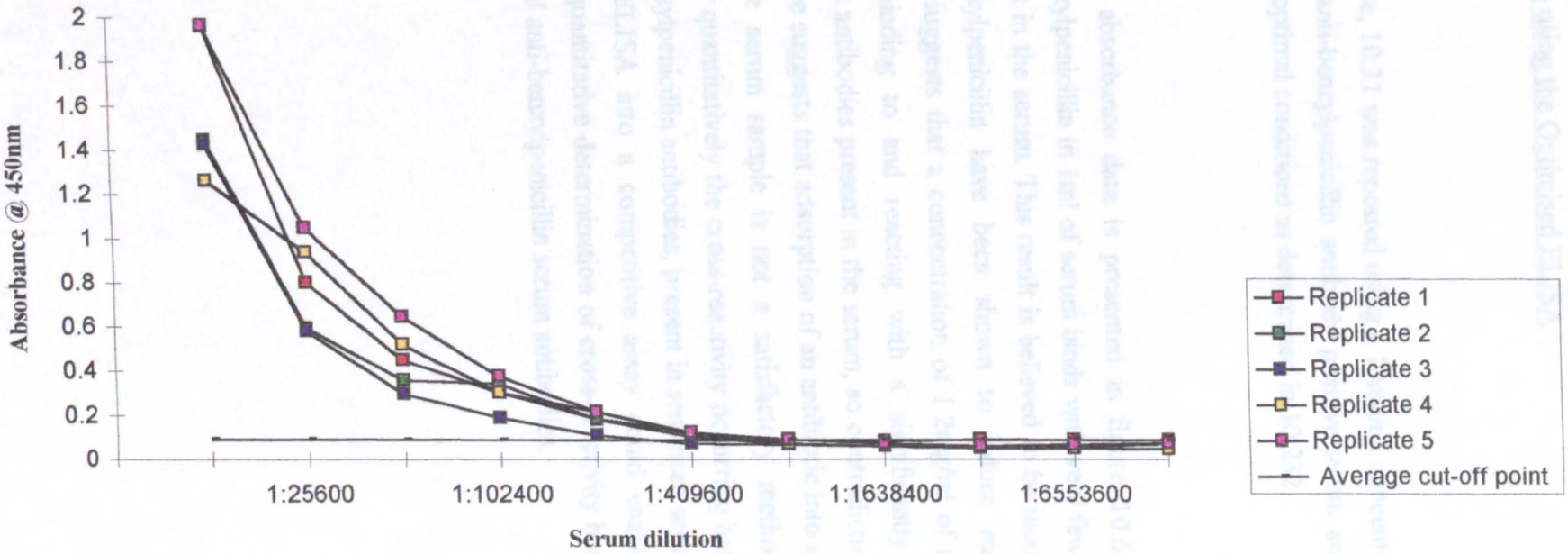


Figure 10.5 Reproducibility of the Optimised BPCC ELISA



10.24 Experiment 4: Adsorption using the Optimised ELISA

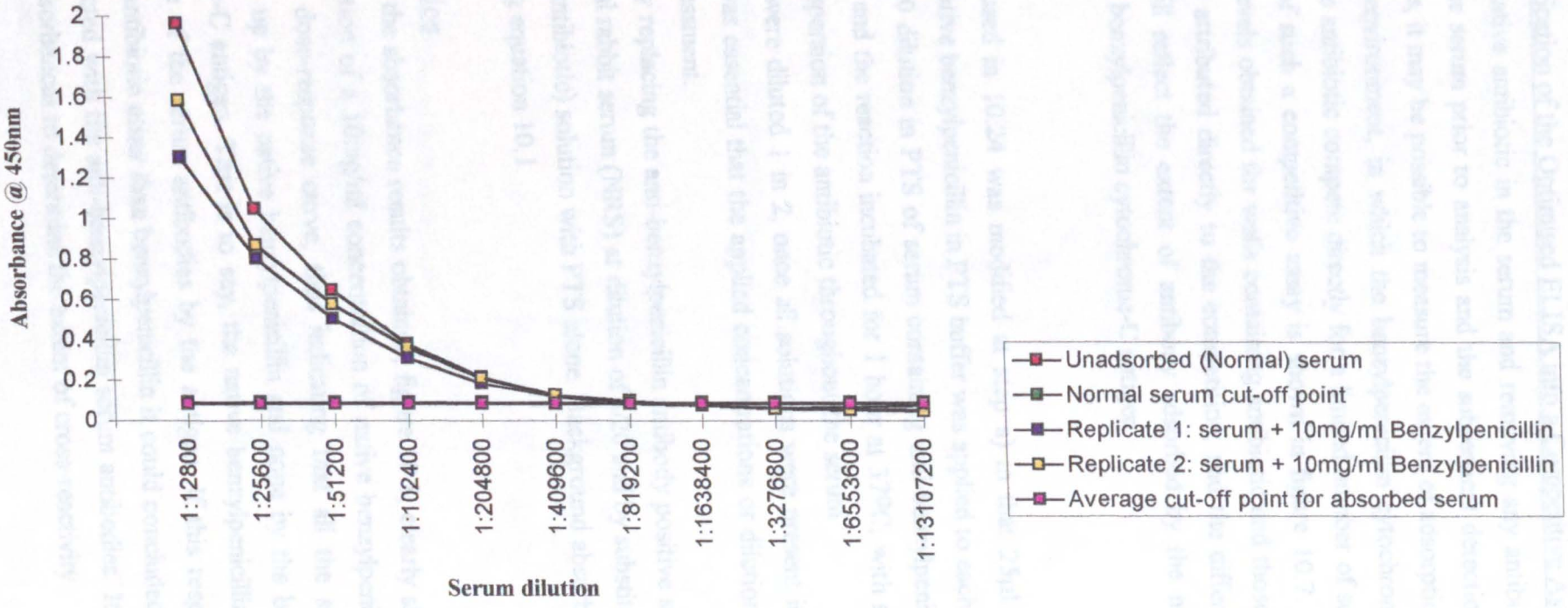
10.241 Method

The previous adsorption procedure, 10.31 was repeated using a 10mg/ml concentration of native benzylpenicillin in the anti-benzylpenicillin antibody positive-serum and the ELISA was performed under the optimal conditions as determined in 10.232.

10.242 Results and Discussion

Graphical representation of the absorbance data is presented in figure 10.6, and suggests that 10mg of native benzylpenicillin in 1ml of serum binds with very few anti-benzylpenicillin antibodies present in the serum. This result is believed to be incorrect, as doses of 1.2mg/ml of benzylpenicillin have been shown to induce massive immunological responses, which suggests that a concentration of 1.2mg/ml of native benzylpenicillin is capable of binding to and reacting with a significantly high proportion of anti-benzylpenicillin antibodies present in the serum, so contradicting the result obtained here. This therefore suggests that adsorption of an antibiotic into a anti-benzylpenicillin antibody positive serum sample is not a satisfactory method for determining either qualitatively or quantitatively the cross-reactivity occurring between a β-lactam antibiotic and anti-benzylpenicillin antibodies, present in sensitised serum. Modification of the optimised ELISA into a competitive assay could enable the development of an assay for the quantitative determination of cross-reactivity between β-lactam antibiotics and polyclonal anti-benzylpenicillin serum antibodies.

Figure 10.6 Adsorption of Benzylpenicillin as Detected by Optimised BPCC ELISA



10.25 Experiment 5: Modification of the Optimised ELISA into a Competitive Assay

Rather than adsorbing the native antibiotic in the serum and removing any antibiotic-antibody complexes from the serum prior to analysis and the subsequent detection of any remaining free antibodies, it may be possible to measure the extent of adsorption as it occurs in a competitive environment, in which the benzylpenicillin cytochrome-C bound antigen and the native antibiotic compete directly for a limited number of serum antibodies. An illustration of such a competitive assay is shown in figure 10.7. Any changes in the absorbance levels obtained for wells containing antibiotic and those not containing antibiotic can be attributed directly to the competition, and the difference between the two values will reflect the extent of antibody adsorbed by the native antibiotic and not the bound benzylpenicillin cytochrome-C antigen.

10.251 Method

The ELISA as previously used in 10.24 was modified at step a) in that 25µl of a 10mg/ml concentration of native benzylpenicillin in PTS buffer was applied to each well followed by 25µl of a 1/6400 dilution in PTS of serum containing anti-benzylpenicillin antibodies ($RB\text{-}BP_{101\text{-}105}$) and the reaction incubated for 1 hour at 37°C, with slight agitation, to ensure equal dispersion of the antibiotic throughout the serum.

As the serum and allergen were diluted 1 in 2, once all solutions were present in the reaction well at step a), it was essential that the applied concentrations or dilution's be double that required for assessment.

Controls were established by replacing the anti-benzylpenicillin antibody positive serum ($RB{:}BP_{101\text{-}105}$) with normal rabbit serum (NRS) at dilution of 1/20 and by substituting the native benzylpenicillin (antibiotic) solution with PTS alone. Background absorbance levels were determined using equation 10.1.

10.252 Results and Discussion

Graphical representation of the absorbance results obtained, figure 10.8, clearly shows that the competitive adsorption of a 10mg/ml concentration of native benzylpenicillin causes a flattening of the dose-response curve, thus indicating that all the serum antibodies have been taken up by the native benzylpenicillin and none by the bound benzylpenicillin cytochrome-C antigen. That is to say, the native benzylpenicillin has completely inhibited uptake of the serum antibodies by the antigen. If this response were to be obtained for an antibiotic other than benzylpenicillin it could concluded that the antibiotic had cross-reacted with the anti-benzylpenicillin serum antibodies. It may also be possible from the absorbances to determine the extent of cross-reactivity.

Figure 10.7 Diagrammatic Representation of Competitive BPCC ELISA for Quantitative Determination of Cross-Reactivity Between β-Lactam Antibiotics and Anti-Benzylpenicillin Serum Antibodies

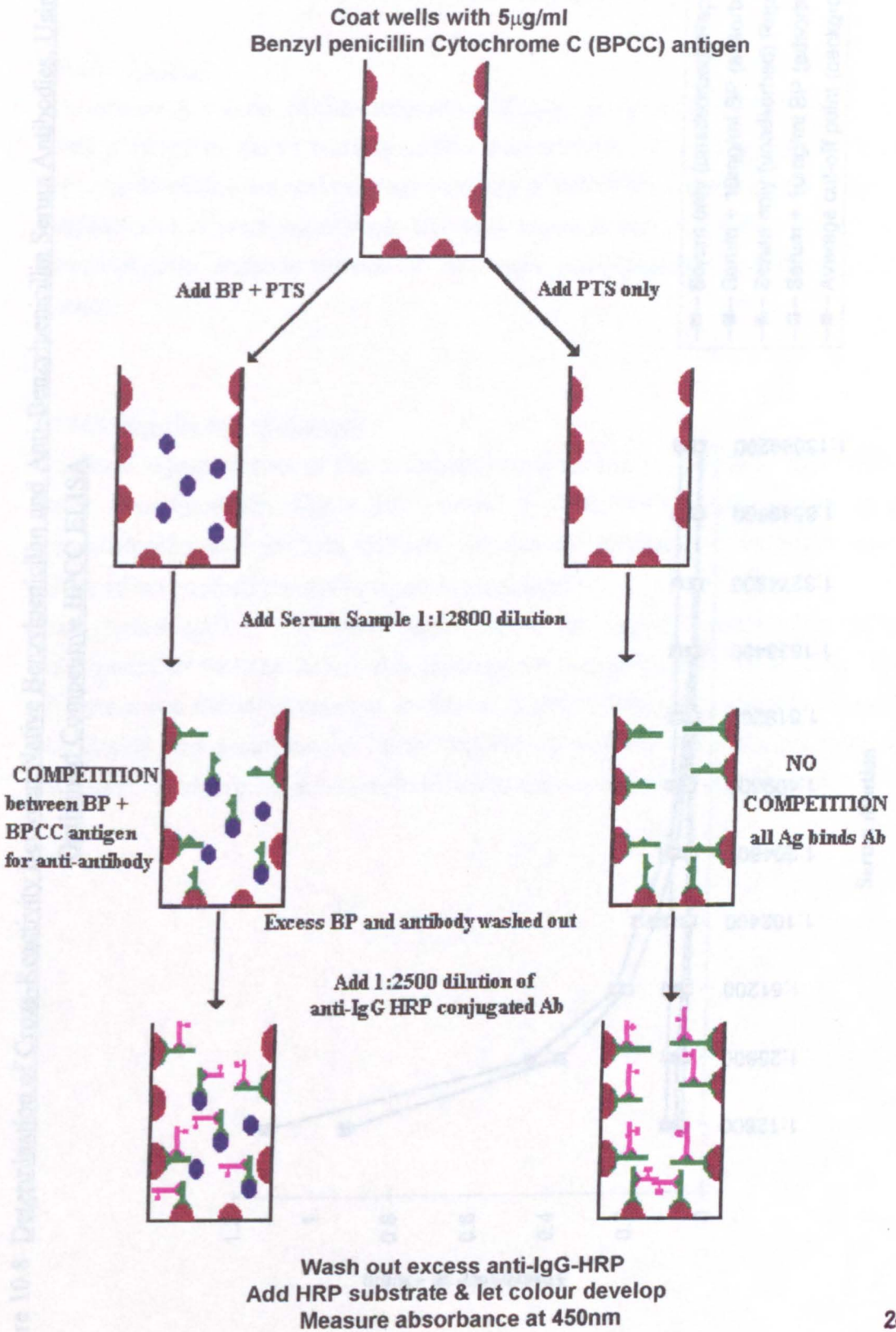
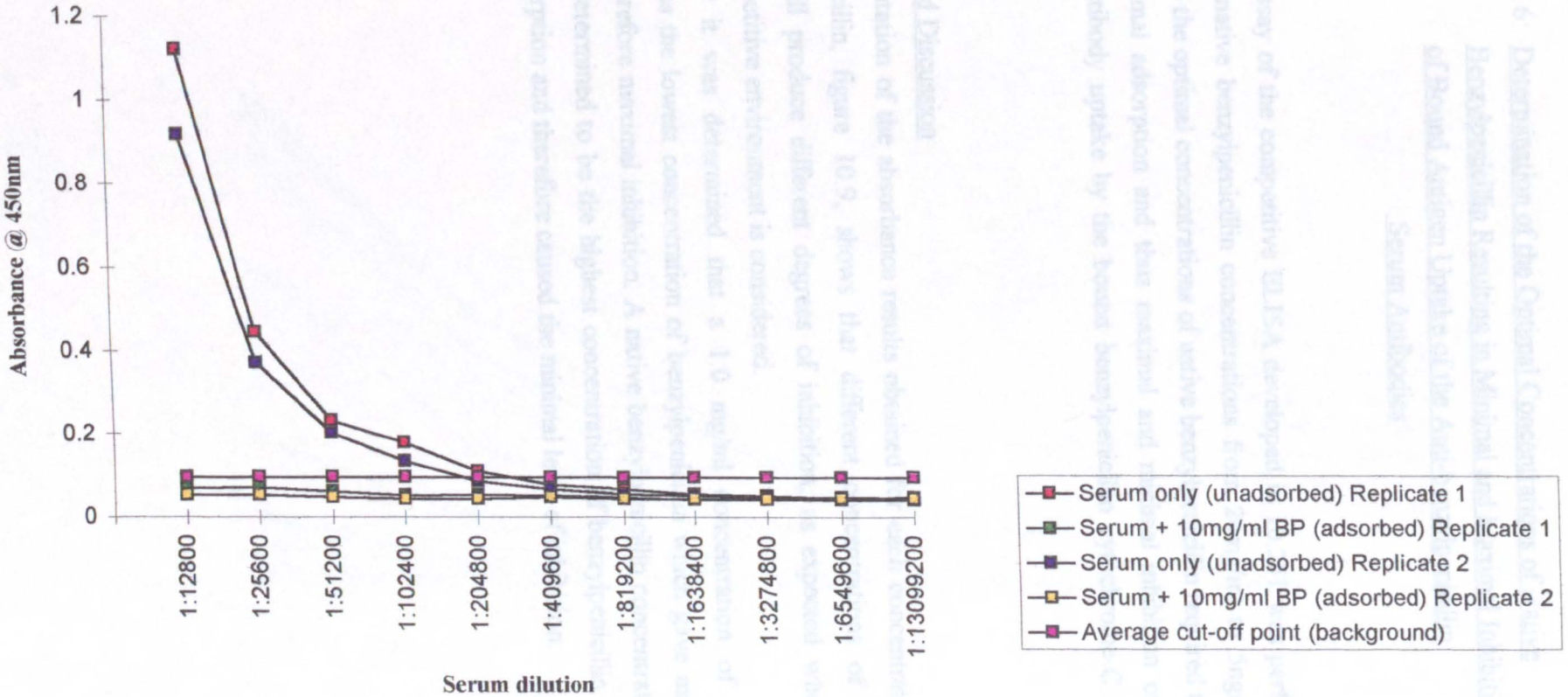


Figure 10.8 Determination of Cross-Reactivity Between Native Benzylpenicillin and Anti-Benzylpenicillin Serum Antibodies, Using the Optimised Competitive BPCC ELISA



10.26 Experiment 6: Determination of the Optimal Concentrations of Native Benzylpenicillin Resulting in Minimal and Maximal Inhibition of Bound Antigen Uptake of the Anti-benzylpenicillin Serum Antibodies

10.261 Method

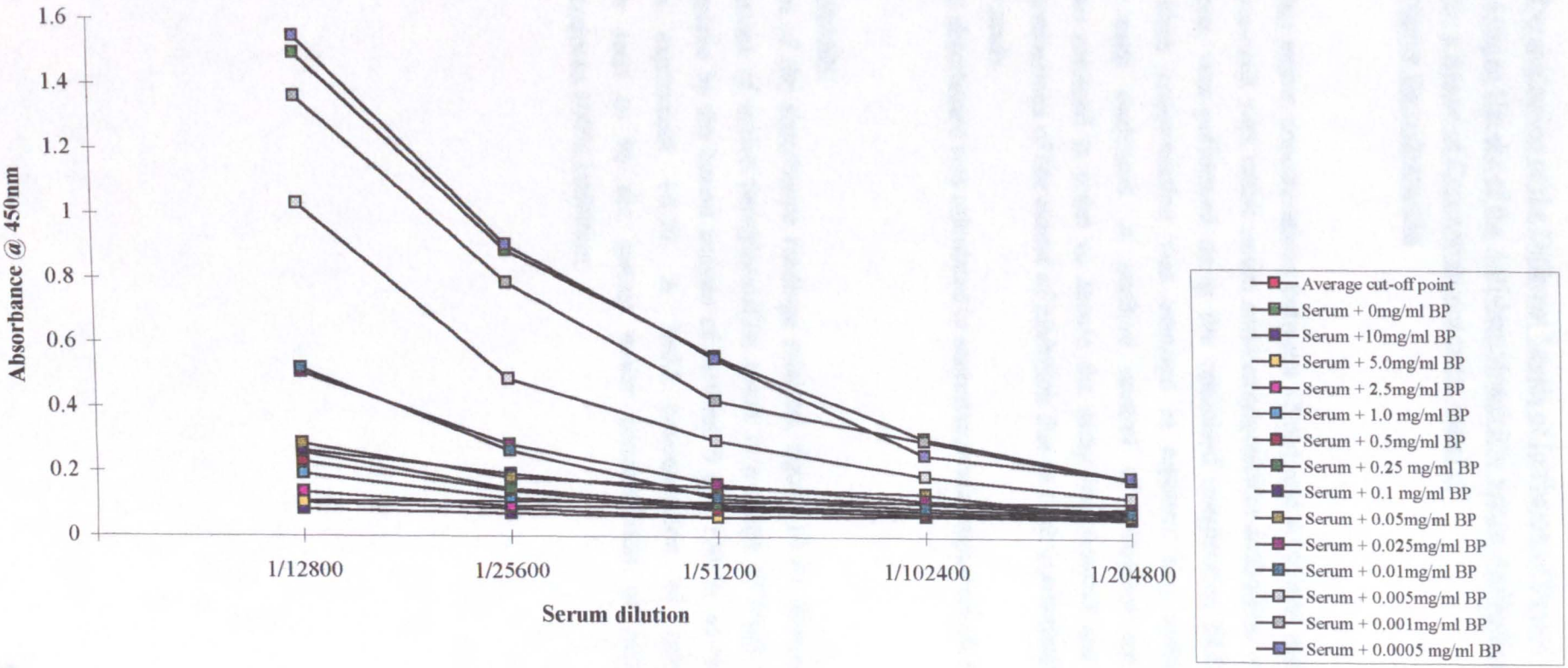
A checkerboard assay of the competitive ELISA developed in 10.251 was performed using a range of native benzylpenicillin concentrations from 20mg/ml to 5ng/ml, in order to determine the optimal concentrations of native benzylpenicillin required to give maximal and minimal adsorption and thus maximal and minimal inhibition of anti-benzylpenicillin antibody uptake by the bound benzylpenicillin cytochrome-C bound antigen.

10.262 Results and Discussion

Graphical representation of the absorbance results obtained for each concentration of native benzylpenicillin, figure 10.9, shows that different concentrations of native benzylpenicillin will produce different degrees of inhibition, as expected when the nature of the competitive environment is considered.

From figure 10.9 it was determined that a 1.0 mg/ml concentration of native benzylpenicillin was the lowest concentration of benzylpenicillin which gave maximal adsorption and therefore maximal inhibition. A native benzylpenicillin concentration of 0.001mg/ml was determined to be the highest concentration of benzylpenicillin which resulted in no adsorption and therefore caused the minimal level of inhibition.

Figure 10.9 Determination of the Concentrations of Native Benzylpenicillin Giving Maximal and Minimal Cross-Reactivity, Using the Optimised Competitive BPCC ELISA



10.27 Experiment 7: Determination of the Different Levels of Inhibition of Bound Antigen Uptake of the Anti-benzylpenicillin Serum Antibodies for a Range of Concentrations(molar units) of Native Benzylpenicillin

10.271 Method

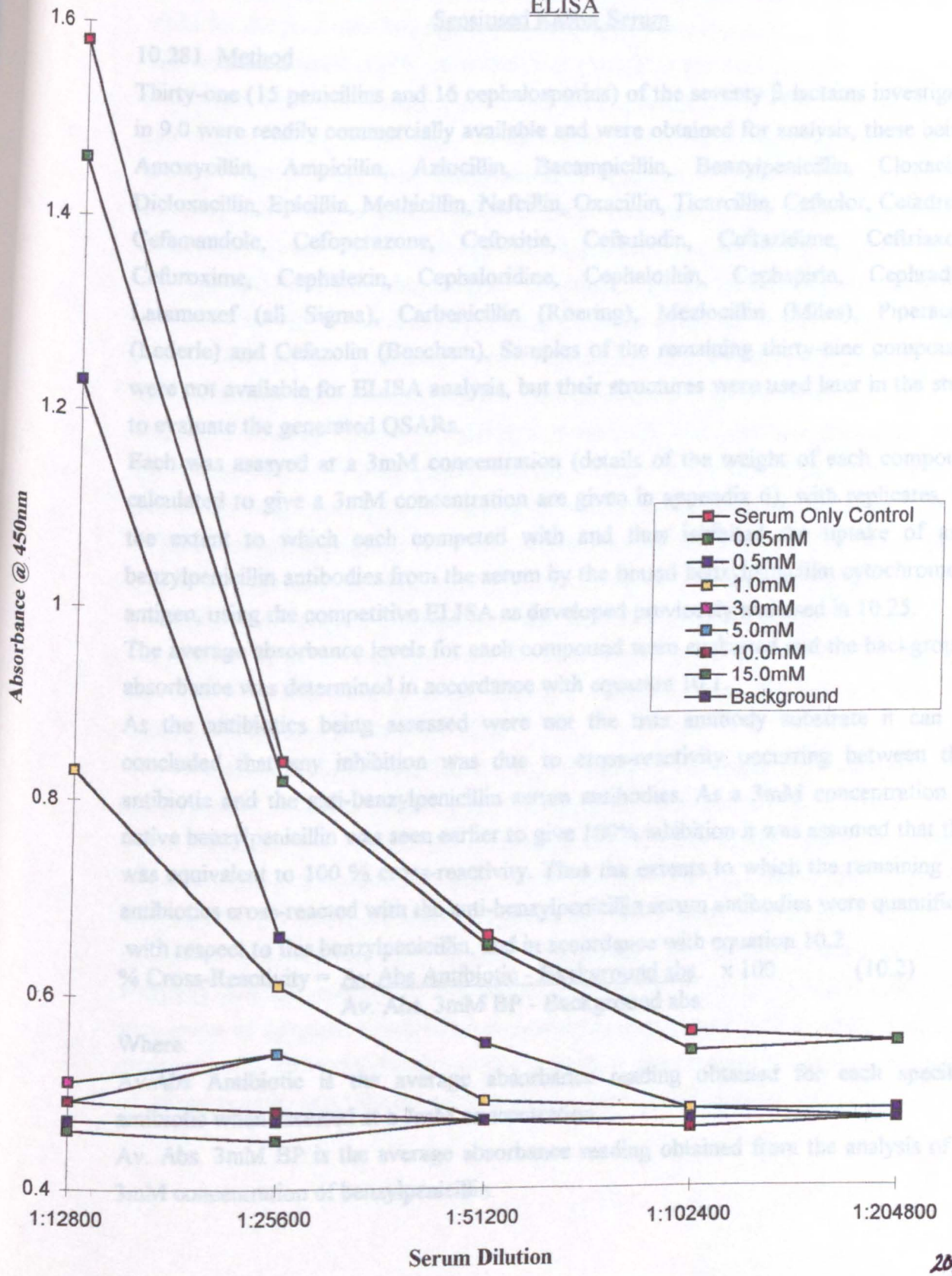
Determination as to what extent concentrations between 15mM and 0.005mM native benzylpenicillin will cross-react with rabbit serum anti-benzylpenicillin antibodies, over range of serum dilutions, was performed using the optimised competitive ELISA developed in 10.26. Each concentration was assessed in replicate and average absorbance levels for each evaluated. A positive control of 1.0mg/ml native benzylpenicillin was also assessed in order to ensure the assay had worked and to enable quantitative determinations of the extent of inhibition due to each concentration of benzylpenicillin to be made.

The level of background absorbance was calculated in accordance with equation 10.1.

10.272 Results and Discussion

Graphical representation of the absorbance readings attained, figure 10.10, illustrate that different concentrations of native benzylpenicillin result in a range of levels of inhibition of antibody uptake by the bound antigen of between 0 and 100 %, as seen with the optimisation experiment 10.26. A 3mM concentration of native benzylpenicillin can be seen to be the lowest molar concentration of native benzylpenicillin which produces 100% inhibition.

Figure 10.10 Graphical Representation of the Different Extents of Adsorption / Cross-Reactivity of Native Benzylpenicillin at Different Concentrations with Anti-Benzylpenicillin Serum Antibodies, Determined by Optimised Competitive BPCC ELISA



10.28 Experiment 8 Quantitation of the Levels of Cross-Reactivity Occurring Between 31 Different β-Lactam Antibiotics, at an Equivalent Molar Concentration, with Polyclonal Anti-benzylpenicillin Antibodies in Sensitised Rabbit Serum

10.281 Method

Thirty-one (15 penicillins and 16 cephalosporins) of the seventy β-lactams investigated in 9.0 were readily commercially available and were obtained for analysis, these being ; Amoxycillin, Ampicillin, Azlocillin, Bacampicillin, Benzylpenicillin, Cloxacillin, Dicloxacillin, Epicillin, Methicillin, Nafcillin, Oxacillin, Ticarcillin, Cefaclor, Cefadroxil, Cefamandole, Cefoperazone, Cefoxitin, Cefsulodin, Ceftazidime, Ceftriaxone, Cefuroxime, Cephalexin, Cephaloridine, Cephalothin, Cephapirin, Cephradine, Latamoxef (all Sigma), Carbenicillin (Roering), Mezlocillin (Miles), Piperacillin (Lederle) and Cefazolin (Beecham). Samples of the remaining thirty-nine compounds were not available for ELISA analysis, but their structures were used later in the study to evaluate the generated QSARs.

Each was assayed at a 3mM concentration (details of the weight of each compound calculated to give a 3mM concentration are given in appendix 6), with replicates, for the extent to which each competed with and thus inhibited the uptake of anti-benzylpenicillin antibodies from the serum by the bound benzylpenicillin cytochrome-C antigen, using the competitive ELISA as developed previously and used in 10.25.

The average absorbance levels for each compound were evaluated and the background absorbance was determined in accordance with equation 10.1.

As the antibiotics being assessed were not the true antibody substrate it can be concluded that any inhibition was due to cross-reactivity occurring between that antibiotic and the anti-benzylpenicillin serum antibodies. As a 3mM concentration of native benzylpenicillin was seen earlier to give 100% inhibition it was assumed that this was equivalent to 100 % cross-reactivity. Thus the extents to which the remaining 30 antibiotics cross-reacted with the anti-benzylpenicillin serum antibodies were quantified with respect to this benzylpenicillin, and in accordance with equation 10.2.

$$\% \text{ Cross-Reactivity} = \frac{\text{Av.Abs Antibiotic - Background abs.}}{\text{Av. Abs. 3mM BP - Background abs.}} \times 100 \qquad (10.2)$$

Where:

Av.Abs Antibiotic is the average absorbance reading obtained for each specific antibiotic when assessed at a 3mM concentration.

Av. Abs. 3mM BP is the average absorbance reading obtained from the analysis of a 3mM concentration of benzylpenicillin.

10.282 Results and Discussion

The percentage cross-reactivities evaluated for the thirty-one β-lactams (15 penicillins and 16 cephalosporins) investigated are presented in table 10.1 and illustrated in figure 10.11 for the penicillins and figure 10.12 for the cephalosporins.

The results obtained clearly show that both penicillins and cephalosporin antibiotics do cross-react with anti-benzylpenicillin antibodies present in pre-sensitised rabbit serum and that they do so to different extents, with carbenicillin showing the greatest level of cross-reactivity (other than Benzylpenicillin itself) at 79.4 %, whilst bacampicillin, cefaclor, cefadroxil, cefazolin, cefuroxime and latamoxef do not appear to cross-react at all. These results therefore support the hypothesis that different β-lactam antibiotics are capable of inducing a large degree of immunologically related responses as a result of cross-reactivity with existing anti-benzylpenicillin serum antibodies [591].

Comparison of the percentage cross-reactivities obtained for the penicillins and cephalosporins shows there to be a distinct difference in the extent to which the respective sub-populations of β-lactams cross-react with anti-benzylpenicillin serum antibodies. The percentage cross-reactivity values for the penicillins span the whole percentage range from 0 to 100, whilst the values for the cephalosporins are all below 15%. These values support the similar observation of Kishiyama and Adelman [588], that there appears to be substantially less cross-reactivity between cephalosporins and penicillins than between the penicillins themselves. The results suggest therefore. that the problem of cross-reactivity exists for both penicillin and cephalosporin antibiotics, but that it is less of a problem with the cephalosporins, as they are 'less' like benzylpenicillin than are the penicillins.

The differential levels of penicillin and cephalosporin cross-reactivity, as shown in this work, present a similar general pattern of incidence, i.e. that on the whole penicillin levels are higher than those of the cephalosporins, as seen with the relative frequencies of cutaneous rash induction following therapy, as shown in 9.2. This suggests therefore that, as both processes are immunologically mediated, some portion of the extent of cutaneous rash exhibited may in fact be attributed to the phenomenon of cross-reactivity and not entirely to the antibiotic itself. In order to try an evaluate the proportion of cutaneous rash (allergic reactions) as reported in 9.2 which is due to cross-reactivity and not the native antibiotic itself, it is necessary to determine the levels of cross-reactivity an antibiotic will induce at its therapeutic dose.

10.29 Experiment 9: Quantitation of the Levels of Cross-Reactivity Occurring Between 31 Different β-Lactam Antibiotics, at Concentrations Equivalent to their Respective Highest Recommended Therapeutic Daily Dose, with Anti-benzylpenicillin Antibodies

A competitive ELISA has been developed which will enable the quantitation of the extent to which different β-lactam antibiotics cross-react with anti-benzylpenicillin serum antibodies. The next step in the study was to determine what portion of the frequency of allergic reactions (cutaneous rash) induced by the therapeutic use of β-lactam antibiotics may be attributed to the phenomenon of cross-reactivity and not directly to the antibiotic itself.

It was decided to evaluate the potential cross-reactivity of different β-lactams at *in vitro* concentrations equivalent to those given therapeutically, thus enabling the results obtained to be related to the clinical incidence of allergic reactions.

The highest recommended therapeutic daily dose (HRTDD) of benzylpenicillin is 1.0g; an equivalent concentration suitable for the quantitation of cross-reactivity by ELISA would be 1.0g/l or 1.0mg/ml. A 1.0mg/ml concentration of benzylpenicillin in fact equates to a 3mM concentration and therefore can be assumed to give a cross-reactivity of 100% in the assay, as shown in 10.27. This extent of benzylpenicillin cross-reactivity with its corresponding serum antibodies corresponds to the responses seen clinically.

10.291 Method

The HRTDD, in grammes, for thirty-one commercially available β-lactam antibiotics (15 penicillins and 16 cephalosporins as in 10.28) were obtained [193,592].

The concentration in mg/ml of antibiotic used in the competitive ELISA was then calculated as HRTDD/1000 mg/ml. Each antibiotic was assayed at this concentration using the competitive ELISA as with 10.28, with replicates, for determination of the extent to which it cross-reacted with anti-benzylpenicillin serum antibodies. The absorbance readings obtained for each antibiotic were averaged, and the background absorbance calculated in accordance with equation 10.1.

The extent to which each antibiotic cross-reacted with the anti-benzylpenicillin serum antibodies was quantified in accordance with equation 10.3, assuming that a 1.0mg/ml concentration of benzylpenicillin resulted in 100 % cross-reactivity.

$$\% \text{ Cross-Reactivity} = \frac{\text{Av.Abs Antibiotic - Background abs.}}{\text{Av. Abs. 3mM BP - Background abs.}} \times 100 \qquad (10.2)$$

Where:

Av.Abs Antibiotic is the average absorbance reading obtained for each specific antibiotic when assessed at a mg/ml concentration equivalent to its HRTDD.

Av. Abs. 1.0mg/ml BP is the average absorbance reading obtained from the analysis of a 1.0mg/ml concentration of benzylpenicillin.

10.292 Results and Discussion

The percentage cross-reactivity results obtained for the 31 β-lactams (15 penicillins and 16 cephalosporins) investigated at concentrations equivalent to their respective HRTDD are given in table 10.1 and illustrated graphically in figure 10.11 for the penicillins and 10.12 for the cephalosporins.

The results show, as with 10.28, that both the penicillin and cephalosporin antibiotics cross-react with anti-benzylpenicillin serum antibodies and that they do so to different extents. The two levels of cross-reactivity evaluated for eighteen antibiotics are similar, given some experimental error, but for ten antibiotics the two levels of cross-reactivity are quite different.

Cirstea et al [591] determined that at low concentrations the extent of amoxycillin cross-reactivity was substantially lower than that of benzylpenicillin, whilst at higher concentrations the extent of cross-reactivity shown by amoxycillin increased, thus reducing the difference between itself and benzylpenicillin, and yet other compounds show no such trend. This phenomenon appears to be the case for the ten compounds in this study whose cross-reactivities at the two test concentrations differ greatly.

The exact reasons for these ten discrepancies are not clear; they may be due in part to the substantially greater HRTDDs of some of these antibiotics, when compared those antibiotics displaying equivalent levels of cross-reactivity at both test concentrations, as detailed in appendix 5; or they may be due to the fact that different rabbits respond differently to benzylpenicillin, during the sensitisation phase (as do humans). The discrepancies therefore, may be a reflection of the varying levels of antibody titre present in the different serum samples used in the study. This problem can be overcome by repeating each antibiotic assessment with a range of rabbit serum samples and determining an average level of cross-reactivity for each test concentration ,thus generating data which are indicative of cross-reactivities across the board, eliminating

individual variation, and not just to a one off response. Other possible reasons for the discrepancies may be the denaturation of the antibodies in the serum sample or experimental error, such as in the weighing out of the compounds. Time did not permit these possibilities to be investigated in this study.

The results presented in 10.29 suggest that different β-lactam antibiotics induce allergic responses to different extents in the clinical environment, as a result of cross-reactivity between the antibiotic and anti-benzylpenicillin polyclonal antibodies present in the serum. The quantitative data produced may be used, in conjunction with the epidemiological data presented in 9.2, to enable the determination of the extent to which an allergic response (cutaneous rash) is due to these cross-reactivity reactions. For example, the penicillin antibiotic azlocillin induces cutaneous rash in 2.9% of the population, with some 34.5% of these reactions being due to the cross-reaction of azlocillin with anti-benzylpenicillin serum antibodies and not directly due to the azlocillin itself.

Although the developed competitive ELISA enables the quantitation of cross-reactivity between a β-lactam antibiotic or any other compound and anti-benzylpenicillin antibodies, it does not enable identification of those physical, chemical or structural properties of the compounds which are responsible for the cross-reactive phenomenon. Analysis of the structure-activity relationships of the process should provide a means of making such identifications.

Table 10.1 Percentage Cross-Reactivities of 31 β-Lactam Antibiotics with Anti-benzylpenicillin Antibodies in Pre-sensitised Rabbit Serum, as Determined by competitive ELISA

	Mode of administration	β-lactam	% Cross-reactivity at concentration equivalent to HRTDD	% Cross-reactivity at 3mM concentration
1	oral	Amoxycillin	43.2 +/- 1.2	9.1 +/- 2.4
2	oral	Ampicillin	31.3 +/- 8.1	47.2 +/- 11.5
3	oral	Azlocillin	34.6 +/- 1.1	19.2 +/- 1.8
4	oral	Bacampicillin	0.0 +/- 4.2	18.6 +/- 9.5
5	oral	Dicloxacillin	12.9 +/- 3.9	12.7 +/- 5.3
6	oral	Epicillin	17.1 +/- 7.1	17.9 +/- 11.5
7	parenteral	Benzylpenicillin	100.0 +/- 4.3	100.0 +/- 4.6
8	parenteral	Carbenicillin	86.6 +/- 9.5	79.4 +/- 5.7
9	parenteral	Cloxacillin	32.6 +/- 3.6	46.2 +/- 14.7
10	parenteral	Methicillin	7.6 +/- 4.6	27.7 +/- 9.5
11	parenteral	Nafcillin	9.8 +/- 2.8	14.2 +/- 5.0
12	parenteral	Oxacillin	29.6 +/- 2.1	24.3 +/- 11.9
13	parenteral	Piperacillin	27.3 +/- 7.3	19.2 +/- 8.8
14	parenteral	Ticarcillin	79.2 +/- 1.0	45.3 +/- 7.3
15	oral	Cefaclor	0.0 +/- 10.4	0.0 +/- 14.3
16	oral	Cefadroxil	0.0 +/- 0.5	0.0 +/- 5.2
17	oral	Cephalexin	14.9 +/- 7.5	19.87 +/- 11.6
18	parenteral	Cefamandole	23.7 +/- 4.8	6.6 +/- 12.2
19	parenteral	Cefazolin	2.8 +/- 6.0	0.0 +/- 5.1
20	parenteral	Cefoperazone	3.2 +/- 1.9	3.5 +/- 1.6
21	parenteral	Cefotaxime	3.5 +/- 2.0	2.6 +/- 0.8
22	parenteral	Cefoxitin	14.3 +/- 4.3	7.0 +/- 9.2
23	parenteral	Cefsulodin	14.0 +/- 0.5	0.8 +/- 8.6
24	parenteral	Ceftazidime	9.53 +/- 7.4	11.29 +/- 1.3
25	parenteral	Ceftriaxone	18.1 +/- 1.5	1.1 +/- 4.1
26	parenteral	Cefuroxime	14.6 +/- 3.2	0.0 +/- 6.2
27	parenteral	Cephaloridine	30.5 +/- 0.2	10.7 +/- 4.2
28	parenteral	Cephalothin	21.7 +/- 5.0	11.2 +/- 7.8
29	parenteral	Cephapirin	14.1 +/- 1.8	12.6 +/- 4.3
30	Parenteral	Cephradine	6.3 +/- 1.9	5.6 +/- 1.2
31	parenteral	Latamoxef	6.3 +/- 0.3	0.0 +/- 2.7

Note:

The +/- figures noted represent the standard deviation of the individual experimentally derived results obtained from the mean percentage cross-reactivities presented in the table.

Figure 10.11 Comparison of the Percentage Cross-Reactivities of Various Penicillin Antibiotics with Rabbit Anti-Benzylpenicillin Serum Antibodies. Determined by Optimised Competitive BPCC ELISA at Two Concentration Levels

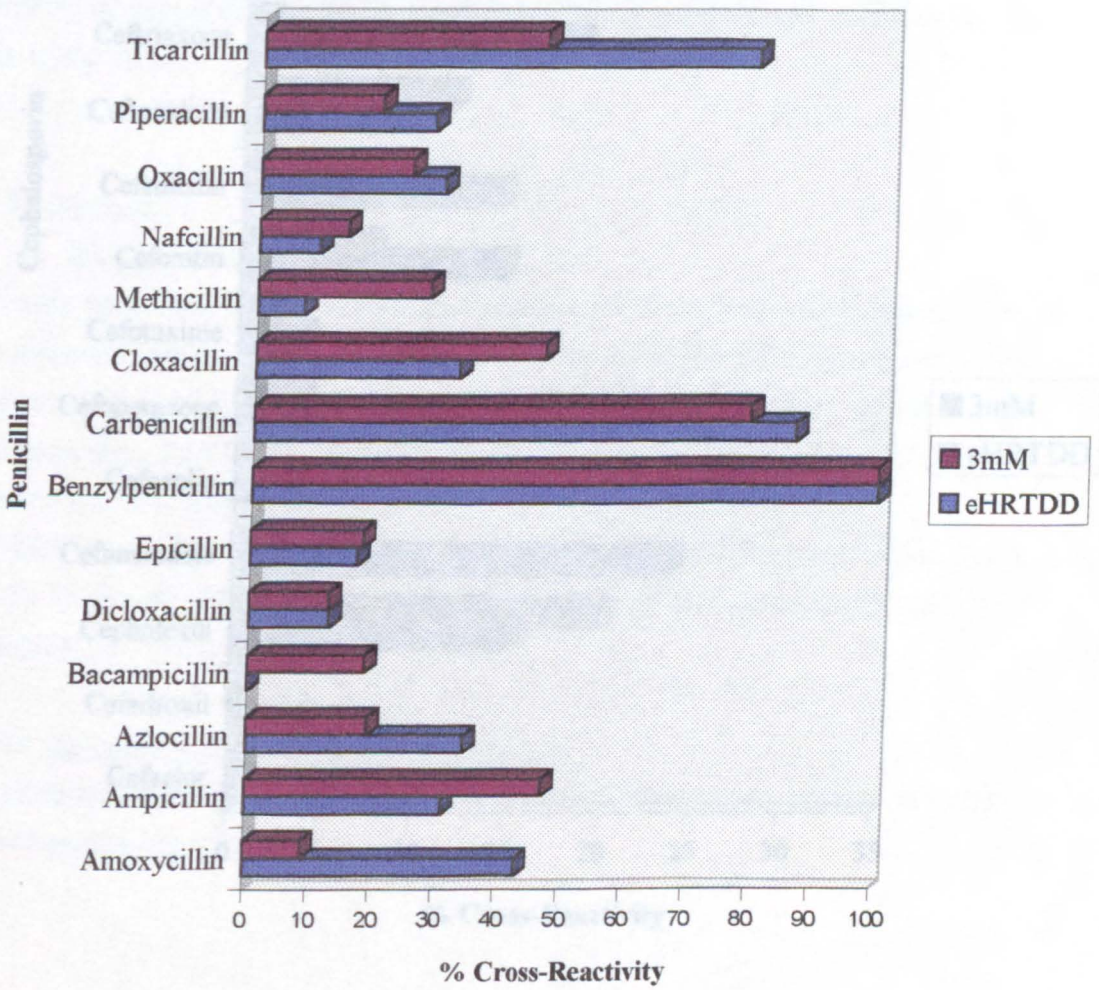
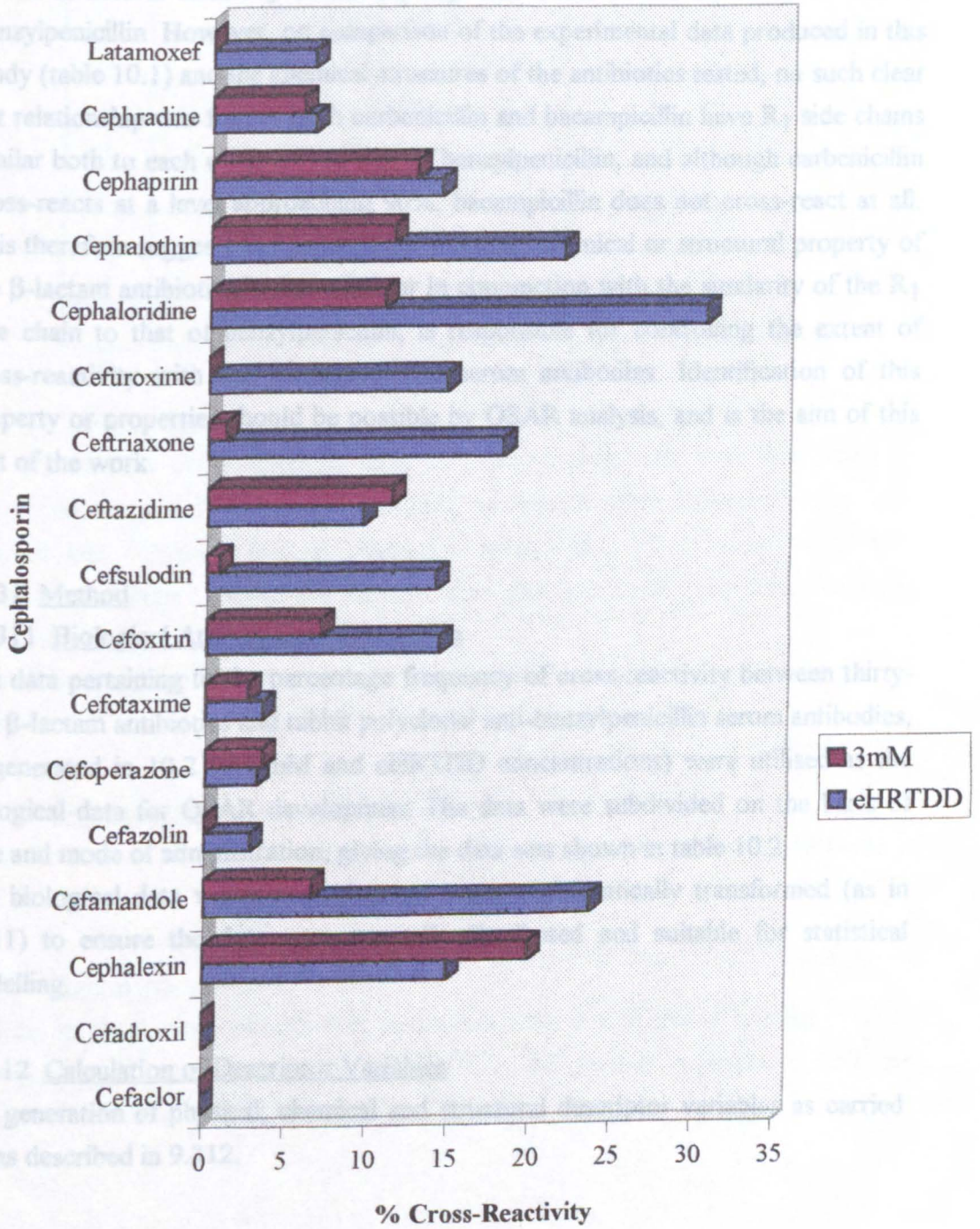


Figure 10.12 Comparison of the Percentage Cross-Reactivities of Various Cephalosporin Antibiotics with Rabbit Anti-Benzylpenicillin Serum Antibodies, Determined by Optimised Competitive BPCC ELISA at Two Concentration Levels



10.3 Quantitative Structure-Activity Relationship Study of the Cross-Reactivity of β-Lactam Antibiotics and Anti-benzylpenicillin Serum Antibodies

Previous workers [34,583,588] have suggested that the extent cross-reactivity between β-lactam antibiotics and anti-benzylpenicillin antibodies is directly related to the structural similarity of the R_1 acyl side chain of the antibiotic to that of benzylpenicillin. However, on comparison of the experimental data produced in this study (table 10.1) and the chemical structures of the antibiotics tested, no such clear cut relationship was found. Both carbenicillin and bacampicillin have R_1 side chains similar both to each other and to that of benzylpenicillin, and although carbenicillin cross-reacts at a level approaching 90%, bacampicillin does not cross-react at all. This therefore suggests that some other physical, chemical or structural property of the β-lactam antibiotics, either alone or in conjunction with the similarity of the R_1 side chain to that of benzylpenicillin, is responsible for controlling the extent of cross-reactivity with anti-benzylpenicillin serum antibodies. Identification of this property or properties should be possible by QSAR analysis, and is the aim of this part of the work.

10.31 Method

10.311 Biological Activity Data Generation

The data pertaining to the percentage frequency of cross-reactivity between thirty-one β-lactam antibiotics and rabbit polyclonal anti-benzylpenicillin serum antibodies, as generated in 10.2 (at 3mM and eHRTDD concentrations) were utilised as the biological data for QSAR development The data were subdivided on the basis of type and mode of administration, giving the data sets shown in table 10.2.

The biological data within each data set were mathematically transformed (as in 9.311) to ensure the data were normally distributed and suitable for statistical modelling.

10.312 Calculation of Descriptor Variables

The generation of physical, chemical and structural descriptor variables as carried out as described in 9.312.

Table 10.2 Data Sets for QSAR Analysis

	Compound	# Compounds in each Data Set. 3mM and eHRTDD
1	All β-Lactams	31
2	All Oral β-Lactams	9
3	All Parenteral β-Lactams	22
4	All Penicillins	14
5	Oral Penicillins	6
6	Parenteral Penicillins	8
7	All Cephalosporins	17
8	Oral Cephalosporins	3
9	Parenteral Cephalosporins	14

10.313 Data Analysis

The square-root cross-reactivity data for the 31 β-lactams were correlated by regression analysis, in the TSAR program, against the three adverse reaction data sets (all ARs, cutaneous rash, GI disruption) generated in 9.2, in order to determine if any relationships between the different data sets, particularly the cross-reactivity and cutaneous rash data, were in existence.

The cross-reactivity data was then further analysed using the procedures described in 9.312.

10.314 Determination of models' significance

Determination of the statistical significance of the generated models was made as in 9.312.

10.315 Data Randomisation

In order to check the possibility that the models were due to chance correlations the biological data were randomised as in 9.312, the models regenerated and the resultant *r* values examined as in 9.312.

10.32 Results and Discussion

10.321 Assessment of the Biological Data

The percentage frequency values of cross-reactivity with anti-benzylpenicillin serum antibodies for the thirty-one β-lactams analysed are given in table 10.1.

Transformation and univariate analysis of the biological data sets (as in table 10.2) generated a range of skewness values and distribution, given in tables 10.3 and 10.4 for the 3mM and eHRTDD reaction concentrations respectively. Examination of each respective transformation for each data set (3mM or eHRTDD concentrations) showed that the square root transformation generated normally distributed data for all data sets.

10.322 Biological Data Correlation Analysis

Regression analysis of the cross-reactivity data, generated in 10.2, and the adverse reaction data, generated in 9.2, showed there to be no significant correlations between the data sets. In 43 of 54 correlations the respective r^2 generated were zero, and the remaining 11 r^2 values were not significant, being below 0.50, as detailed in table 10.5. This indicates that the cross-reactivity data are not directly related to any of the adverse reaction data. One would, however, expect to find some partial relationship between the cross-reactivity and the cutaneous rash data as both are an indication of the level of induction of equivalent immunological responses. The lack of a correlation may be due to the fact that the cutaneous rash data does not relate exclusively to immunologically mediated responses but also types of rash initiated by non-immunological reaction mechanisms.

10.323 Regression Analysis

A total of eighty-four physico-chemical and structural descriptors (as listed in appendix 3) were generated and entered into the TSAR data base along with the square-root transformed cross-reactivity data. A summation of the final results attained from regression analysis (including a description of the preliminary validation plots) and from cross-validation analysis for the eighteen models generated are given in table 10.6. It can clearly be seen, from these results, that a statistically significant QSAR has been developed for each of the respective data sets, at each reaction concentration. Three of the models lack predictive validity, because they were produced using data pertaining to only very few compounds; the data therefore, produced a disproportionate bias in the model generation procedure.

The incorporation of further compounds into the model generation procedure for these three models would almost certainly improve predictive validity whilst maintaining statistical significance.

The QSARs generated are presented in models 10.1 to 10.9 for the cross-reactivities at 3mM antibiotic concentrations and in models 10.10 to 10.18 for cross-reactivities of the antibiotic at a concentration equivalent to its highest recommended therapeutic daily dose (HRTDD).

A list of the descriptor variables used in each of the generated models is presented in table 10.7.

Appendix 7 lists the compounds, biological, physico-chemical and structural parameter values, actual, predicted and residual square-root cross-reactivity values, the correlation matrices, and representations of the two validity plots for each model.

Table 10.3 Univariate Skewness Values Generated Following Various Mathematical Transformations of the 3mM Cross-Reactivity Data

Data set	Skewness value (Sk) for the respective transformation of:				
	%Xr	Log_{10} %Xr	$\sqrt{}$%Xr	$\%Xr^2$	$1/\%_{Xr}$
All β-lactams	1.86	-1.594	0.519	3.076	22.478
All oral β-lactams	0.867	-1.100	-0.374	1.869	1.120
All parenteral β-lactams	1.621	-1.613	0.645	2.463	3.822
All penicillins	1.206	0.373	0.813	1.809	0.628
All oral penicillins	1.097	0.519	0.862	1.301	0.280
All parenteral penicillins	0.680	0.142	0.431	1.042	0.471
All cephalosporins	0.892	-0.760	0.166	2.030	1.352
All oral cephalosporins	0.385	0.385	0.385	0.385	-0.385
All parenteral cephalosporins	0.329	-1.159	-0.143	0.851	2.645

%Xr is the percentage cross-reactivity with anti-benzylpenicillin serum antibodies as determined experimentally by competitive ELISA (10.2).

The square root transformation generated normally distributed data for all of the sub-data sets and was therefore used in the generation of QSARs pertaining to the frequency of cross-reactivity responses at a 3mM concentration of each β-lactam antibiotic with anti-benzylpenicillin serum antibodies.

Table 10.4 Univariate Skewness Values Generated Following Mathematical Transformations of the eHRTDD Cross-Reactivity Data

Data set	Skewness value (Sk) for the respective transformation of:				
	%Xr	Log_{10} %Xr	$\sqrt{}$%Xr	%Xr^2	$^1/_{\%Xr}$
All β-lactams	1.689	-1.839	0.400	2.501	2.470
All oral β-lactams	0.375	0.375	-0.127	0.863	0.375
All parenteral β-lactams	1.501	-0.924	0.802	1.920	3.640
All penicillins	0.859	-2.380	0.038	1.316	2.978
All oral penicillins	0.616	-1.495	-0.359	1.331	1.620
All parenteral penicillins	0.589	-0.052	0.342	0.745	0.706
All cephalosporins	0.274	-1.513	-0.533	1.185	1.945
All oral cephalosporins	0.385	0.385	0.385	0.385	-0.385
All parenteral cephalosporins	0.130	-1.567	-0.644	0.941	2.539

%Xr is the percentage cross-reactivity with anti-benzylpenicillin serum antibodies as determined experimentally by competitive ELISA (10.2).

The square root transformation generated normally distributed data for all of the sub-data sets and was therefore used in the generation of QSARs pertaining to the frequency of cross-reactivity responses of β-lactams at a concentration equivalent to the compounds highest recommended therapeutic daily dose, with anti-benzylpenicillin serum antibodies.

Table 10.5 Regression Coefficient Values Obtained from the Cross Correlation Analysis of the Cross-Reactivity and Adverse Reaction Data

The table presented only details those correlations of biological data which produced a regression coefficient of greater than zero.
All of the omitted correlations gave r^2 values of zero and presentation of this data was felt not to be relevant.

	All βLs All ARs	Oral βLs All ARs	Oral βLs Rash	Parent. Pens. Rash	Parent. Pens. GI	All Cephs. All ARs	Oral Cephs. All ARs
HRTDD % Xr	-	0.21	-	0.22	0.39	0.15	0.20
3mM %Xr	0.16	0.49	0.42	-	0.23	0.30	0.17

Table 10.6 Summation of Regression Statistics, Plots and Cross Validation Results

Data Set	Biological Response	n	# params	# outliers	r	r^2	s	plot 1	plot 2	r $(CV)^2$
All β-lactams	3mM XR (∫)	31	4	0	0.805	0.649	1.67	sk	homo	0.418
	eHRTDD XR (∫)		4	0	0.889	0.790	1.22	lin	homo	0.688
Oral β-lactams	3mM XR (∫)	9	2	2	0.935	0.874	1.08	sk	homo	0.165
	eHRTDD XR (∫)		2	0	0.892	0.795	1.36	sk	homo	0.365
Parenteral β-lactams	3mM XR (∫)	22	2	1	0.791	0.626	1.80	sk	homo	0.399
	eHRTDD XR (∫)		2	1	0.814	0.662	1.57	lin	homo	0.618
All Penicillins	3mM XR (∫)	14	2	3	0.904	0.818	0.98	lin	homo	0.742
	eHRTDD XR (∫)		2	0	0.930	0.866	1.08	lin	homo	0.716
Oral Penicillins	3mM XR (∫)	6	1	2	0.924	0.853	0.68	sk	homo	-0.568
	eHRTDD XR (∫)		1	0	0.933	0.870	0.95	lin	homo	0.755
Parenteral Penicillins	3mM XR (∫)	8	2	1	0.954	0.911	0.77	lin	hetro	0.605
	eHRTDD XR (∫)		1	0	0.930	0.866	1.10	lin	homo	0.700
All Cephs	3mM XR (∫)	17	2	1	0.818	0.670	0.97	lin	homo	0.557
	eHRTDD XR (∫)		2	3	0.927	0.860	0.68	lin	homo	0.819
Oral Cephs.	3mM XR (∫)	3	1	0	0.823	0.677	1.02	sk	homo	0.285
	eHRTDD XR (∫)		1	0	0.945	0.893	1.00	sk	hetro	-1.041
Parenteral Cephs.	3mM XR (∫)	14	2	1	0.870	0.756	0.76	lin	homo	0.707
	eHRTDD XR (∫)		2	1	0.921	0.848	0.62	lin	hetro	0.639

Key:

sk - data are skewed about the line of best fit, so the error distribution is not normal.

lin - data are linear, so the distribution of error is normal.

hetro - data are heteroskedastic about the zero line, so there is a large scope for variation and the model is not a good fit with the data.

homo - data are homoskedastic about the zero line, there is even distribution of variation and the model is a good fit with the data used.

Table 10.7 Summary of the Descriptor Variables Used in the QSARs Generated

Biological Response	Parameters used in the QSAR models for each respective data set								
	All β-lactams	All Oral β-lactams	All Parenteral β-lactams	All Penicillins	Oral Penicillins	Parenteral Penicillins	All Cephalosporins	Oral Cephalosporins	Parenteral Cephalosporins
3mM XR (↶)	Dipole Vector X Sterimol B1c=o Accessible surface area Admin. ID	Sterimol B1c=0 Accessible surface area	Surface area Dipole vector X	Surface area Dipole vector X	Surface area	Surface area Dipole vector X	Dipole vector X Dipole vector Y	Dipole vector Y	Dipole vector X Dipole vector Y
eHRTDD XR (↶)	Surface area E_{HOMO} Sterimol L(CH3) Admin. ID	E_{HOMO} Sterimol L(CH3)	Dipole vector X Sterimol B4(C=O)	# Methyl grps. Admin. ID	# Methyl grps.	# R1 Methyl grps.	Shape Similarity to benzylpenicillin Sterimol B4(C=O)	Sterimol B4(C=O)	Sterimol B4(C=O) Dipole vector X

10.3231 QSARs of the Frequency of Cross-Reactivity of β-Lactam Antibiotics at 3mM Concentration with Anti-benzylpenicillin Serum Antibodies

10.32311 All β-Lactam Antibiotics

The square-root transformed data has a skewness value of 0.159 indicating normal distribution and that the data are suitable for modelling. Stepwise regression against seventy physico-chemical and structural descriptors (the remaining 14 descriptors applied only to the cephalosporins, so there incorporation here was not felt to be relevant) highlighted a number which correlated with the transformed cross-reactivity data. Regression analysis of the 3mM cross-reactivity data with combinations of the most significant descriptor variables, highlighted by stepwise regression, yielded the statistically significant and predictively valid model 10.1, which described 65% of the information within the biological data.

No compounds were found to be acting as an outlier.

Model 10.1

$\sqrt{XR}$ due to all β-lactams at 3mM conc.

$$= -0.223(0.151)DVx - 1.677(0.413)VB_{1c=o} - 5.208(4.574)ASA + 1.525(0.881)Admin. + 6.114(3.422)$$

$n=31$ $r=0.805$ $r^2=0.649$ $s=1.665$ $F=11.08$ probability $=1.64^{-5}$ $r(CV)^2=0.418$

Where:

DVx is the dipole moment in vector X.

$VB_{1c=o}$ is the Sterimol B_1 width parameter for the whole molecule as measured from the carbonyl oxygen of the β-lactam ring.

ASA is the surface area of the molecule accessible to a water molecule, determined in COSMIC.

Admin. ID is an indicator variable pertaining to the mode of administration of the β-lactam. A value of 3 indicates an oral compound and a value of 4 a parenteral compound.

The model (10.1) presents a negative relationship of cross-reactivity between β-lactam antibiotics and anti-benzylpenicillin antibodies with structural shape and charge separation. This suggests therefore that the phenomenon of cross-reactivity between β-lactam antibiotics and anti-benzylpenicillin serum immunoglobulins is dependent on the ability of the antibiotic to enter a shape specific receptor site on the immunoglobulins and the ability of the antibiotic, once in the receptor, to interact and bind via charge-charge interactions, thus initiating the immunological response. The model suggests that the whole structure of the β-lactam antibiotic is significant in controlling whether it cross-reacts with anti-benzylpenicillin serum antibodies, thus causing the induction of an adverse immunological response. This partially supports the work of Smith et al [589] who proposed that cross-reactivity is due to the similarity of the bicyclic core structures of the penicillins and cephalosporins, but contradicts the theories of side chain dependent penicillin-cephalosporin cross-reactivity, as proposed by Batchelor [584], Shibata [585] and Allemenos [582]. This latter theory may, however, be true when the cross-reactivities of specific types of β-lactam (penicillins or cephalosporins) are examined in isolation.

The model also indicates that cross-reactivity is dependent upon the differential mode of administration of the β-lactam antibiotic, suggesting that oral and parenteral antibiotics may be recognised by different types of immunoglobulins or possess slightly different properties which govern their ability to enter a specific receptor site and to bind, so inducing a cross-reactive immune response.

Differences between the different types of antibiotics (penicillins and cephalosporins) and between the oral and parenterally administered compounds may account for the vagueness of the descriptors and in the low level of statistical significance and predictive validity. Development of QSARs for oral or parenteral antibiotics and for penicillins and cephalosporins specifically may yield more significant models.

10.32312 All Oral β-Lactam Antibiotics

The square-root transformed data presented a skewness value of -0.374, which indicates that the data are normally distributed and so suitable for modelling. Regression analysis of the transformed cross-reactivity data against combinations of the descriptor parameters highlighted as significant for all β-lactams (10.32211) yielded the statistically significant and predictively valid model 10.2, which modelled 87% of the variance contained in the biological data.

Two antibiotics amoxycillin and epicillin were found to be behaving as outliers. Amoxycillin on the grounds of the large range of experimental results and error presented both in this study and in those of others [591]. It was felt that the results obtained were neither reliable nor accurate and that their incorporation would not be conducive to successful QSAR development. Epicillin was determined to be acting as an outlier for the reasons given previously in 9.3222.

Model 10.2

$\sqrt{}$ XR due to all oral β-lactams at 3mM conc. $= -2.599(1.413)VB_{1c=o} - 8.740(5.425)\frac{ASA}{1000} + 15.93(2.396)$

n= 7 r=0.935 r^2=0.874 s=1.077 F=13.06 probability =0.006 $r(CV)^2$=0.165

As with model 10.1 an inverse relationship between the extent of cross-reactivity and the shape of the respective oral β-lactam antibiotic is indicated, thus supporting the previous proposal that cross-reactivity is dependent on the binding of the antibiotic in a shape-specific receptor site present on one or more serum anti-benzylpenicillin immunoglobulins. Two shape parameters may be required to compensate for the differing shapes of receptor sites on different types of immunoglobulin or to compensate for dissimilarities in the receptor sites for penicillin and cephalosporin antibiotics. It appears, due to the lack of significance of a relevant descriptor in the model, that the distribution of charge within oral antibiotics is not a major factor in controlling the cross-reactivity potential of oral antibiotics.

10.32313 All Parenteral β-Lactam Antibiotics

The skewness value for the square-root transformed data is 0.645 indicating that the data are normally distributed and so suitable for modelling. Regression analysis of the biological data against combinations of the descriptor parameters highlighted as significant for all β-lactams (10.32211) and other parameters felt to be of relevance to parenteral compounds, yielded the statistically significant and predictively valid model 10.3, which modelled 63% of the information within the biological data.

Cefazolin was determined to be an outlier on the grounds that it is the only parenteral β-lactam antibiotic which has a 5-membered heterocyclic ring, and an R_1 side chain which comprises 4 nitrogen atoms. It was felt that this distribution of nitrogen atoms adversely effects the charge distribution within the X vector, thus introducing an error or bias factor into the model generation procedure.

Model 10.3

$\sqrt{}$XR due to all parenteral β-lactams at 3mM conc. = -0.187(0.052)SA - 0.493(0.232)DVx + 10.050(1.585)

n=21 r=0.791 r^2=0.626 s=1.798 F=13.41 probability =2.72^{-4} $r(CV)^2$=0.399

Where: SA is the total surface area of the antibiotic, determined in TSAR.

The descriptor variables included in model 10.3, as with model 10.1, indicate an inverse relationship between surface area and internal charge distribution of the parenteral antibiotics and cross-reactivity. This suggests that the overall size of the parenteral antibiotic may control access of the parenteral antibiotic into a benzylpenicillin shape dependent binding site on an immunoglobulin. The smaller the overall size of the parenteral antibiotic, the more readily it will be able to enter the receptor site. The charge separation within the parenteral antibiotic may act to control the extent of binding within the receptor site. The lower the charge separation, in the X vector, the greater the interaction between the antibiotic and the receptor surface and so the greater is the extent of immunological response initiated. The two properties in conjunction will govern both the possibility and the resultant extent of cross-reactive stimulation of the immunologic mechanisms and thus the degree of adverse response initiated by parenterally administered β-lactam antibiotics.

10.32314 All Penicillin Antibiotics

The square-root data have a skewness value of 0.813 indicating them to be normally distributed and so suitable for modelling. Regression of the transformed cross-reactivity data against those descriptors previously indicated to be relevant in cross-reactivity (model 10.1) yielded model 10.4, which was both statistically significant and predictively valid and which modelled 82% of the variance in the biological data.

Three compounds amoxycillin, epicillin and nafcillin were found to be behaving as outliers. Amoxycillin and epicillin for the reasons given in 10.32211. Nafcillin was determined to be an outlier as it is the only penicillin tested which contains a naphthalene ring in the R_1 side chain, the size of which may restrict access into the immunoglobulin receptor site, to a larger degree than a measurement of surface area would predict.

Model 10.4

$\sqrt{}$XR due to all penicillins at 3mM conc.

$$= -3.927(0.702)\frac{SA}{100} - 8.15(1.00)\frac{DVx}{10} + 16.84(2.52)$$

n=14 r=0.904 r^2=0.818 s=0.979 F=17.99 probability =4.87^{-4} $r(CV)^2$=0.742

Model 10.4, as model 10.1, indicates that an inverse relationship exists between the extent of cross-reactivity exhibited with anti-benzylpenicillin serum antibodies and the overall size and vectorial charge separation of the penicillin antibiotics. This supports the proposed hypothesis (10.32211) that cross-reactivity results from the binding of the antibiotic within a shape-specific receptor present on the anti-benzylpenicillin serum immunoglobulins, in that the smaller the size and X-vectorial charge separation of the penicillin antibiotic the more readily it can enter and be held in the receptor site and thus initiate the immunological responses associated with allergy (as detailed in 7.23).

10.32315 Oral Penicillin Antibiotics

The square-root data are normally distributed, with a skewness value of 0.862 and thus are suitable for modelling.

Amoxycillin and epicillin are both oral penicillins and were removed from the data set prior to regression analysis as outliers for the reasons as given in 10.32211.

Regression analysis of the cross-reactivity data with respect to the descriptors highlighted in 10.32214 yielded the statistically significant model 10.5, which modelled 85% of the information contained within the biological data. The model, however, lacks predictive validity as a result of the small number of compounds in the model generation set and the resultant bias towards fitting with the descriptors. This is also reflected in the fact that the probability value is relatively high. It may be therefore that this model, although presenting descriptors as highlighted previously, is due to chance correlation and so is not a valid QSAR. However, the presence in the model of the descriptor pertaining to surface area, as highlighted in other related models (models 10.2 and 10.4) and of the same sign (negative) and the lack of a charge descriptor (as with model 10.2), suggests that this model is significant in modelling the cross-reactivity of oral penicillins and that the model therefore was not derived by chance. The model supports the theory, as in 10.32212, that oral antibiotics interact via non-charge dependent interactions in order to initiate the immunological response.

The incorporation of cross-reactivity data for further oral penicillins and the regeneration of the model will indicate whether model 10.5 is due to chance or merely lacks predictive validity because of the low numbers of compounds used.

Model 10.5

$\sqrt{XR}$ due to oral penicillins at 3mM conc. $= -0.81(2.658)\frac{SA}{100} + 16.15(9.336)$

n=4 r=0.924 r^2=0.853 s=0.676 F=11.64 probability =0.042 $r(CV)^2$= -0.568

10.32316 Parenteral Penicillin Antibiotics

The square-root data for the parenteral penicillins are normally distributed (Sk= 0.431) and as such are suitable for modelling.

Nafcillin was eliminated from the data set as an outlier for the reasons given previously in 10.32214.

Regression analysis was performed using those descriptors highlighted in 10.32214 and yielded model 10.6, which was both statistically significant and predictively valid and which modelled 91% of the variance within the biological data.

Model 10.6

$\sqrt{}$ XR due to parenteral penicillins at 3mM conc. $= -0.408(0.139)\frac{SA}{10} - 0.833(0.225)DVx + 17.69(4.82)$

n=7 r=0.954 r^2=0.911 s=0.766 F=20.46 probability =0.002 $r(CV)^2$=0.605

As with models 10.3 and 10.4 this model proposes that cross-reactivity due to parenteral penicillin antibiotics is inversely related both to the surface area and the X-vectorial charge separation of the compound. This is consistent with the previously proposed receptor binding hypothesis (10.232211).

10.32317 All Cephalosporin Antibiotics

The square-root data for all the cephalosporins are normally distributed (Sk=0.166) and as such are suitable for modelling. Regression analysis of the data against those descriptors highlighted in 10.3211, yielded the statistically significant and predictively valid model 10.7, which described 67% of the variance contained within the data.

Ceftriaxone was found to be an outlier because with respect to the other cephalosporins tested it has a high proportion of nitrogen atoms and carbonyl oxygens, which it was felt, adversely influence the charge distribution within the molecule, thus disrupting the ability to fit the biological data and the relevant parameters accurately.

Model 10.7

$\sqrt{}$XR due to all cephalosporins at 3mM conc. = -0.196(0.065)DVx + 0.512(0.088)DVy + 0.556(0.145)

n=16 r=0.818 r^2=0.670 s=0.974 F=11.16 probability =0.002 $r(CV)^2$=0.557

Where: DVy is the dipole moment in vector Y.

The model proposes that, unlike the penicillin antibiotics (models 10.4,10.5 and 10.6), the structural size / shape of the cephalosporin antibiotics is not a major factor influencing cross-reactivity with anti-benzylpenicillin serum antibodies, but that it is the charge separation within the molecules which controls the extent of cross-reactivity, as indicated in model 10.1. There is an inverse relationship between cross-reactivity and the X-vectorial charge separation, as seen in models 10.1, 10.3, 10.4 and 10.6, and a positive relationship with the Y-vectorial charge separation.

The cephalosporins, by virtue of the presence of an R_2 side chain, are significantly larger than the penicillins and therefore it may not be possible for the whole cephalosporin molecule to enter the anti-benzylpenicillin immunoglobulin receptor sites. Therefore cross-reactivity may be brought about by the attraction of the cephalosporin to a region on the immunoglobulin adjacent to the receptor pocket and the holding of the cephalosporin there via charge-charge interactions, the extent of binding being governed by the Y-vectorial charge separation in the molecule; this may possibly relate to the charge distribution in the R_2 side chain. It was not possible to ascertain, from the software used, which region of the molecule constituted the specific dipole vectors. One can only assume that they are comparable regions for all the antibiotics and that they control interactions with or near to the immunoglobulin receptor sites. These interactions may then enable the remaining regions of the cephalosporin (the side chain [34,582,583,584,585,588] and / or the bicyclic core [589]) partially to enter the receptor pocket and initiate the immunological reaction mechanisms via X-vectorially controlled charge- charge interactions , as proposed by models 10.1 and 10.4.

Should the cephalosporin bind in an incompatible orientation, entry of the R_1 side chain or nuclear core into the receptor pocket will not be possible, thus no cross-reactive response will be initiated. Alternatively if the relevant region of the cephalosporin involved in the X-vectorial charge separation is sufficiently distant from the receptor surface the response will be weak if at all. This hit and miss receptor site activation may account for the lower levels of cross-reactivity seen for the cephalosporins with respect to those of the penicillins (as given in table 10.1), which are on the whole capable of complete entry into the receptor pocket.

10.32318 Oral Cephalosporin Antibiotics

The square-root data have a skewnes of 0.385, which suggests normal distribution and thus that the data are suitable for modelling.

As only three oral cephalosporins were used in the model generation it was felt that no compound could be justified as being an outlier.

Regression analysis using those descriptor variables proposed as significant in 10.32217 resulted in model 10.8, which effectively modelled 68% of the biological variance and was statistically significant. However as with 10.32215 (model 10.5) the model lacked predictive validity as a result of the small number of compounds used in its generation.

Model 10.8

$\sqrt{}$ XR due to oral cephalosporins at 3mM conc. = 1.233(1.754)DVy - 1.236(4.679)

n=3 r=0.823 r^2=0.677 s=1.023 F=2.096 probability =0.285 $r(CV)^2$= -1.363

The model suggests that the extent of oral cephalosporin cross-reactivity with anti-benzylpenicillin antibodies is directly related to the Y-vectorial charge separation, as discussed for model 10.7. The model, as with those other models derived specifically for oral compounds (models 10.2 and 10.5), indicates that the X-vectorial charge separation plays no part in the initiation of immunological responses.

10.32319 Parenteral Cephalosporin Antibiotics

The skewness value of the square-root data is -0.143, indicating normal distribution and therefore that the data are suitable for modelling.

Ceftriaxone was removed from the data set for the reasons as given in 10.32217.

Regression analysis of the transformed biological data those descriptors highlighted in 10.32217, yielded model 10.9, which was both statistically significant and predictively valid and which modelled 76% of the information in the biological data.

Model 10.9

$\sqrt{}$XR due to all cephalosporins at 3mM conc. = -0.187(0.022)DVx + 0.428(0.065)DVy + 0.887(0.166)

n=14 r=0.870 r^2=0.756 s=0.755 F=12.40 probability =0.002 $r(CV)^2$=0.707

The model indicates that the cross-reactivity of parenteral cephalosporins with anti-benzylpenicillin serum antibodies is governed by the charge separation within the molecule, as with model 10.7.

10.3232 QSARs of Cross-Reactivity Frequencies of β-Lactam Antibiotics at Concentrations Equivalent to Their Highest Recommended Therapeutic Daily Dose (eHRTDD) with Anti-benzylpenicillin Serum Antibodies

10.32321 All β-Lactam Antibiotics

The square root transformation of the biological data presented a skewness value of 0.400, showing them to be normally distributed and so suitable for modelling. Stepwise regression against seventy chosen descriptor variables indicated that a numbers of parameters were related to the biological data. Regression analysis against combinations of the six primary descriptors, highlighted by stepwise regression, produced the statistically significant and predictively valid regression model 10.10, which modelled 79% of the variance in the biological data.
No compounds were found to be acting as outliers.

Model 10.10

$\sqrt{}$ XR due to All β-lactam antibiotics at eHRTDD

$$= -1.963(0.496)\frac{SA}{100} - 5.466(0.647)E_{HOMO} + 5.194(0.398)VL_{(CH3)} - 53.60(6.499)$$

n=31 r=0.889 r^2=0.790 s=1.215 F=23.56 probability =9.339^{-9} $r(CV)^2$=0.688

Where: E_{HOMO} is the HOMO energy of the molecule.
$VL_{(CH3)}$ is the Sterimol length measurement of the whole molecule, as measured from a hydrogen of the first methyl group of the thiazolidine or dihydrothiazoline ring common to all the antibiotics.

As with model 10.1, the model indicates that cross-reactivity of all β-lactam antibiotics is inversely proportional to the size and charge of the respective antibiotic. As with the model for 3mM concentration (model 10.1) the surface area of the antibiotic is significant; however, the vectorial charge separation and the Sterimol B_3 width (measured from the carbonyl oxygen of the β-lactam ring) parameters of model 10.1 have been replaced in this model by E_{HOMO} and the Sterimol length of the molecule (measured from the a methyl group hydrogen common to the bicyclic cores of penicillins and cephalosporins). Although different descriptors are highlighted in the model they essentially indicate the same properties to be of importance in cross-reactivity, as those in model 10.1, and thus support the proposed theory of receptor binding. The reasoning for the different descriptors being indicated probably lies in the fact that the relative concentrations of the antibiotics at their equivalent highest therapeutic daily dose vary considerably from that of 3mM (as shown in appendix 6), and thus generating a model using the same descriptors is unlikely. The fact that the descriptors highlighted are equivalent gives great significance to models 10.1 and 10.10.

10.32322 All Oral β-Lactam Antibiotics

The square-root transformed biological data have a skewness value of -0.127, thus indicating that they are normally distributed and therefore suitable for regression analysis. No compound was found to be acting as an outlier and so all nine compounds in the data set were used in the model generation process. Regression analysis with combinations of those parameters highlighted in 10.32221 (model 10.10), yielded model 10.11 which was both statistically significant and predictively valid and which modelled 80% of the variance in the biological data.

Model 10.11

$\sqrt{}$ XR due to oral β-lactam antibiotics at eHRTDD $= - 0.550(0.221)(E_{HOMO} \times 10) + 0.504(0.051)VL_{(CH3)} - 50.99(19.90)$

n=9 r=0.892 r^2=0.795 s=1.364 F=11.64 probability =0.004 $r(CV)^2$=0.365

The model indicates, as with previous models 10.1 and 10.10, that cross-reactivity potential is inversely related to the energy of the molecule. However, previously for oral antibiotics (models 10.2, 10.5 and 10.9), energy has not been highlighted as significant, and it has been an inverse relationship to shape which has been proposed as the controlling property. In this model the shape property is still evident but in this case the relationship is positive, i.e. the longer the molecule the greater the cross-reactivity potential. The change of relationship (negative to positive) is an unexpected manifestation, but could possibly be attributed to the fact that the Sterimol length measured in this parameter reflects the ease with which the functional group of the R_1 side chain can enter the receptor pocket without hindrance from the bicyclic core. This would therefore, support the R_1 side chain dependence theories, proposed by Batchelor [584], Shibata [585] and Allemenos [582], which previous models (10.1) seemed to contradict.

Previous energy descriptors have related specifically to charge interactions, but HOMO is a more general energy term and may relate to the ease with which the antibiotic may interact at the receptor surface via electron donation, and so although not previously highlighted the parameter still fits in with the proposed receptor binding hypothesis put forward in 10.3221.

10.32323 All Parenteral β-Lactam Antibiotics

The skewness value of the square root transformed data was 0.802, indicating them to be normally distributed and so suitable for regression analysis. Nafcillin was determined to be behaving as an outlier for the reasons as detailed in 10.32214. Regression analysis was performed using combinations of those descriptor variables highlighted as being relevant in 10.32221 (model 10.10) and those descriptors previously highlighted as being relevant to parenteral antibiotics. Model 10.12, which was statistically significant and predictively valid, describing 66% of the biological variance, was produced.

Model 10.12

$\sqrt{}$XR due to parenteral β-lactam antibiotics at eHRTDD $= - 0.599(0.050)DVx + 0.977(0.122)VB_{4c=o} + 13.55(1.089)$

$n=21$ $r=0.814$ $r^2=0.662$ $s=1.566$ $F=14.71$ probability $=1.96^{-4}$ $r(CV)^2=0.618$

Where: $VB_{4c=o}$ is a measure of the B_4 width of the molecule, as determined from the carbonyl oxygen of the β-lactam ring.

The model indicates, as did models 10.3 and 10.10, that the properties of size / shape and charge distribution within the molecule, are important in controlling the cross-reactivity potential of a parenteral antibiotic, as discussed in 10.3221. The Sterimol B4 width (as measured from the carbonyl oxygen of the β-lactam ring) may be equivalent to the Sterimol length (L) of model 10.11, in that it measures the distance of the R_1 functional group from the bicyclic nucleus of the parenteral antibiotic, and both are considered to model shape. Thus, it reflects the ease of entry of the parenteral antibiotic into the receptor site, so supporting the proposed R_1 dependence theories of cross-reactivity [582,584,585]. The model indicates, as have previous models (10.3, 10.6, 10.9), that the charge distribution in the parenteral molecules' X vector has a significant, negative relationship with cross-reactivity.

10.32324 All Penicillin Antibiotics

The square root transformation of the biological data gave a skewness value of 0.038, indicating them to be normally distributed and so suitable for modelling. Regression analysis against those descriptors previously highlighted to be significant in cross-reactivity (10.3221 and 10.3222), yielded the statistically significant and predictively valid model 10.13, which described 87% of the variance in the data. No compound was found to be acting as outlier.

Model 10.13

$\sqrt{}$ XR due to all penicillin antibiotics at eHRTDD = -3.140(0.346)#CH_3 + 2.780(0.980)Admin. + 3.818(3.743)

n=14 r=0.930 r^2=0.866 s=1.079 F=35.49 probability =5.40^{-6} $r(CV)^2$=0.716

Where: #CH_3 is the number of methyl groups within the whole molecule.
Admin. is an indicator variable pertaining to the mode of administration of the penicillin, where 3 indicates oral and 4 parenteral administration.

The model proposes that for the penicillin antibiotics there is a negative relationship between the cross-reactivity potential of a compound and the number of methyl groups in its structure, i.e. the more methyl groups the lower the cross-reactivity. The number of methyl groups may reflect two general properties of the penicillins; the hydrophobicity and / or the extent of branching and thus the size, shape or flexibility. (It was not possible to yield any valid relationships through the correlation of #CH_3 groups with either shape or hydrophobicity parameters alone). If the latter property is the significant property being modelled, this would support the theories of cross-reactivity being dependent on the nature of the R_1 side chain, because any variation in the number of methyl groups within a penicillin antibiotic occurs in the R_1 side chain, whilst other parts of the structure do not normally alter. Thus any increase in the number of methyl groups may act to alter the shape of the substituent so making it unable to enter the immunoglobulin receptor site, or may act to shield the reactive centres of the substituent, so restricting binding and initiation of the immunological response. The model also proposes, as in 10.32211, that the mode of administration of the penicillin (oral or parenteral) influences the cross-reactivity potential. This suggests that oral and parenteral penicillins may cross-react with anti-benzylpenicillin antibodies via slightly different mechanisms, which are controlled by slightly different molecular properties.

10.32325 Oral Penicillin Antibiotics

Univariate statistical analysis showed the square root transformed data to have a skewness value of -0.359, indicating that they are normally distributed and suitable for statistical modelling. Regression analysis against the primary descriptor signified in model 10.13, yielded model 10.14, which was both statistically significant and predictively valid, modelling some 87% of the variance.

No compound was found to be acting as an outlier.

Model 10.14

$\sqrt{}$ XR due to oral penicillins at eHRTDD $= -2.614(0.518)\#CH_3 - 10.85(1.165)$

n=6 r=0.933 r^2=0.870 s=0.947 F=26.67 probability =0.004 $r(CV)^2$=0.755

The model indicates, as with model 10.13, that there is a negative relationship between the number of methyl groups in the oral penicillin and its cross-reactivity potential. If, as discussed with model 10.13, this is a reflection of the molecular shape of the oral penicillin then this model indicates that similar properties to those the equivalent 3mM model (10.5) are controlling the biological response.

9.32326 Parenteral Penicillin Antibiotics

The skewness value of 0.342 attained for the parenteral penicillin square-root transformed data showed them to be normally distributed and so suitable for regression analysis. Regression analysis of the transformed cross-reactivity data against combinations of the descriptor variable highlighted in model 10.13, produced the statistically significant and predictively valid model 10.15, which modelled 87% of the information in the biological data.

No compound was found to be acting as an outlier.

Model 10.15

$\sqrt{}$ XR due to parenteral penicillins at eHRTDD $= -3.666(0.891)\#R_1CH_3 + 9.059(0.299)$

$n=8$ $r=0.930$ $r^2=0.866$ $s=1.102$ $F=38.70$ probability $=4.362^{-4}$ $r(CV)^2=0.700$

Where: $\#R_1CH_3$ is equivalent to the number of methyl groups within the R_1 substituent of the penicillin.

The model indicates a negative relationship between the number of methyl groups in the R_1 side chain of the parenteral penicillins and their cross-reactivity potential. The more methyl groups present, the higher is the degree of branching and the possibility of functional group shielding, so the lower is the possibility of the parenteral antibiotic interacting at the receptor surface. This model adds further support to the proposed theories of cross-reactivity being dependent on the nature of the R_1 side chain [34,583,588].

10.32327 All Cephalosporin Antibiotics

The square root transformed data are normally distributed, having a skewness value of - 0.533, and so are suitable for statistical modelling. Regression analysis against combinations of those descriptors indicated in 10.32221 as being significant generated the statistically significant and predictively valid regression model 10.16, which modelled 86% of the variance in the data.

Three cephalosporins within the data set were found to be outliers. Ceftriaxone for the reasons detailed in 10.32217. Cephalexin and cefamandole were classified as outliers because of the cephalosporins experimentally tested, their respective HRTDDs far exceed those of the other cephalosporins (detailed in appendix 5). It was felt therefore their incorporation would introduce a high bias into the data and so the results obtained could be distorted.

Model 10.16

$\sqrt{}$ XR due to all cephalosporins at eHRTDD $= 31.34(2.106)SSBp + 9.96(0.76)VB_{4c=o} - 34.11(2.042)$

$n=14$ $r=0.927$ $r^2=0.860$ $s=0.675$ $F=30.75$ probability $=1.89^{-8}$ $r(CV)^2=0.819$

Where: SSBp is a measure of the shape similarity of the antibiotic to the sensitising antibiotic benzylpenicillin.

The model indicates that the cross-reactivity potential is directly related to the shape of the cephalosporin antibiotics, as proposed previously (models 10.1 and 10.10). This, however, contradicts the equivalent 3mM model 10.7, which indicates that cephalosporin cross-reactivity is dependent on charge separation within the molecule. This model proposes that the shape similarity of the cephalosporins to benzylpenicillin is significant. This property is biologically significant as the receptor-bearing immunoglobulins were raised against benzylpenicillin and so it could be assumed that if a cephalosporin were to fit the receptor it should bear some similarity to benzylpenicillin. In the case of the penicillin antibiotics, which all closely resemble each other in shape, fitting into the receptor poses little difficulty; it is interaction at the receptor surface which is the limiting factor. As in this model for the cephalosporins, an additional shape parameter (Sterimol B_4 width) is required to describe the ability to interact at the relevant receptor surface. If, as previously discussed, this B_4 parameter relates to the size and / or shape of the R_1 side chain then this model too supports the theories of R_1 dependence of cross-reactivity.

10.32328 Oral Cephalosporin Antibiotics

The skewness value obtained (0.385) showed the square-root transformed data to be normally distributed, and hence suitable for statistical analysis, but as only three compounds were present in the data set this was expected. Regression analysis against those physico-chemical parameters previously suggested (model 10.16) as being relevant, enabled the generation of the statistically significant model 10.17, which described 89% of the biological variance. As with model 10.8 the model lacks predictive validity because of the small number of compounds used in its generation.

No compound could be justified as being an outlier.

Model 10.17

$\sqrt{}$XR due to oral cephalosporins at eHRTDD $= + 1.86(1.53)VB_{4C=O} - 12.40(10.82)$

n=3 r=0.945 r^2=0.893 s=1.004 F=8.35 probability =0.102 $r(CV)^2$= -1.041

The model indicates, as with the previous model of oral specificity (model 10.11), that cross-reactivity is related only to the size / shape of the oral antibiotic, and this instance to the Sterimol B_4 width of the cephalosporin, as discussed previously (models 10.12, 10.16).

10.32329 Parenteral Cephalosporin Antibiotics

A skewness value of -0.644 suggests a normal distribution and indicates that the square-root transformed data are suitable for statistical modelling. Regression analysis produced model 10.18 which was statistical significant and predictively valid and which described 85% of the biological variance contained within the data. Latamoxef was found to be acting as an outlier; the reason for this was believed to be that this compound in the only parenteral cephalosporin in which the dihydrothiazoline ring sulphur atom has been substituted with an oxygen, a substitution known to affect the structure-activity properties of the cephalosporins, as detailed previously in 2.4.

Model 10.18

$\sqrt{}$XR due to parenteral cephalosporins at eHRTDD $= - 0.224(0.097)DVx + 3.190(0.142)VB_{4c=o} - 9.917(1.432)$

$n=13$ $r=0.921$ $r^2=0.848$ $s=0.616$ $F=25.03$ probability $=2.36^{-4}$ $r(CV)^2=0.639$

The model indicates, as have previous parenteral models (models 10.3,10.6,10.12) that cross-reactivity due to parenteral antibiotics is controlled both by a positive relationship with the shape of the compounds and by a negative relationship with the charge separation in the X vector of the molecule. The biological significance of parameters indicated VB_4 and DVx have been discussed previously with regard to model 10.12.

10.333 Data Randomisation

The biological data with respect to each of the 18 models generated were randomised 15 times and the regression analysis with the specific significant descriptors repeated. Examination of the resultant regression coefficients, table 10.8, showed that for each randomised model the value of r were significantly less than that obtained for the original model. This indicates that the original models were not generated by chance, but reflect the property responsible for initiating and controlling cross-reactivity between the specific class of β-lactam antibiotic and anti-benzylpenicillin serum antibodies.

Table 10.7 Regression Coefficient Values Obtained from Randomised Data Analysis

Model	r value														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10.1	0.16	0.01	0.00	0.06	0.00	0.06	0.00	0.00	0.07	0.02	0.05	0.03	0.06	0.02	0.06
10.2	0.02	0.01	0.16	0.00	0.00	0.01	0.16	0.00	0.00	0.17	0.01	0.05	0.06	0.12	0.09
10.3	0.05	0.08	0.10	0.15	0.15	0.02	0.00	0.00	0.00	0.01	0.15	0.03	0.12	0.00	0.03
10.4	0.09	0.21	0.00	0.21	0.03	0.01	0.18	0.01	0.03	0.01	0.16	0.13	0.09	0.02	0.00
10.5	0.10	0.00	0.03	0.07	0.01	0.01	0.00	0.00	0.06	0.00	0.14	0.08	0.01	0.10	0.04
10.6	0.02	0.17	0.10	0.01	0.01	0.06	0.01	0.02	0.08	0.02	0.20	0.12	0.02	0.01	0.10
10.7	0.00	0.08	0.02	0.03	0.09	0.00	0.01	0.00	0.01	0.21	0.18	0.14	0.00	0.13	0.01
10.8	0.00	0.00	0.00	0.00	0.00	0.13	0.08	0.18	0.00	0.18	0.02	0.12	0.10	0.17	0.00
10.9	0.06	0.11	0.00	0.01	0.02	0.09	0.07	0.14	0.07	0.01	0.11	0.02	0.06	0.00	0.00
10.10	0.14	0.12	0.03	0.12	0.00	0.00	0.05	0.19	0.05	0.16	0.02	0.03	0.02	0.00	0.00
10.11	0.10	0.00	0.00	0.00	0.00	0.16	0.01	0.16	0.06	0.14	0.07	0.04	0.09	0.06	0.02
10.12	0.03	0.01	0.10	0.13	0.04	0.02	0.00	0.03	0.12	0.00	0.00	0.08	0.01	0.05	0.12
10.13	0.14	0.00	0.09	0.02	0.13	0.00	0.11	0.20	0.00	0.06	0.08	0.09	0.13	0.03	0.02
10.14	0.10	0.00	0.11	0.00	0.00	0.03	0.00	0.00	0.09	0.00	0.06	0.00	0.06	0.12	0.04
10.15	0.14	0.01	0.00	0.00	0.14	0.06	0.11	0.00	0.00	0.00	0.13	0.02	0.01	0.00	0.00
10.16	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.09	0.04	0.01	0.00	0.00	0.03	0.00	0.01
10.17	0.18	0.00	0.00	0.00	0.01	0.00	0.14	0.02	0.04	0.00	0.00	0.00	0.00	0.03	0.11
10.18	0.00	0.00	0.02	0.05	0.16	0.07	0.05	0.06	0.09	0.10	0.04	0.02	0.01	0.06	0.13

10.4 QSAR Prediction of the Extent of Cross-Reactivity Occurring Between Untested β-Lactam Antibiotics and Anti-benzylpenicillin Serum Antibodies

10.41 Methods

Due to certain compounds not being commercially available not all of the β-lactam antibiotics previously examined in this study (9.0) were assessed experimentally, using the developed ELISA, for the extent with which they cross-reacted with anti-benzylpenicillin serum antibodies. The necessary physico-chemical and structural descriptors were calculated for these untested compounds to enable the developed QSARs (10.3) to be used to predict each compounds cross-reactivity potential, both at a 3mM concentration and at a concentration eHRTDD.

10.42 Results and Discussion

The structures of the compounds assessed are given in appendix 1. The relevant descriptor variable values generated and the predicted cross-reactivity potentials, as evaluated using the relevant QSARs for each of the penicillins are given in 10.421 and for each of the cephalosporins in 10.422.

In those cases where the mode of administration of the antibiotic was not known, several models were used to evaluate the several frequency values for the same adverse reaction type.

The predicted values, 10.421 and 10.422 show that the HRTDD models, particularly those pertaining to the penicillins, used descriptor variables that are integers, so the predicted cross-reactivity values are only semi-quantitative (high, medium or low), whereas the 3mM predicted values span the entire range of values set by the experimental determinations. Therefore, the 3mM models enable more accurate predictions of cross-reactivity potential to be made. The eHRTDD models do have their use in that they yield descriptors which can be related to the biological situation, and which support previous theories of cross-reactivity.

Although the cross-reactivities predicted in tables 10.421 and 10.422 cannot be verified at present, they nevertheless serve as a guide to the likelihood of cross-reactivity occurring.

10.421 Prediction of the Extent of Cross-Reactivity Occurring Between Untested Penicillin Antibiotics and Anti-benzylpenicillin Serum Antibodies

Penicillin	Descriptor values				XR at 3mM		XR at eHRTDD	
	SA	#CH_3	DVz	DVx	$\sqrt{}$	%	$\sqrt{}$	%
Orals								
Amdinocillin-pivoxil	308.4	5	--	--	5.98	35.76	-2.22	4.93
Azidocillin	296.9	2	--	--	6.30	39.66	5.62	31.58
Cardinacillin	406.2	2	--	--	2.67	7.12	5.62	31.58
Carfecillin	368.0	2	--	--	3.94	14.33	5.62	31.58
Cyclacillin	300.1	2	--	--	6.19	38.31	5.62	31.58
Lenampicillin	365.2	3	--	--	4.03	16.24	3.01	9.05
Penicillin-V	308.3	2	--	--	5.92	35.03	5.62	31.58
Phenbenicillin	321.6	3	--	--	5.48	29.99	3.01	9.05
Pivampicillin	409.3	5	--	--	2.57	6.59	-2.22	4.93
Talampicillin	382.5	2	--	--	3.46	11.95	5.62	31.58
Parenterals		in R_1						
Apalcillin	409.7	0	-3.36	--	3.77	14.22	9.06	82.07
Flucloxacillin	357.4	1	-2.25	--	6.19	38.33	5.39	29.08
Mezlocillin	421.1	1	1.12	--	-1.44	2.07	5.39	29.08
Sulbenicillin	340.2	0	2.81	--	1.47	2.16	9.06	82.07
Temocillin	397.4	0	0.82	--	0.80	0.64	9.06	82.07
Admin. Unknown								
BLP-1654 all pens.	351.3	2	1.54	5.86	orl.- 0.94 parent.- -1.73	0.87 3.00	5.88 8.66	34.55 74.96
-oral pen.		2		--	4.49	20.16	5.62	31.61
parenteral pen.		0		--	2.08	4.33	9.06	82.07

10.422 Prediction of the Extent of Cross-Reactivity Occurring Between Untested Cephalosporin Antibiotics and Anti-benzylpenicillin Antibodies

Cephalosporin	Descriptor values			XR at 3mM		XR at eHRTDD	
	DVy	$VB_{4c=o}$	DVy	√	%	√	%
Orals							
Cefaloglycine	-0.04	9.01	--	1.28	1.65	4.38	19.18
Cefatrizine	-0.27	6.31	--	-1.57	2.47	-0.64	0.41
Cefixime	3.33	10.20	--	2.87	8.24	6.60	43.56
Cefmenoxime	-0.56	9.93	--	-1.93	3.73	6.10	37.21
Cefpiramide	-1.41	13.33	--	-2.98	8.88	12.43	100
Cepodoxime-proxetil	6.29	9.71	--	6.52	42.51	5.69	32.38
Cefprozil	4.84	10.03	--	4.73	22.37	6.29	39.56
Cefroxadine	2.30	6.28	--	1.60	2.56	-0.70	0.49
Cefteram	0.22	9.75	--	-0.97	0.94	5.77	33.29
Ceftibuten	1.59	9.96	--	0.72	0.52	6.16	37.95
Cefuroxime-axetil	-0.55	9.10	--	-1.91	3.65	4.55	20.70
Cefuzonam	2.52	9.86	--	1.87	3.50	5.97	35.64
Cephamycin	-0.07	9.72	--	-1.32	1.74	5.71	32.60
T-2588	1.70	10.72	--	0.86	0.74	7.57	57.31
Parenterals							
Cefacetrile	-1.22	8.01	3.53	-0.29	0.08	-4.95	24.50
Cefepriome	2.80	9.96	7.02	0.78	0.61	-10.08	100
Cefmetazole	2.26	9.50	0.32	1.80	3.24	-5.12	26.21
Cefminox	-2.77	9.48	-3.30	0.32	0.10	-3.88	15.05
Cefodizime	4.88	10.95	4.26	2.49	6.20	-3.78	14.29
Cefonicid	-5.09	8.78	-0.84	-1.13	1.28	-7.06	49.84
Ceforanide	-2.74	6.59	1.09	-2.28	5.20	-4.27	18.23
Cefotetan	3.05	9.02	-5.73	3.27	10.69	-5.70	32.49
Cefotiam	5.51	8.58	4.82	2.35	5.52	-5.53	30.58
Ceftizoxime	2.2	9.99	3.09	1.25	1.56	-3.34	11.16
Flomoxef	4.62	8.90	0.73	2.73	7.44	-4.70	22.09

10.5 Conclusions

A highly sensitive, reproducible competitive ELISA has been developed for the quantitative determination of cross-reactivity occurring between β-lactam antibiotics and anti-benzylpenicillin serum antibodies. Use of this assay clearly shows that cross-reactivity between different β-lactams and anti-benzylpenicillin antibodies does occur *in vitro* and that different β-lactams cross-react to different extents. If such cross-reactions occur *in vivo* in pre-sensitised patients, they will initiate the immune system and may bring about hypersensitivity type reactions, the magnitude of which will be governed by the level of cross-reactivity occurring. The design of new β-lactam antibiotics will need to address and overcome this problem. The developed ELISA and subsequent results generated are concerned with cross-reactivity of the β-lactam antibiotics and polyclonal anti-benzylpenicillin antibodies in pre-sensitised rabbit serum; for this reason extrapolation of the data to the human clinical environment must be approached with caution, as the rabbit serum may contain different antibodies or antibodies of different specificity. The rabbit is currently the species of choice, in the pharmaceutical industry, for pre-clinical immunogenicity assessment, and it was for this reason, and so that different immunogenicity studies could be related that the ELISA for determination of cross-reactivity was developed with rabbit serum and not human sera. Pedersen-Bjergaard [593], demonstrated the phenomenon of cross-reactivity between cephalothin and anti-benzylpenicillin antibodies in human sera, so it should be relatively easy to modify the developed ELISA to asses human polyclonal serum anti-benzylpenicillin antibodies cross-reactivity. This modification could be achieved by substituting the ELISA components of coating antigen, anti-rabbit IgG horse radish peroxidase conjugate for equivalent human specific components. The ELISA may need re-optimising. Thus assessment of the β-lactams with benzylpenicillin pre-sensitised human sera should enable generation of data which can be extrapolated more confidently to the clinical situation and related to the incidence of allergic response following therapy, in such a way that it will be possible to determine what extent of an allergic response is due to the drug itself and what is due to cross-reactivity. Replacing the coating antigen with one specific for another antibiotic, e.g. ampicillin cytochrome-C, will enable determination of cross-reactivities occurring with other serum anti-antibodies.

The QSARs developed enable the prediction of the cross-reactivity potential of new β-lactam antibiotics to be made, and clearly show that cross-reactivity is a shape-dependent phenomenon. The models suggest that on the whole it is the nature of

the R_1 side chain which controls the extent of cross-reactivity, thus supporting the previous theories proposed by Blanca [34,583], Kishiyama [588], Allemenos [582], Batchelor [584] and Shibata [585].

Although the descriptors in each model are not identical they can each be related to the antibody-antigen reaction scenario, the immunological reaction mechanism behind cross-reactivity. The reasons for these different descriptors being selected may reflect the polyclonal nature of the serum investigated, in that the serum consists of a variety of immunoglobulin types, each of which may present a slightly different epitope (receptor site); thus, in order to model these slightly different receptors general descriptors are chosen.

Modification of the ELISA to detect specific serum monoclonal antibody cross-reactivities, and the regeneration of the QSARs, in conjunction with receptor modelling techniques, may enable identification of the specific immunoglobulin types involved in cross-reactivity and determination of the more specific antibiotic molecular properties responsible for receptor binding, and hence the initiation of a cross-reactive response.

Determination of the immunoglobulin types involved in cross-reactivity reactions of different classes of antibiotic (penicillin or cephalosporin, oral or parenteral) will enable conclusions to be drawn as to the type of allergic reaction induced (immediate or delayed hypersensitivity) by specific compounds to be made *in vitro*, a technique which will be of importance in drug design, but which currently is in its infancy.

11.0 Determination of Differential Cytokine Secretion Patterns from Sensitised Lymphocytes, Following β-Lactam Antibiotic Simulation

11.1 Introduction

The previous experimental protocols and QSARs developed in this study, 9.0 and 10.0, enable quantitative predictions to be made as to the extent to which a β-lactam antibiotic will induce an allergic reaction (cutaneous rash) directly or via cross-reactivity with anti-benzylpenicillin serum antibodies, but they do not permit distinctions as to the type of allergic response initiated (immediate or delayed hypersensitivity) by the respective β-lactam antibiotic.

There is increasing evidence that different classes of allergen, e.g. β-lactam antibiotics, provoke different immune responses which are consistent with the preferential stimulation of T_{helper} 1 and T_{helper} 2 lymphocyte sub-populations [594,595], as discussed in 6.5. Recent advances in the understanding of these T_{helper} cells and their unique cytokine secretion patterns, in conjunction with the knowledge of the function of the specific cytokines, as discussed previously in 6.6, have increased knowledge of the role cytokines play in the induction and elicitation of an allergic response and in determining the nature and severity of the response [596] and have led to the development of methods for the detection of immunologic responses induced by various chemicals.

Immediate and delayed hypersensitivity reactions can be differentiated on the basis of the cytokines secreted by the T_{helper} lymphocytes following exposure to a specific compound, e.g. an antibiotic. Th1 cells, if simulated, secrete interleukin-2 (IL2) which causes initiation of delayed hypersensitivity reactions, whereas stimulation of Th2 cells leads to the secretion of interleukin-4 (IL4) which causes the initiation of immediate hypersensitivity reactions. Roux-Lambard and Steiner [597] have stated that 'the measurement of differential cytokine levels in biological fluids may be helpful in staging and prognosis of a disease'. As far as chemically induced immunological reactions are concerned therefore, the immunotoxic potential of a chemical agent, both the type and the extent of response induced, can be predicted by the quantitation of the specific cytokine (IL2 and IL4) secretions from T-lymphocytes following exposure [598,599].

Analysis of cytokine production can be applied to at least three situations which are relevant to immunotoxicity testing; Firstly, the analysis can be conducted *in vivo* on exposed animals, and tissues such as spleen, thymus and lymph nodes can be removed and gross analysis for specific cytokines can be conducted. Secondly, analysis can be performed on *ex vivo* material; tissue and cells taken form exposed animals can be stimulated or manipulated *in vitro* to determine the effects of pre-

exposure *in vivo* on the ability of cells to generate characteristic patterns of cytokine production. Finally, analysis can be performed exclusively *in vitro*, where defined populations of cells can be pre-incubated with a test compound before stimulation and cytokine analysis [600]. Since cytokine expression is essential for the development of immune responses both *in vivo* and *in vitro*, it is possible to establish patterns of expression for *in vivo* immune activation. The effect of test chemicals on these characteristic patterns of cytokine expression can then be monitored, following *in vivo* and *in vitro* exposure.

There are commercially available immunoglobulins and substrate systems which permit the differential detection of cytokines by ELISA, thus enabling distinction between the T_{helper} clones involved and giving an indication as to the direction of the immune response activated by a specific compound. Under these conditions analysis of cytokines becomes a quantitative marker of immunotoxicity.

It is the aim of this study to modify existing ELISA systems in order to develop highly sensitive assays for the quantitation of IL2 and IL4 levels secreted by lymph node cells, and then to use these assays in conjunction with the mouse lymph node assay (mLNA) in an attempt to devise a protocol for the quantitative determination of IL2 and IL4 secretions, following β-lactam antibiotic exposure. This should allow determination of whether a specific antibiotic induces an immediate or delayed hypersensitivity reaction.

It may in the future be possible to perform QSAR analysis on the quantited cytokine secretion patterns secreted following stimulation, in order to determine if any specific physico-chemical or structural properties are responsible for controlling cytokine release and if so how do they relate to those already determined to be important in controlling the elicitaion of an adverse reaction or cross-reactivity.

Interpretation of these data in conjunction with those of allergenicity and cross-reactivity, previously generated in 9.0 and 10.0, may enable determination of a more complete immunotoxicological profile of the β-lactams, i.e. is cross-reactivity a Th1 and / or Th2 phenomenon?, which compound initiates which type immunological response (Th1 or Th2)?, what specific clinical symptoms are developed in response to the different immunological responses?, and what proportion of the immunological response, if any, is a result of cross-reactivity?.

11.2 Development of Sensitive ELISAs for the Detection of IL2 and IL4

11.21 Test Rationale

The cytokines IL2 and IL4 are differentially secreted by Th1 and Th2 sub-populations following their relative 'switching on' by a xenobiotic, and therefore quantitation of the levels of each cytokine secreted following exposure may enable prediction of the type of immune response (immediate or delayed hypersensitivity) initiated. Mitchelmore [601] and Flint [602] have developed reliable ELISAs for the quantitative detection of standard concentrations of IL2 and IL4 in solution. These assays, however, are not sensitive enough for the quantitative detection of the very low concentrations of IL2 and IL4 or of any slight changes in concentration occurring in biological fluids, such as occurs following xenobiotic challenge. Application of the ELISA optimisation techniques used previously in 10.2, to the IL2 and IL4 ELISA procedures may enable quantitative determination of theses biological cytokine levels to be made.

11.211 IL2 ELISA Standard Protocol

Wells of an Immunlon4 flat bottomed plate (Dynatech) were coated by addition of 50µl of 1µg/ml rat IGg2a anti-mouse IL2 antibody (Pharmigen 18161D clone JE56-1A12) prepared in coating buffer (0.05M carbonate bicarbonate buffer pH9.6) (Sigma C3041) and incubated in a Stuart Scientific incubator model S118 for 2 hours at 37°C. Prior to each subsequent step the wells were washed three times with PTS (Phosphate buffered saline pH7.4 (Sigma P3813) + 0.01% Tween 20 (polyoxyethylenesorbitan-monolaurate) (Sigma P1379)), using a Dynatech MRW automatic plate washer. The reactive sites within the reaction wells were then blocked by the addition of 200µl of 10mM phosphate buffered saline containing 10% bovine serum albumin at pH7.4 (Sigma P-3688), and incubated for 1.5 hours at 37°C.

The antigen-coated wells were subsequently incubated with;

- 50µl of 25ng/ml recombinant mouse IL2 in PTS buffer (Genzyme MIL-2) for 1 hour at 37°C
- 50µl of 2.5µg/ml biotinylated rat anti-mouse IL2 (Pharmingen 18172D clone JES6-5H4) for 1 hour at room temperature (RT).
- 100µl 2.5mg/ml Avidin peroxidase conjugated antibody (Vector Labs. A-2004) for 0.5 hours at RT.
- 100µl 3,3',5,5' tetramethylbenzidine liquid substrate (TMB) (Sigma T-8540) at RT until sufficient colour had developed. The reaction was stopped with 100µl 0.5M H_2SO_4. Absorbance readings were then taken at 450nm using a BioTek EL900 plate reader.

11.212 IL4 ELISA Standard Protocol

Wells of an Immunlon4 flat bottomed plate (Dynatech) were coated by addition of 50µl of 4µg/ml rat IGg2b anti-mouse IL4 antibody (Pharmigen 18031D clone BVD4-1D11) prepared in coating buffer (0.05M carbonate bicarbonate buffer pH9.6) (Sigma C3041) and incubated in a Stuart Scientific incubator model S118 for 2 hours at 37°C. Prior to each subsequent step the wells were washed three times with PTS (Phosphate buffered saline pH7.4 (Sigma P3813) + 0.01% Tween 20 (polyoxyethylenesorbitan-monolaurate) (Sigma P1379)), using a Dynatech MRW automatic plate washer. The reactive sites within the reaction wells were then blocked by the addition of 200µl of 10mM phosphate buffered saline containing 10% bovine serum albumin at pH7.4 (Sigma P-3688), and incubated for 1.5 hours at 37°C.

The antigen-coated wells were subsequently incubated with;

- 50µl of 2µg/ml recombinant mouse IL4 in PTS buffer (Pharmingen 19231W) for 1 hour at 37°C
- 50µl of 2µg/ml biotinylated rat anti-mouse IL4 (Sigma I-1390) for 1 hour at RT.
- 100µl 2.5mg/ml Avidin peroxidase conjugated antibody (Vector Labs. A-2004) for 0.5 hours at RT.
- 100µl 3,3',5,5' tetramethylbenzidine liquid substrate (TMB) (Sigma T-8540) at RT until sufficient colour had developed. The reaction was stopped with 100µl 0.5M H_2SO_4. Absorbance readings were then taken at 450nm using a BioTek EL900 plate reader.

Controls for both ELISAs were established by replacing the recombinant mouse IL2 or IL4 with PTS buffer only. Background absorbance levels were determined using equation 10.1.

The dose-response curves generated by both ELISAs are presented in figure 11.1 for the IL2 ELISA and figure 11.2 for the IL4 ELISA.

The technical data sheets for both the IL2 and IL4 ELISA antibodies are given in appendix 8.

Figure 11.1 Dose-Response Curve generated by Standard IL2 ELISA
(Adapted from Mitchelmore [601] and Flint [602])

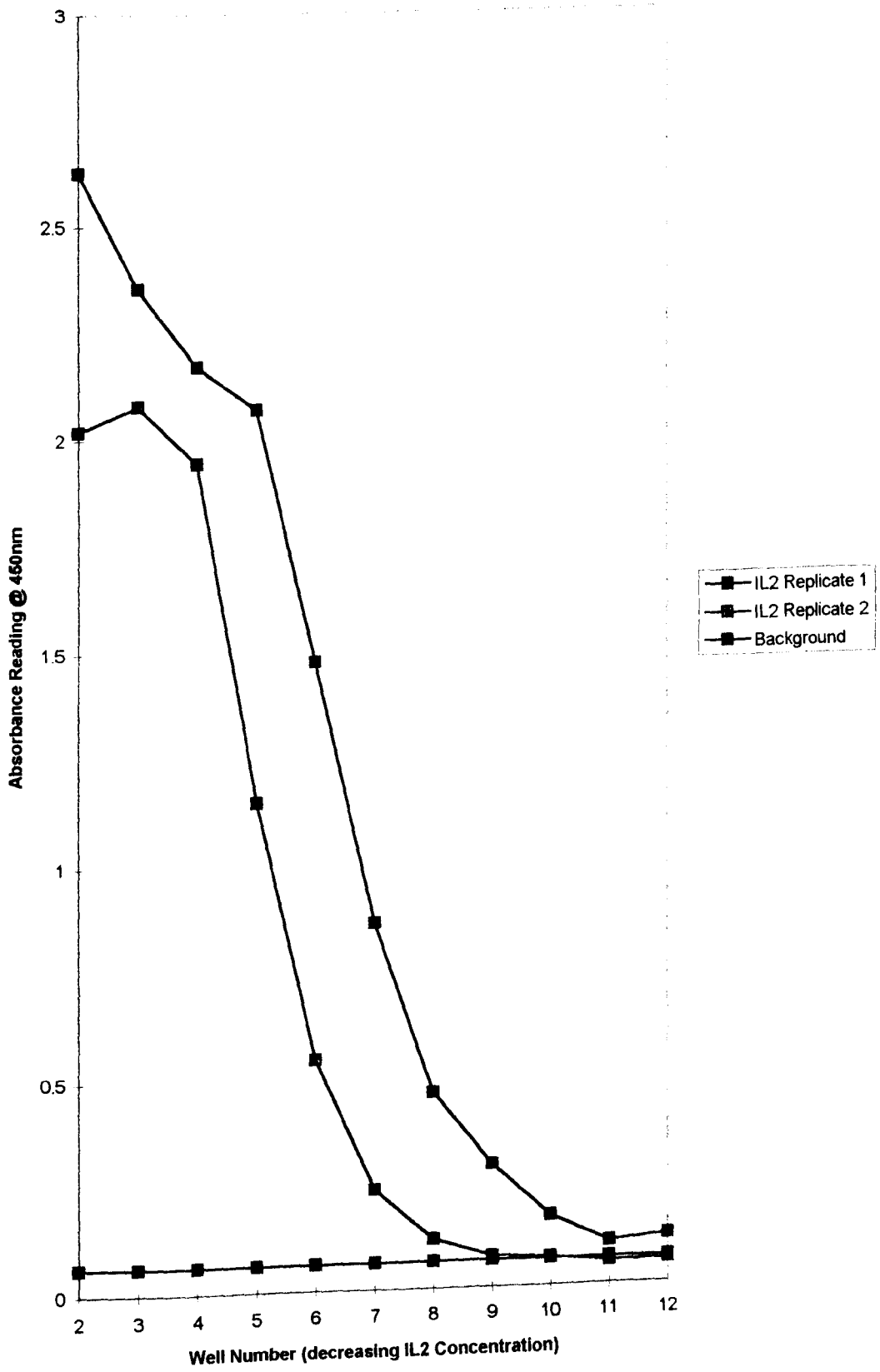
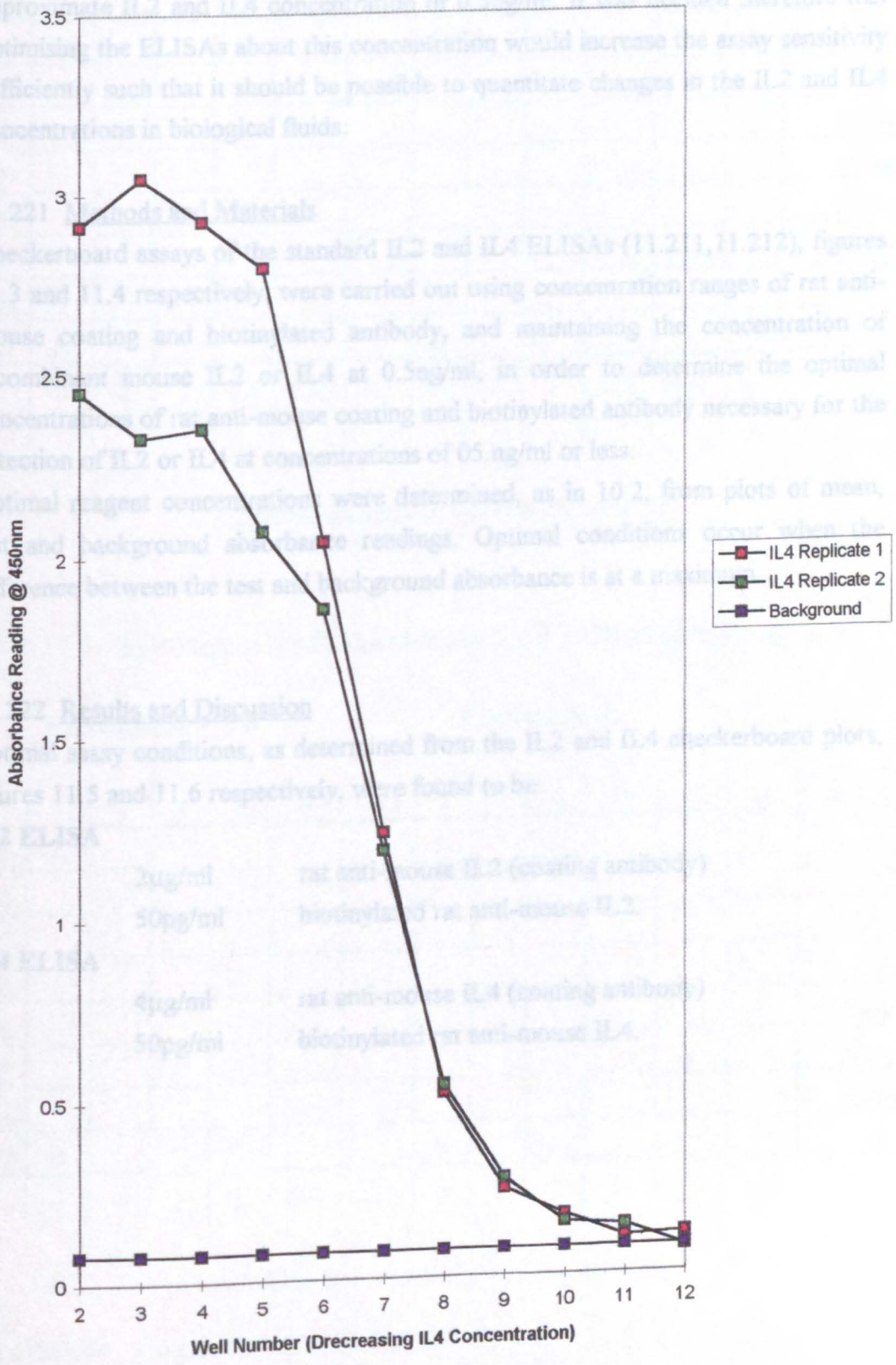


Figure 11.2 Dose-Response Curve generated by Standard IL2 ELISA
(Adapted from Mitchelmore [601] and Flint [602])



11.22 Experiment 1: Increasing the Sensitivity of the IL2 and IL4 ELISAs

Examination of the dose response curves generated by the IL2 and IL4 ELISAs, figures 11.1 and 11.2 respectively, of Mitchelmore [601] and Flint [602], indicated that the tail-end of the respective dose-response relationships starts to occur at approximate IL2 and IL4 concentration of 0.5ng/ml. It was decided therefore that optimising the ELISAs about this concentration would increase the assay sensitivity sufficiently such that it should be possible to quantitate changes in the IL2 and IL4 concentrations in biological fluids.

11.221 Methods and Materials

Checkerboard assays of the standard IL2 and IL4 ELISAs (11.211,11.212), figures 11.3 and 11.4 respectively, were carried out using concentration ranges of rat anti-mouse coating and biotinylated antibody, and maintaining the concentration of recombinant mouse IL2 or IL4 at 0.5ng/ml, in order to determine the optimal concentrations of rat anti-mouse coating and biotinylated antibody necessary for the detection of IL2 or IL4 at concentrations of 05.ng/ml or less.

Optimal reagent concentrations were determined, as in 10.2, from plots of mean, test and background absorbance readings. Optimal conditions occur when the difference between the test and background absorbance is at a maximum.

11.222 Results and Discussion

Optimal assay conditions, as determined from the IL2 and IL4 checkerboard plots, figures 11.5 and 11.6 respectively, were found to be:

IL2 ELISA

2μg/ml	rat anti-mouse IL2 (coating antibody)
50pg/ml	biotinylated rat anti-mouse IL2.

IL4 ELISA

4μg/ml	rat anti-mouse IL4 (coating antibody)
50pg/ml	biotinylated rat anti-mouse IL4.

Figure 11.3 Illustration of the Plate Layout for the IL2 Checkerboard ELISA

Recombinant mIL2 Blocking buffer

	1	2	3	4	5	6	7	8	9	10	11	12	Biot.raIL2
A	B						B						0
B													1µg/ml
C													400ng/ml
D													200ng/ml
E													100ng/ml
F													40ng/ml
G													20ng/ml
H	B						B						0
	0	2	1	0.5	0.25	0.1	0	2	1	0.5	0.25	0.1	

µg/ml rat anti-mouse IL2 coating antibody

Figure 11.4 Illustration of the Plate Layout for the IL4 Checkerboard ELISA

Recombinant mIL4 Blocking buffer

	1	2	3	4	5	6	7	8	9	10	11	12	Biot.raIL4
A	B						B						0
B													4µg/ml
C													2µg/ml
D													1µg/ml
E													500ng/ml
F													200ng/ml
G													100ng/ml
H	B						B						0
	0	4	2	1	0.5	0.25	0	4	2	1	0.5	0.25	

µg/ml rat anti-mouse IL4 coating antibody

KEY:

Biot.raIL2/4 - biotinylated rat anti-mouse IL2 or IL4

B - control blank well

Figure 11.5 Optimisation Plot Attained from the IL2 Checkerboard ELISA

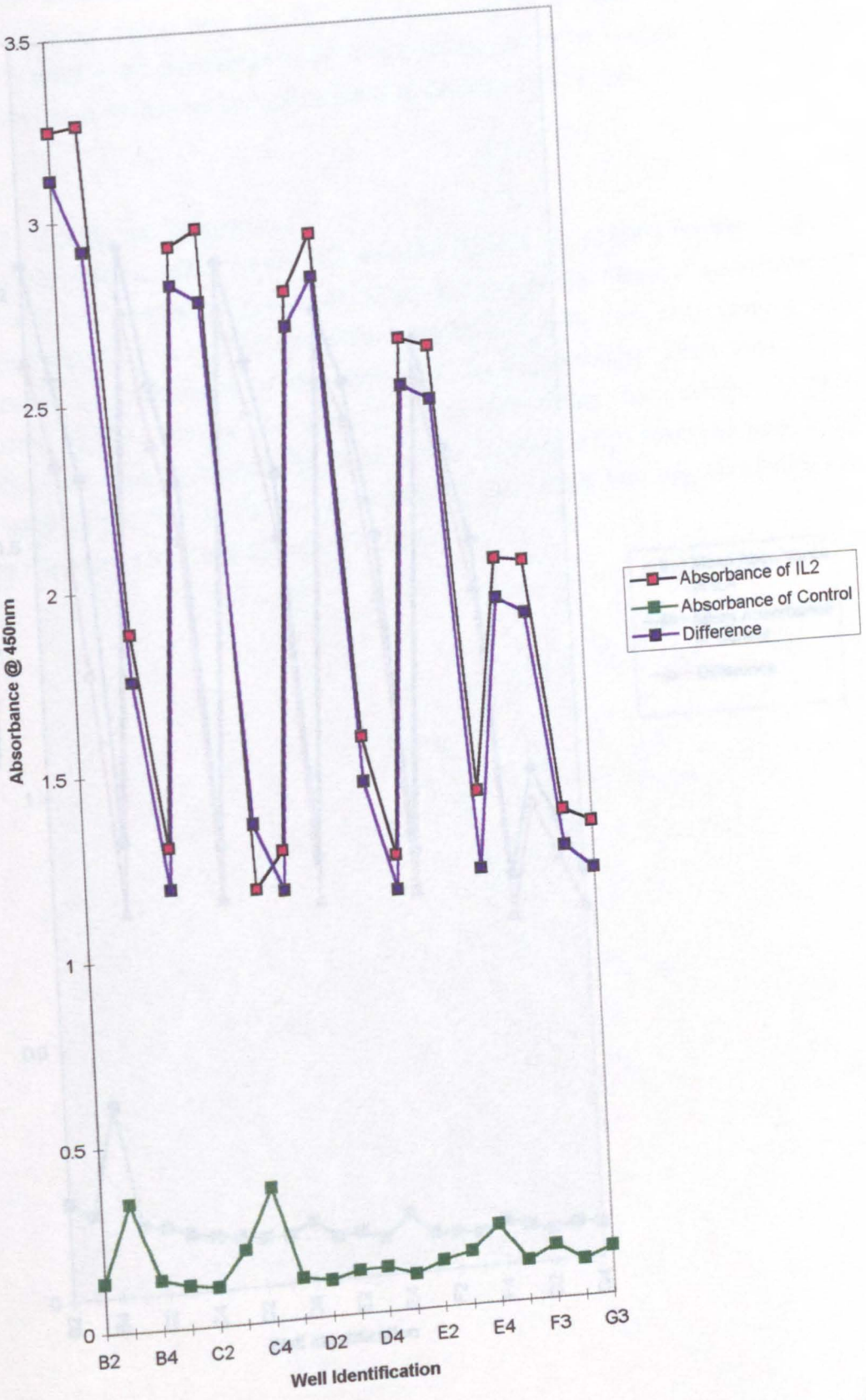
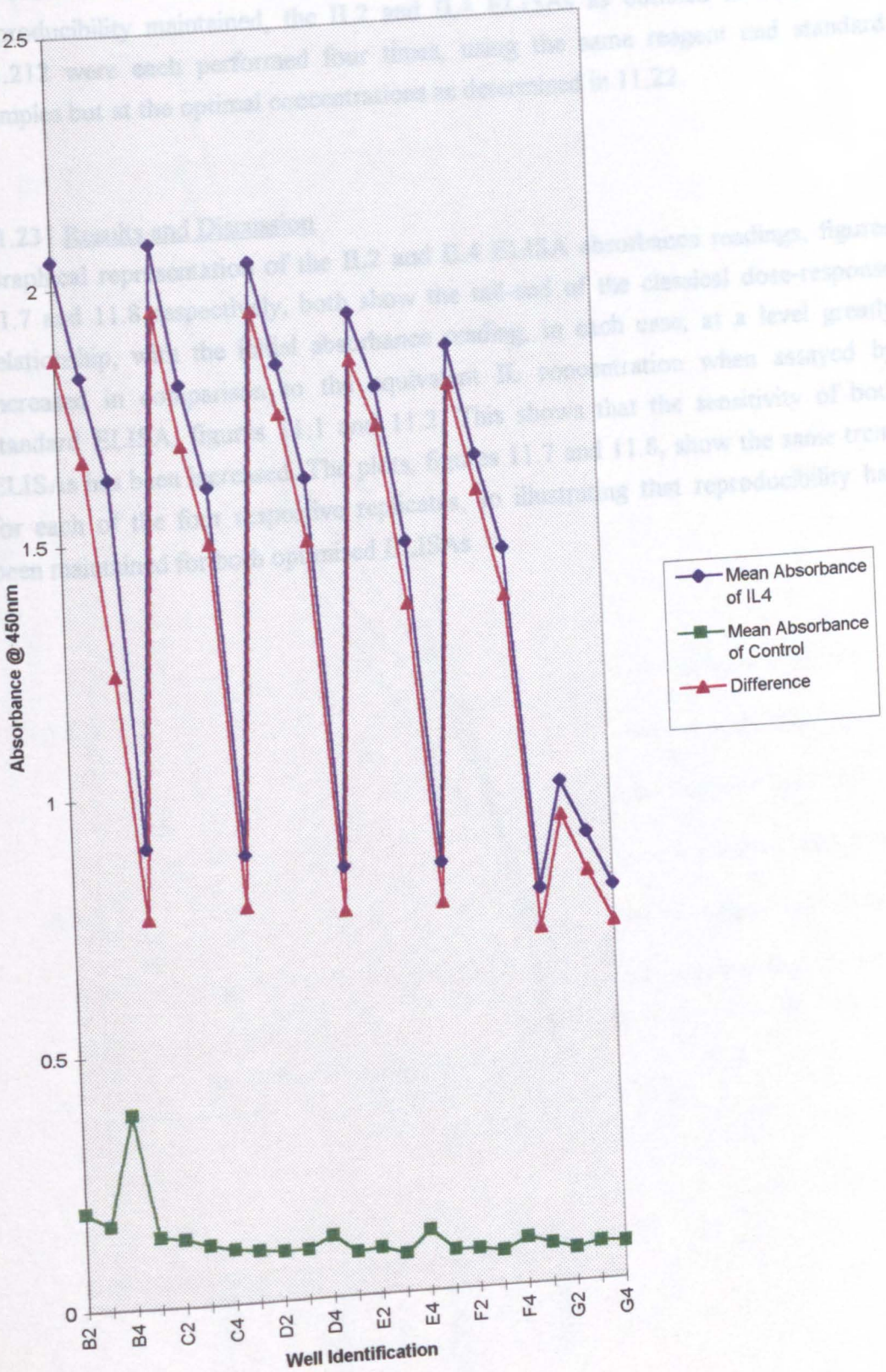


Figure 11.6 Optimisation Plot Attained from the IL4 Checkerboard ELISA



11.23 Experiment 2: Reproducibility of the Optimised IL2 and IL4 ELISAs

11.231 Methods and Materials

In order to ensure the sensitivity of the ELISAs had been increased and assay reproducibility maintained, the IL2 and IL4 ELISAs as outlined in 11.211 and 11.212 were each performed four times, using the same reagent and standard samples but at the optimal concentrations as determined in 11.22.

11.231 Results and Discussion

Graphical representation of the IL2 and IL4 ELISA absorbance readings, figures 11.7 and 11.8 respectively, both show the tail-end of the classical dose-response relationship, with the initial absorbance reading, in each case, at a level greatly increased in comparison to the equivalent IL concentration when assayed by standard ELISA, figures 11.1 and 11.2. This shows that the sensitivity of both ELISAs has been increased. The plots, figures 11.7 and 11.8, show the same trend for each of the four respective replicates, so illustrating that reproducibility had been maintained for both optimised ELISAs.

Figure 11.7 Graphical Representation of the Increased Sensitivity and Reproducibility of the Optimised IL2 ELISA

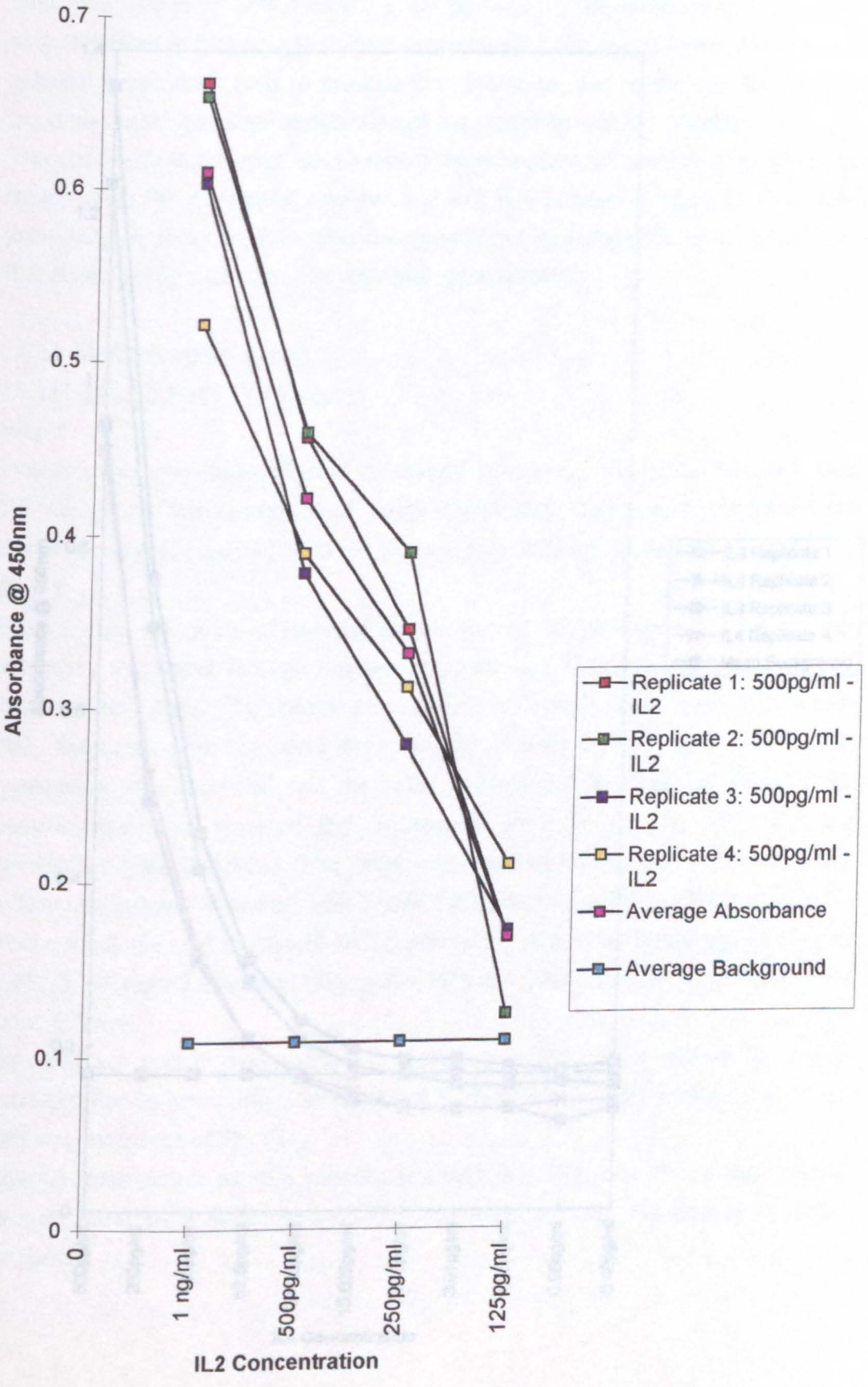
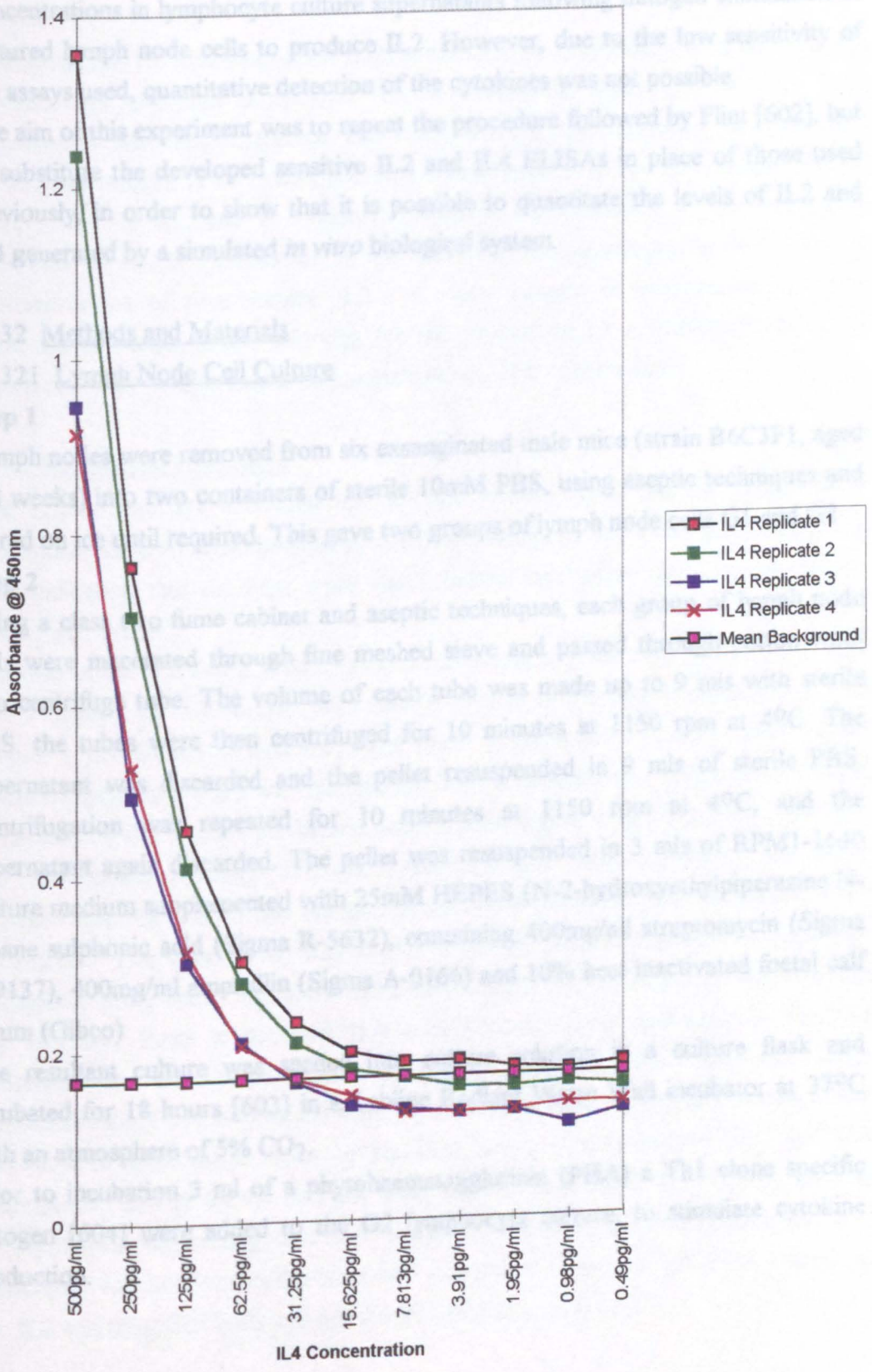


Figure 11.8 Graphical Representation of the Increased Sensitivity and Reproducibility of the Optimised IL4 ELISA



11.3 Determination of IL2 and IL4 Levels in Lymphocyte Culture Supernatant, Following *In Vitro* Stimulation

11.31 Test Rationale

Flint [602] has previously used IL2 / IL4 ELISAs for the detection of IL2 and IL4 concentrations in lymphocyte culture supernatants following mitogen stimulation of cultured lymph node cells to produce IL2. However, due to the low sensitivity of the assays used, quantitative detection of the cytokines was not possible.

The aim of this experiment was to repeat the procedure followed by Flint [602], but to substitute the developed sensitive IL2 and IL4 ELISAs in place of those used previously, in order to show that it is possible to quantitate the levels of IL2 and IL4 generated by a simulated *in vitro* biological system.

11.32 Methods and Materials

11.321 Lymph Node Cell Culture

Step 1

Lymph nodes were removed from six exsanginated male mice (strain B6C3F1, aged 7-8 weeks) into two containers of sterile 10mM PBS, using aseptic techniques and stored on ice until required. This gave two groups of lymph node cells G1 and G2.

Step 2

Using a class two fume cabinet and aseptic techniques, each group of lymph node cells were macerated through fine meshed sieve and passed through cotton wool into centrifuge tube. The volume of each tube was made up to 9 mls with sterile PBS. the tubes were then centrifuged for 10 minutes at 1150 rpm at 4°C. The supernatant was discarded and the pellet resuspended in 9 mls of sterile PBS. Centrifugation was repeated for 10 minutes at 1150 rpm at 4°C, and the supernatant again discarded. The pellet was resuspended in 3 mls of RPMI-1640 culture medium supplemented with 25mM HEPES (N-2-hydroxyethylpiperazine N-ethane sulphonic acid (Sigma R-5632), containing 400mg/ml streptomycin (Sigma S-9137), 400mg/ml ampicillin (Sigma A-0166) and 10% heat inactivated foetal calf serum (Gibco)

The resultant culture was seeded into culture solution in a culture flask and incubated for 18 hours [603] in a Labline Radiant Warm Wall incubator at 37°C with an atmosphere of 5% CO_2.

Prior to incubation 3 ml of a phytohaemataggluinin (PHA) a Th1 clone specific mitogen [604] were added to the G2 lymphocyte culture, to stimulate cytokine production.

Step 3 - Cell viability test

10µl of culture solution were removed from the culture flask pre and post incubation and added to 10µl of 0.4% trypan blue dye (Sigma T-8154). The cell solution was then applied to a haemocytometer and clear viable cells counted.

Step 4

Following the 18 hour incubation each of the culture solutions was transferred into a centrifuge tube and centrifuged for 10 minutes at 2500 rpm at 4°C. Each supernatant was then extracted and assayed using the optimised IL2 and IL4 ELISAs developed in 11.2 using the supernatant in place of the recombinant IL2 / 4 sample, for its IL2 and IL4 content. Samples were assayed in four replicates. However to enable quantitation of the IL2 /4 in the supernatant standard known concentrations of recombinant IL2 / 4 were assayed in conjunction with the supernatant samples, thus allowing for the production of a standard curve from which the IL2 / 4 supernatant concentrations could be determined.

11.33 Results and Discussion

The trypan blue dye exclusion test for cell viability, both pre and post culture incubation, showed an abundance of clear cells when viewed under the microscope, thus indicating that the cells were viable before incubation and retained viability during incubation.

Analysis of the IL2 and IL4 ELISA absorbance levels, given in table 11.1, indicated that a quantity of IL2 and IL4 was present in each of the twelve replicates of each culture supernatant (G1 and G2). The mean concentration of IL2 in the treated culture supernatant (G2) was quantitated from a standard IL2 concentration curve generated by IL2 ELISA performed concurrently, figure 11.9. (Table 11.2 presents the absorbance readings for each standard IL2 concentration). The mean concentration of IL2 in the stimulated culture supernatant was evaluated to be 1.55ng/ml.. The mean level IL2 in the untreated culture supernatant (G1) was negligible, being below the lower limit of the standard curve, so quantitation was not possible. From a standard IL4 concentration curve (absorbance readings presented in table 11.3), figure 11.10, it was determined that the mean concentration of IL4 in both G1 and G2 was negligible, occurring at levels below the limits of quantitation from the standard curve. Comparison of the mean IL4 absorbance readings for G1 and G2, table 11.1, showed the levels of absorbance to be equivalent, suggesting that IL4 secretion remained constant throughout the incubation and assay procedures. It was concluded therefore that PHA stimulates the IL2 secreting Th1 cells and not the Th2 IL4 secreting cells.

Table 11.1 Absorbance Readings at 450nm Obtained Following IL2 and IL4 ELISA of Stimulated and Unstimulated Lymphocyte Culture Supernatants

Replicate #	IL2			IL4		
	Background	Group 1	Group 2	Background	Group 1	Group 2
1	0.056	0.086	0.747	0.048	0.073	0.074
2	0.051	0.082	0.836	0.050	0.074	0.073
3	0.055	0.084	0.759	0.049	0.073	0.076
4	0.055	0.084	0.861	0.049	0.075	0.075
5	0.054	0.084	0.743	0.051	0.079	0.076
6	0.058	0.086	0.855	0.051	0.079	0.078
7	0.078	0.084	0.641	0.050	0.105	0.096
8	0.065	0.080	0.0680	0.046	0.107	0.101
9	0.064	0.099	0.570	0.051	0.109	0.094
10	0.063	0.079	0.0710	0.048	0.101	0.100
11	0.066	0.085	0.619	0.048	0.096	0.093
12	0.051	0.086	0.732	0.047	0.103	0.102
mean	0.058	0.085	0.729	0.049	0.090	0.087

Table 11.2 Absorbance Readings at 450nm for Standard IL2Concentrations Evaluated by IL2 Optimised ELISA

IL2 concentration	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
2ng/ml	1.010	0.947	0.871	0.892	0.930
1ng/ml	0.712	0.515	0.428	0.453	0.527
500pg/ml	0.294	0.286	0.238	0.280	0.275
250pg/ml	0.179	0.180	0.201	0.299	0.215
125pg/ml	0.131	0.130	0.145	0.134	0.135
62.5pg/ml	0.101	0.101	0.178	0.109	0.122
31.25pg/ml	0.099	0.098	0.133	0.101	0.103
15.63pg/ml	0.087	0.082	0.106	0.092	0.092

Table 11.3 Absorbance Readings at 450nm for Standard IL4Concentrations Evaluated by IL4 Optimised ELISA

IL4 concentration	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
2ng/ml	1.832	1.879	1.988	2.070	1.942
1ng/ml	1.217	1.242	1.180	1.251	1.223
500pg/ml	0.770	0.742	0.706	0.613	0.708
250pg/ml	0.456	0.447	0.391	0.391	0.421
125pg/ml	0.261	0.271	0.252	0.238	0.256
62.5pg/ml	0.175	0.170	0.184	0.179	0.177
31.25pg/ml	0.133	0.135	0.159	0.140	0.142
15.63pg/ml	0.111	0.112	0.140	0.150	0.128
7.82pg/ml	0.108	0.099	0.139	0.125	0.118
3.91pg/ml	0.096	0.108	0.140	0.134	0.199

Figure 11.9 IL2 Standard Concentration Curve and Determination of the Average IL2 Concentration Present in PHA Stimulated Lymphocyte Culture Supernatant

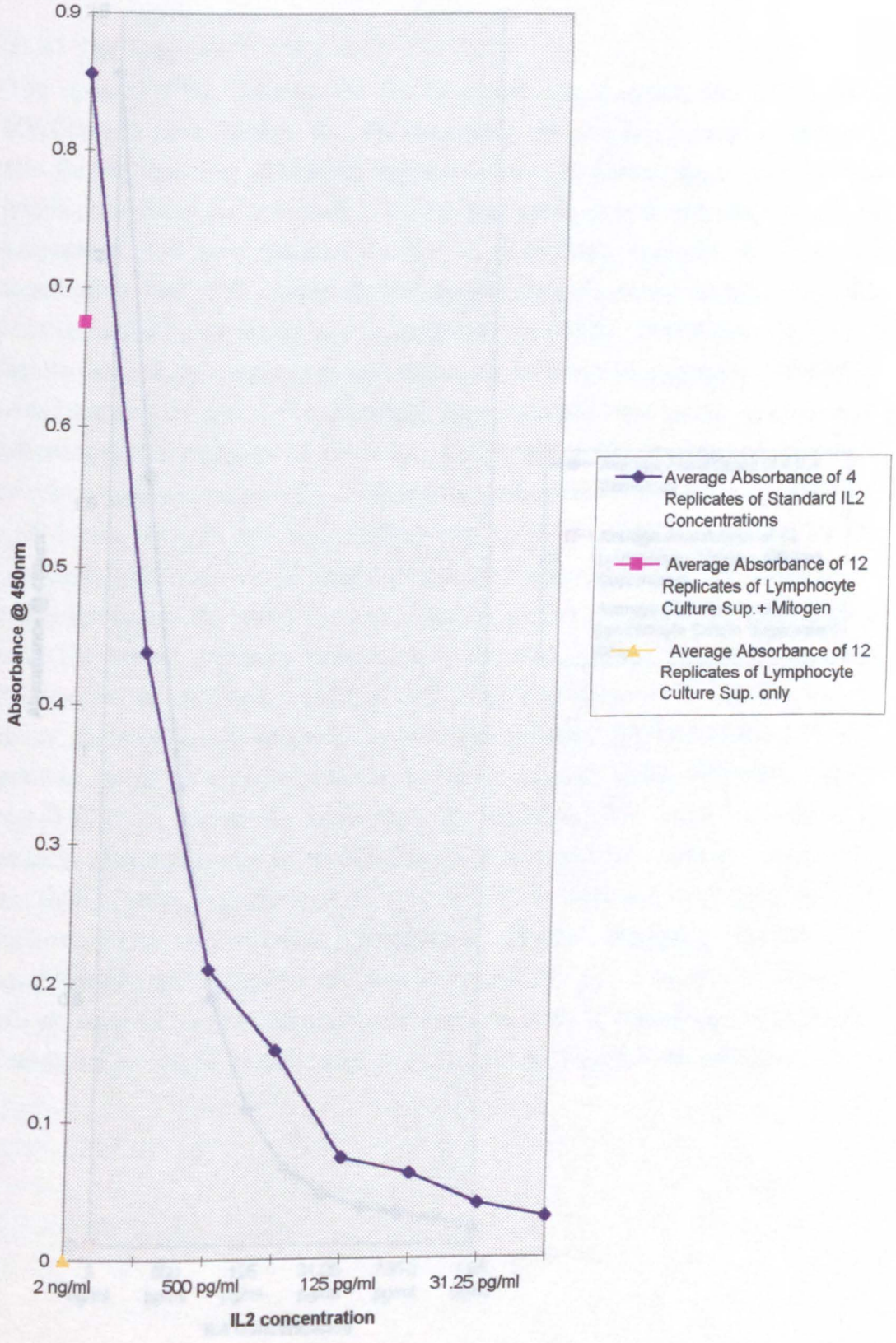
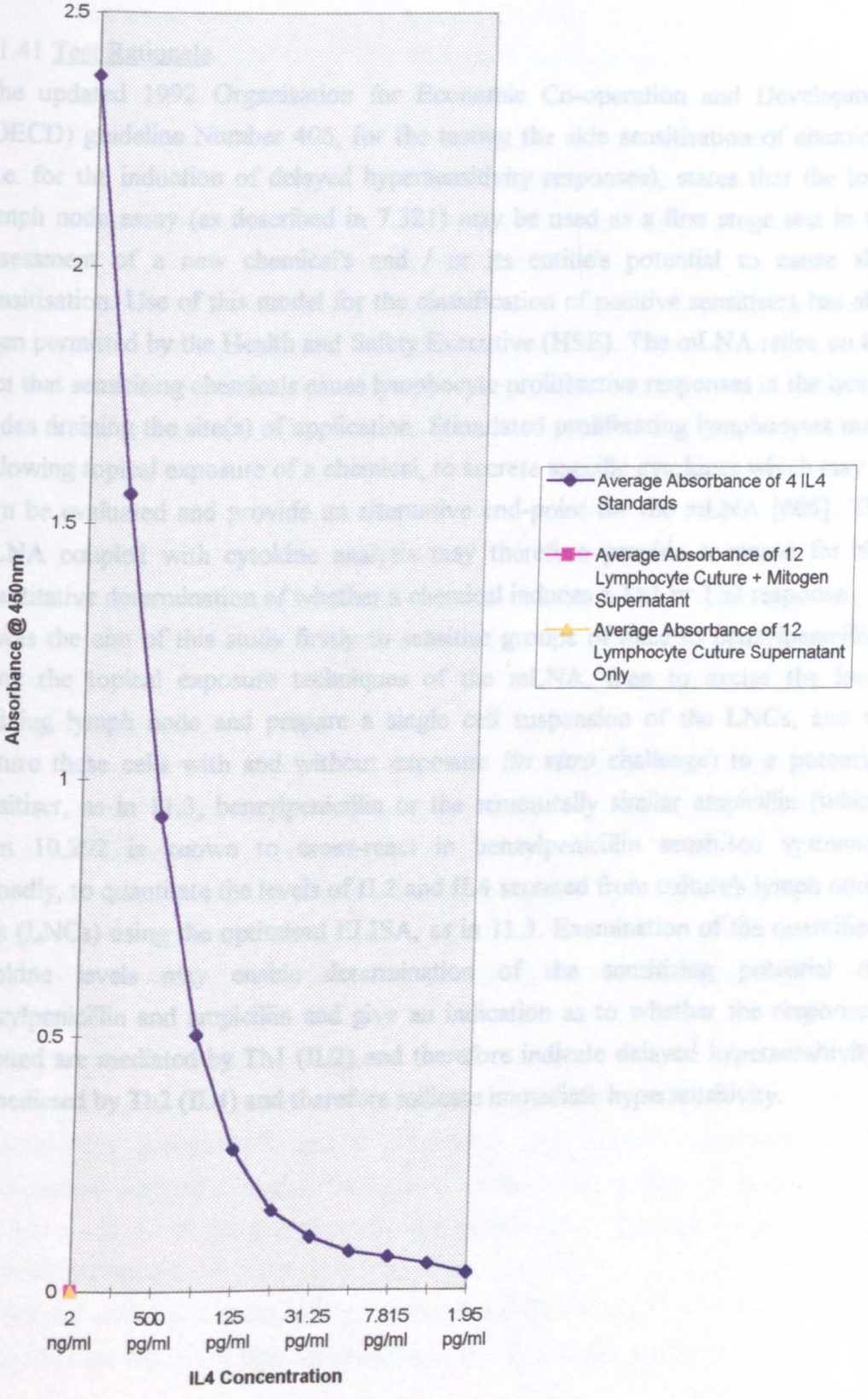


Figure 11.10 IL4 Standard Concentration Curve and Determination of the Average IL4 Concentration Present in PHA Stimulated Lymphocyte Culture Supernatant



11.4 Investigation into a Modified Murine Local Lymph Node Assay (mLNA), using Benzylpenicillin, Assessing Cytokine Release following *In Vitro* Challenge of Cultured Lymphocytes with Benzylpenicillin and Assessment of Cross-Reacivity with Ampicillin

11.41 Test Rationale

The updated 1992 Organisation for Economic Co-operation and Development (OECD) guideline Number 406, for the testing the skin sensitisation of chemicals (i.e. for the induction of delayed hypersensitivity responses), states that the local lymph node assay (as described in 7.321) may be used as a first stage test in the assessment of a new chemical's and / or its entitie's potential to cause skin sensitisation. Use of this model for the classification of positive sensitisers has also been permitted by the Health and Safety Executive (HSE). The mLNA relies on the fact that sensitising chemicals cause lymphocyte proliferative responses in the lymph nodes draining the site(s) of application. Stimulated proliferating lymphocytes may, following topical exposure of a chemical, to secrete specific cytokines which may in turn be evaluated and provide an alternative end-point for the mLNA [605]. The mLNA coupled with cytokine analysis may therefore provide a means for the quantitative determination of whether a chemical induces a Th1 or Th2 response.

It was the aim of this study firstly to sensitise groups of mice to benzylpenicillin, using the topical exposure techniques of the mLNA, then to excise the local draining lymph node and prepare a single cell suspension of the LNCs, and to culture these cells with and without exposure (*in vitro* challenge) to a potential sensitiser, as in 11.3, benzylpenicillin or the structurally similar ampicillin (which from 10.292 is known to cross-react in benzylpenicillin sensitised systems); secondly, to quantitate the levels of IL2 and IL4 secreted from culture's lymph node cells (LNCs) using the optimised ELISA, as in 11.3. Examination of the quantified cytokine levels may enable determination of the sensitising potential of benzylpenicillin and ampicillin and give an indication as to whether the responses initiated are mediated by Th1 (IL2) and therefore indicate delayed hypersensitivity or mediated by Th2 (IL4) and therefore indicate immediate hypersensitivity.

11.42 Methods and Materials

11.421 Murine Local Lymph Node Assay

11.4211 Animals

Source	Small Animal Breeding Unit, GRD.
Species & strain	Mouse B6C3F1
Number for study	50 males (plus 4 spares)
Age on Day 0 of study	7-8 weeks
Diet	Rat & Mouse #1 expanded diet (SDS Ltd.) and water supplied *ab libitum.*
Accommodation	M1 cages with disposable cage inserts and 'Alpha-dri' bedding.

The mice were randomly allocated to cages (one mouse per cage to minimise cross-contamination and fighting) and treatment groups using specialised ramdomisation computer software (PADMAN) and were uniquely identified by an animal number tattooed on the tail. The animals were allowed to acclimatise to their experimental environment for 5 days.

11.4212 Dosing

The benzylpenicillin sensitising dosage was determined from the previous work of Stejskal et al [606], who used penicillin antibiotics in their work investigating skin sensitisation.

Dosage Preparation:

- a 4:1 ratio ethanol / polyethylene glycol (EPG), was prepared by combining 8mls of 95 % ethanol (BDH 28303) and 2ml propylene glycol (Sigma P-1009), this is the vehicle for compound application to the mice.
- 15% benzylpenicillin in EPG was prepared by dissolving 0.6g of benzylpenicillin (Sigma Pen-NA) in 4ml of EPG.
- 10% benzylpenicillin was prepared by mixing 2ml of 15% benzylpenicillin with 1ml of EPG.

Dosing Regimen and Observations

The study comprised four treatment groups, as detailed in table 11.4.

Animals from groups 2, 3 and 4 received a 25µl topical application of the appropriate dosing solution onto the internal surface of the pinnae, on days 0, 1 and 2 of the study. Compound application was performed by Bio-Resources at GRD, witnessed by myself.

All the animals were examined twice daily, by Bio-Resources, throughout the duration of the study, for signs of ill health or reaction to the treatment.

The individual body weights of all animals pre-treatment, on day 0, and post-treatment, on day 5, were recorded, as any significant variation may indicate a toxic effect.

Table 11.4 mLNA Dosage Groups

Group No.	Colour Code	Dosage	No. Mice	ID Nos.
1	white	untreated	10	1-10
2	yellow	Ethanol / Propylene glycol (EPG)	10	11-20
3	blue	10% (w.v) Benzylpenicillin in EPG	15	21-35
4	red	15% (w.v) Benzylpenicillin in EPG	15	36-50

Termination:

On day 5 of the study all the mice were sacrificed by vena cava venepuncture exsanguination under deep anaesthesia (isoflurane), performed by Bio-resources at GRD, witnessed by myself.

The serum from each mouse was collected into individually labelled containers and retained for serological analysis (11.424).

Immediately following termination, the lymph nodes draining the dosing sites were aseptically excised from all mice and placed into specifically labelled containers of pure RPMI-1640 culture medium (Sigma R-5632). The lymph nodes from five mice within each dosing group were pooled in order to give 10 experimental groups for lymphocyte culture, as detailed in table 11.5. The lymph node excisions were performed by Miss Su Evans (Immunotoxicology Section GRD), myself assisting.

11.422 LNC Single Cell Culture

Culturing of the excised LNCs was carried out as in 10.3 (as ampicillin was to be assayed it was not applied to the culture medium as in 10.3), with the addition of PHA, 100μg/ml benzylpenicilln and 100μg/ml ampicillin being added to the specific experimental groups detailed in table 11.5, prior to culturing.

Cell viability was determined as in 10.3 by trypan blue dye exclusion. The number of lymphocytes in the culture was evaluated pre and post culturing, by the Haematology Department, GRD, using a H1 Techicon Analyser.

11.423 Quantitation of Culture Supernatant IL2 and IL4 Levels

The concentration of IL2 and IL4 in the culture supernatant of each experimental group was determined by ELISA as in 10.3.

Table 11.5 Experimental Groups for Lymphocyte Culture

Mouse ID	Experimental Group No.	Dosage	*in vitro* Challenge
1-5	1A	untreated	untreated
6-10	1B	untreated	1:1000 PHA
11-15	2A	EPG	100μg/ml BP
16-20	2B	EPG	100μg/ml ampicillin
21-25	3A	10% BP in EPG	untreated
26-30	3B	10% BP in EPG	100μg/ml BP
31-35	3C	10% BP in EPG	100μg/ml ampicillin
36-40	4A	15% BP in EPG	untreated
41-45	4B	15% BP in EPG	100μg/ml BP
46-50	4C	15% BP in EPG	100μg/ml ampicillin

11.424 Determination of Serum Antibody Titres

The serum samples collected from mice, during exsanguination, in the same dosing group were pooled into 4 test groups.

The IgG antibody titre of each serum group was carried out using the standard BPCC ELISA described 10.211, but replacing the anti-rabbit IgG conjugated horse radish peroxidase antibody with an equivalent concentration of anti-mouse IgG conjugated horse radish peroxidase (HRP) antibody (Sigma A-4416) and replacing the positive rabbit sera with the pooled serum samples.

Note: Three rows on the respective ELISA were treated with IgG positive rabbit serum, NRS and PTS alone, and hence the anti-rabbit-IgG HRP antibody was used in these rows. This was done to provide a control showing that the ELISA was functioning normally.

11.43 Results and Discussion

11.431 Observations and Body Weights

No adverse observations or abnormalities were observed for any animal during the course of the study.

The animal body weights pre and post treatment are presented in table 11.6, and show no dramatic changes to have occurred, therefore indicating that all animals were in good health throughout the study.

Table 11.6 Pre and Post Treatment Animal Body Weights

Animal Number	Pre-treatment Body Weight (g)	Post-treatment Body Weight (g)	Body Weight Increase (g)
01	29.7	30.0	0.3
02	30.2	31.3	1.1
03	28.7	29.4	0.7
04	30.1	31.0	0.9
05	29.1	31.1	2.0
06	28.8	29.7	0.9
07	30.0	31.1	1.1
08	26.8	27.0	0.2
09	29.9	31.1	1.2
10	29.9	31.4	1.5
11	26.9	27.8	0.9
12	25.1	27.0	1.9
13	25.2	27.5	2.3
14	26.3	27.1	0.8
15	25.4	26.1	0.7
16	22.9	24.4	1.5
17	23.2	24.1	0.9
18	24.7	26.1	1.4
19	24.1	25.8	1.7
20	27.5	28.7	1.2
21	23.8	25.6	1.8

Continued over page

Table 11.6 continued

Animal Number	Pre-treatment Body Weight (g)	Post-treatment Body Weight (g)	Body Weight Increase (g)
22	23.4	25.4	2.0
23	25.1	26.7	1.6
24	25.7	27.3	1.6
25	26.2	27.6	1.4
26	24.4	25.5	1.1
27	25.6	27.0	1.4
28	23.7	24.1	0.4
29	26.5	26.9	0.4
30	24.6	25.7	1.1
31	23.7	24.4	0.7
32	24.9	25.7	0.8
33	24.3	25.0	0.7
34	23.8	25.3	1.5
35	25.4	26.2	0.8
36	23.4	24.2	0.8
37	23.0	24.5	1.5
38	24.1	25.7	1.6
39	23.4	24.9	1.5
40	27.1	28.8	1.7
41	25.3	27.2	1.9
42	22.5	23.7	1.2
43	24.1	24.6	0.5
44	23.4	23.7	0.3
45	20.8	21.2	0.4
46	25.5	26.6	1.1
47	22.8	24.2	1.4
48	25.8	27.2	1.4
49	24.1	25.9	1.8
50	22.4	23.7	1.3

11.432 Lymphocyte Culture and Serological Analysis

The concentrations of IL2 and IL4 in each tested culture supernatant were determined from standard curves produced by ELISA concurrently, figures 11.11 and 11.12 respectively. The percentage of viable cells and lymphocytes were counted pre and post culturing and the concentrations of IL2 and IL4 determined in each respective experimental group as presented in table 11.7.

Table 11.7 Table of the Lymphocyte Culture and Serological Analysis Results

Test Group	Pre-Culture Lymphocyte Count ^{10} / l	Post-Culture Lymphocyte Count ^{10} / l	Pre-Culture % Cell Viability	Post-Culture % Cell Viability	IL2 Conc. pg/ml	IL4 Conc. pg/ml	IgG Titre
1A	0.66	0.20	64.3	90.0	0.0	3.5	0
1B	2.77	0.54	70.9	3.5	0.0	0.0	0
2A	3.82	2.73	62.5	77.4	0.0	0.0	0
2B	3.29	0.83	66.7	40.0	0.0	3.7	0
3A	3.43	1.59	52.4	38.9	<3.9	<3.9	0
3B	5.91	8.43	66.7	23.5	3.9	<3.9	0
3C	1.91	0.57	73.3	57.1	<3.9	<3.9	0
4A	1.84	0.85	60.0	37.5	40	42	0
4B	1.59	0.54	66.7	53.3	200	<3.9	0
4C	3.42	2.85	50.0	55.0	<3.9	0.0	0

Examination of the lymphocyte counts clearly shows that, in each case, during the incubation period of cell culturing the cell numbers diminished, rather than proliferated as was expected. This may be due to some lack of essential nutrient in the culture medium, the self degradation of the cells or due to a reduced CO_2 supply. The cell numbers obtained are moderate, as is the viability of these cells; these two factors together may explain why such low cytokine levels, compared with 11.3, were obtained. That is, if half of the low number cells present are non-viable, and thus non-cytokine secreting, only a very small number of cells remain to secrete the cytokines, and subsequently the cytokine levels are low. It is also apparent from examination of the lymphocyte cell counts that the numbers of cells varied significantly from group to group. This inability to standardise cell populations is an common problem when culturing cells and reflects the inconsistency in numbers of lymph nodes it is possible to excise from different mice. This variation in cell numbers therefore leads to errors in the calculation index and restricts the accuracy of comparisons made between the results obtained from different cell populations.

The post-culturing cell viabilities are relatively low (below 75%) and this may be due to the excessive time (3 hours) between animal termination, lymph node excision and culturing, which itself was a legacy of the protocol and the large number of animals used. During the time between excision and culturing, it is reasonable to assume that some of the LNCs would have died. In future studies of this type it may be more pertinent to terminate, excise and culture one test group at a time, i.e. only 5 mice, thus reducing the delay and restricting the likelihood of excessive cell death.

The results obtained from Group 1A indicate that no quantifiable amount of IL2 and only a negligible amount of IL4 were present in the culture supernatant. These levels were as expected as this group was the negative untreated control group for the study.

Group 1B is essentially a repeat of the previously performed PHA lymphocyte stimulation, 11.3, and should therefore have provided a positive control for the study. However, upon examination of the cytokine levels evaluated it is apparent that some fault has occurred, in that the IL2 concentration, rather than being of the magnitude of 1.55ng/ml as obtained in 11.3, was below the level of quantitation. This limited IL2 secretion is explained by the fact that for this test group the cell viability was extremely low at only 3.5%, thus there were only a few cells viable enough to be stimulated and to secrete IL2. The concentrations of IL4 were also below the limits of quantifiable detection. As previously mentioned in 11.3, PHA appears to stimulate only the IL2 secreting Th1 cell sub-population, therefore the

experiment contained no positive control for the stimulation of IL4 secreting Th2 cells. Suitable candidate chemicals for such an IL4 control in future work could be oxazalone or 2,4-dinitrochlorobenzene (DNCB), both of which are known Th2 stimulators [607].

Group's 2A and 2B are EPG vehicle and challenge control groups, which, by virtue of the lack of IL2 and negligible levels of IL4 detected, demonstrated that the EPG was not responsible for sensitising the LNCs and that without prior sensitisation neither benzylpenicillin nor ampicillin generated a challenge response.

Group 3A presented a low lymphocyte and viability count, which may be why the levels of IL2 and IL4 obtained were both negligible. However, The fact, that the IL2 concentration, when compared to that of Group 1A, appeared to have increased marginally and the IL4 levels were unaltered, suggests that the 10% benzylpenicillin solution may possibly have caused a mild sensitisation of the draining LNCs.

Group 3B generated marginally greater concentrations of IL2, with little change in the IL4 levels, thus suggesting that the sensitised environment presented the *in vitro* challenge with benzylpenicillin stimulated the secretion of more IL2. The low cell numbers and low viability explain why IL2 levels were not of a higher magnitude.

Group 3C presented IL2 and IL4 levels no different from those of Group 3A, suggesting therefore that, unlike benzylpenicillin, the challenge of mildly sensitised lymphocytes with ampicillin does not result in further lymphocyte stimulation and so neither the IL2 or IL4 secretion is increased.

Group 4A cytokine levels clearly show that a topical application of 15% benzylpenicillin results in LNC sensitisation and induces the secretion of IL2 and IL4.

Group 4B, in comparison to the cytokine levels of Group 4A, shows a greatly increased IL2 concentration, of a magnitude equivalent to that of the PHA stimulated IL2 production in 11.3, whilst the IL4 concentration is significantly reduced. This indicates therefore that the challenge of a benzylpenicillin-sensitised system with benzylpenicillin results in the stimulation of the IL2 secreting Th1 lymphocyte sub-populations to the detriment of IL4 secretion. Thus benzylpenicillin may possibly have induced delayed type hypersensitivity reactions, as has been proposed previously [608]. It has been proposed [609] that the possible reason behind why the concentration of IL4 secreted is diminished in both Groups 3B and 4B, is that increased concentrations of IL2 act via negative feedback mechanisms to suppress the secretion of IL4 and via immunosuppressive mechanisms to down regulate the production of Th2 cells whilst also acting via positive feedback mechanisms to enhance the differentiation of virgin Th cells into IL2 secreting Th1

clones. Group 4C, as with Group 3C, presents levels of IL2 and IL4 equivalent to the respective A groupings, thus further indicating that ampicillin does not act *in vitro* to stimulate either IL2 or IL4 production.

The lack of an IgG titre for any of the test groups suggests, at first glance, that the application of benzylpenicillin may not have induced the sensitisation of the LNCs. However, as previously discussed, the altering of IL2 and IL4 levels in test groups 3 and 4 indicates that a sensitisation and stimulatory response were occurring. The apparent lack of any IgG may be a result of the five day dosing regimen not being long enough to stimulate a significant IgG response. Furuhama et al [610] propose that in order to stimulate an IgG response a post-dosing period of twenty days is required. Kimber [611] proposes that for the five day dosing regimen of the mLNA it would be more meaningful to evaluate the levels of the immunoglobulins which are stimulated, i.e. IgM or IgE. IgE is specifically involved in Th2 immediate hypersensitivity whilst IgM production is a result of Th1 stimulation. Therefore the evaluation of both these immunoglobulins would provide a rough indication as to the direction of the induced immune response.

Figure 11.11 IL2 Standard Curve and Determination of IL2 content of Lymphocyte Culture Supernatant Sample

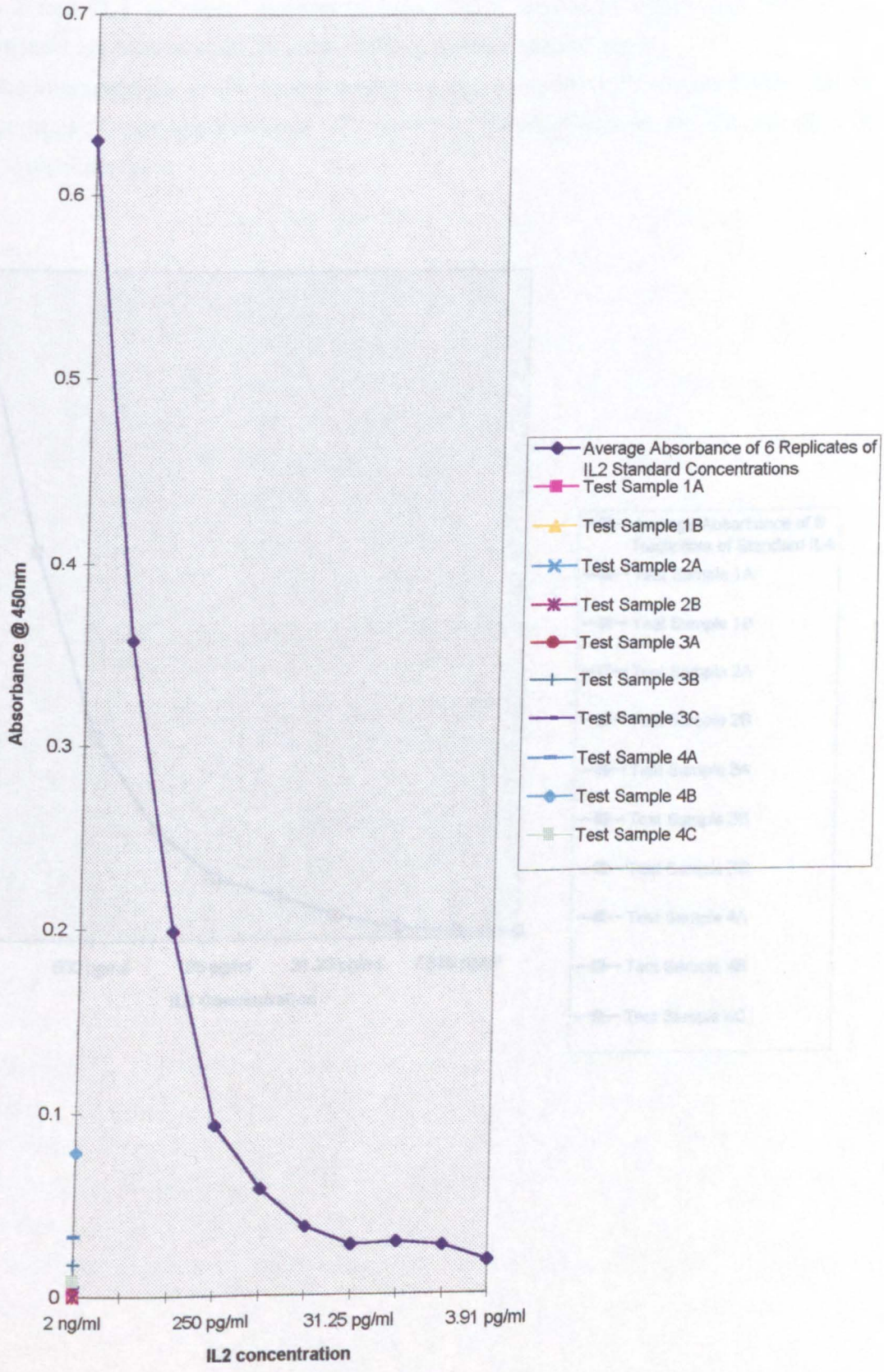
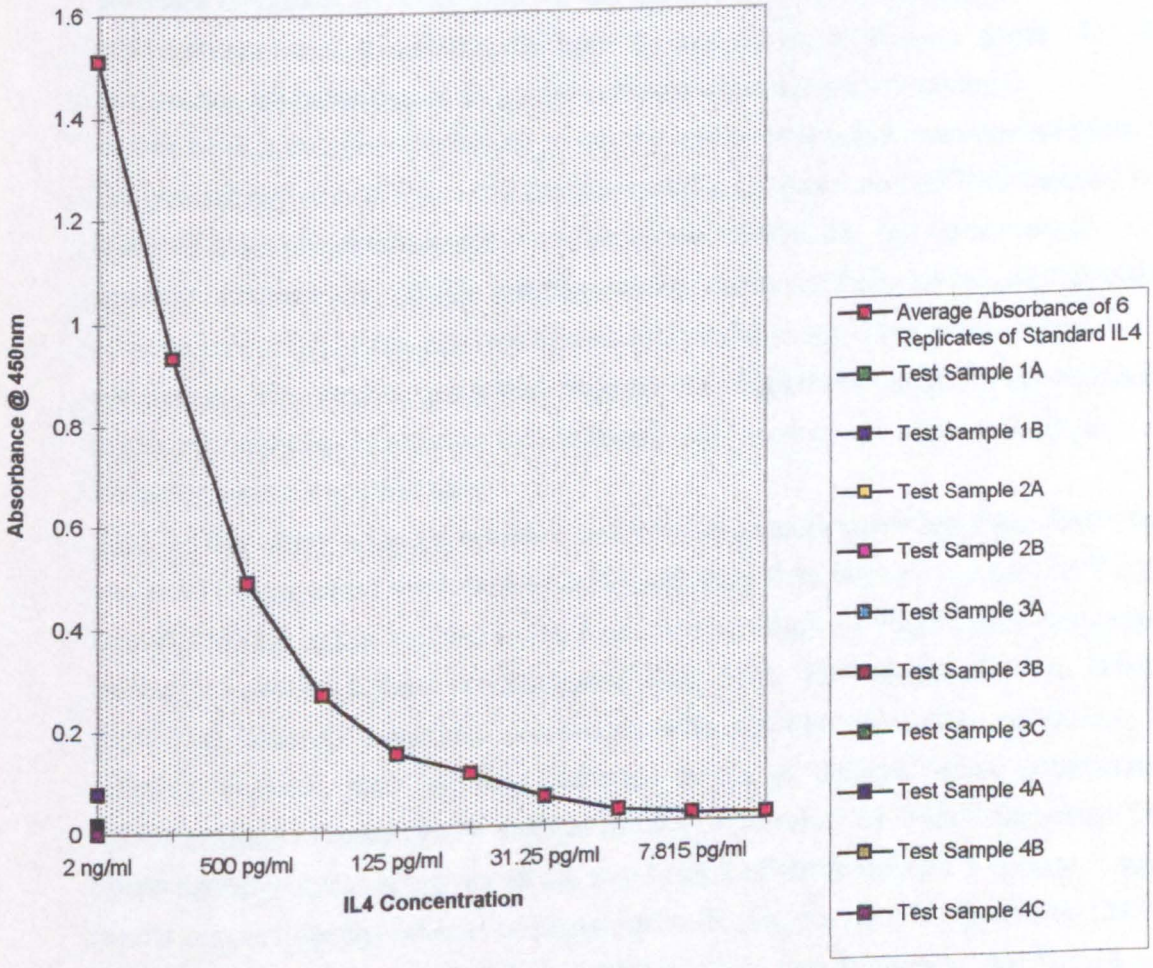


Figure 11.12 IL4 Standard Curve and Determination of IL4 content of Lymphocyte Culture Supernatant Sample



11.5 Conclusion

An assay has been developed which enables the quantitation of IL2 and IL4 levels in cultured lymphocyte supernatants following mLNA. Further modification and optimisation of the assay protocol should enable the quantitative determination of IL2 and IL4 secretion patterns induced by a sensitising agent and how these patterns are altered upon *in vitro* challenge with a second agent.

The interpretation of the such quantitative measurements of cytokine levels may in future pay important dividends with respect to the identification and classification of chemical allergens.

12.0 General Conclusion

In parallel with the expanding needs of immunotoxicological evaluation there is a need to develop new approaches and new *in vitro* models [612]. One such novel approach is via mechanistic studies, which relate immunotoxicity to the physical, chemical and / or structural properties of the immunotoxicants themselves, i.e. QSAR. Mechanistic studies of immunotoxicants in the past have proved to be extremely complex [613], but with the recent advances in computing technology such studies are now possible.

In this work, it has been possible, using the highly sophisticated and dedicated software of QSAR to bring together the approaches of epidemiology, immunology and computational chemistry, in such a way as to produce a means for the mechanistic investigation of β-lactam antibiotic immunotoxic potential.

In this work a database pertaining to the frequency of adverse reactions exhibited in the general population following the therapeutic administration of 70 β-lactams and some of their physical, chemical and structural properties, has been compiled and used to develop 27 QSAR models, which mathematically relate the potential adverse induction of the immune system to the shape and charge separation of the antibiotics. The results generated support the hypotheses that immunologically mediated adverse reactions are initiated via binding to and stimulation of immunoglobulin receptor sites.

Also, in this study a highly sensitive and reliable immunoassay has been developed and used to quantitate the extent by which different β-lactams cross-react with anti-benzylpenicillin serum antibodies, both at concentrations of 3mM and at equivalent to their respective highest recommended daily dose. The results of which clearly show that different β-lactams cross-react with anti-benzylpenicillin antibodies to differing degrees, thus initiating different levels of immunological stimulation. QSAR analysis of these results enabled the development of 18 models that relate the phenomenon of cross-reactivity to the size / shape of the respective β-lactam. These results support the hypotheses of cross-reactivity, as proposed by Batchelor [584], Shibata [585], Allemenos [583] and Blanca [34], that β-lactams cross-react by binding to specific epitopes on specific pre-developed immunoglobulins.

The complexity and diversity of the immunotoxicologically mediated responses prevents exact determination of the specific immunocomponents involved in each reaction type and of the reaction type induced.

Receptor modelling may provide information as to the nature of the epitopic site(s) involved.

Protein binding or haptenisation of the β-lactams is a pre-requisite for allergenicity, however, due to the constraints of the software available, it was not possible to model the protein bound β-lactams or their metabolites. Investigation of these derivatives may allow for more specific QSARs or be developed.
Also, in this study a new *in vitro* approach for the determination of the direction of stimulation of the induced immunological response (immediate or delayed hypersensitivity) by the β-lactams, directly or via cross-reactivity has been developed. The approach involves the quantitative determination of the levels of the IL2 and IL4 cytokines in culture supernatant, secreted following *in vitro* challenge of sensitised or unsensitised lymphocytes by a specific β-lactam antibiotic.

The use of the QSAR's developed, in this study, in conjunction with the immunological assays provide a reliable means for the mechanistic assessment of the potential allergenicity, cross-reactivity and the direction of immunological stimulation of new or untested β-lactam antibiotics.
Use of this information in conjunction with other related data, e.g. antimicrobial structure-activity relationships, efficacy and general toxicity, in the early stages of new compound design and development will enable the pharmaceutical industry to produce the necessary, new b-lactams antibiotics at a fraction of the time and cost, whilst maintaining efficacy and minimising toxicity.

13.0 References

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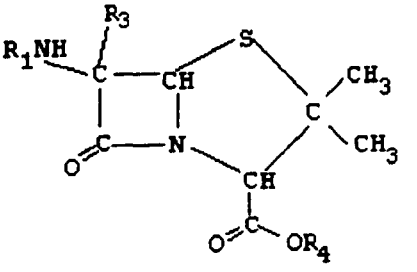
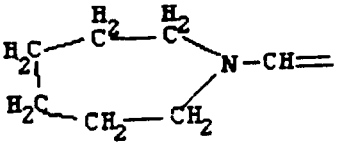
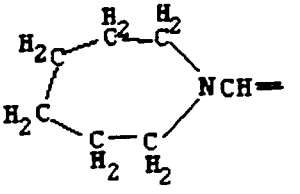
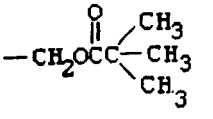
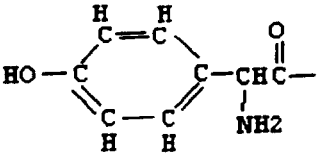
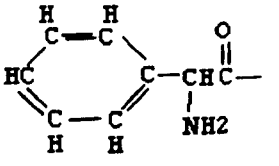
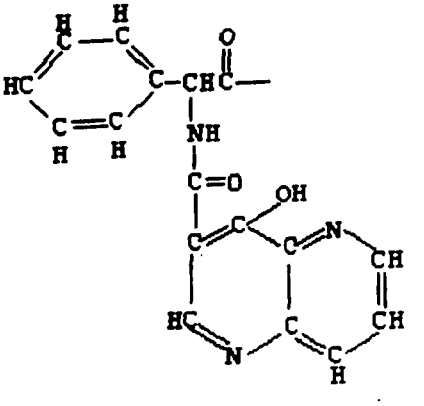
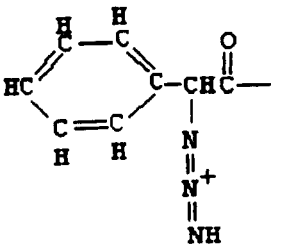
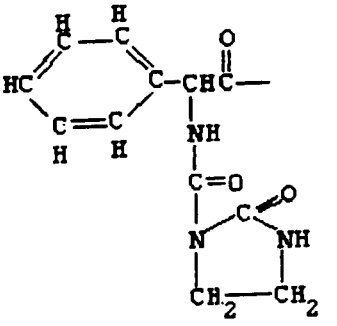
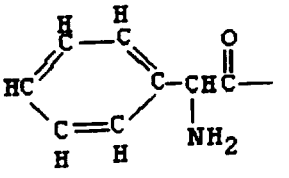
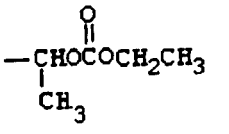
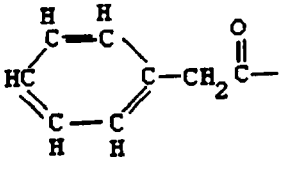
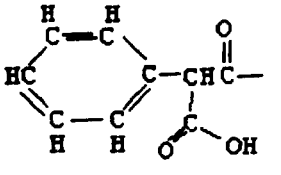
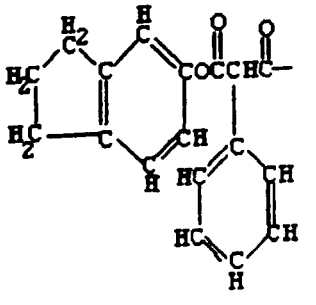
APPENDIX 1 Structures of 70 β-Lactam Antibiotics

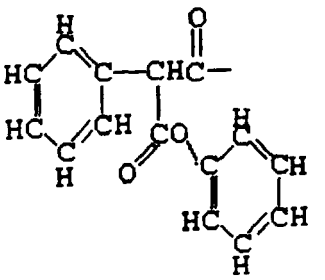
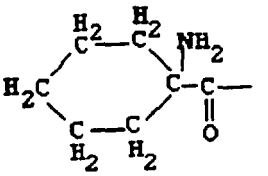
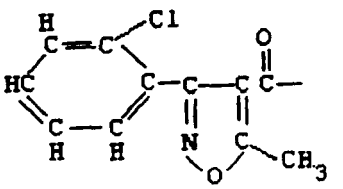
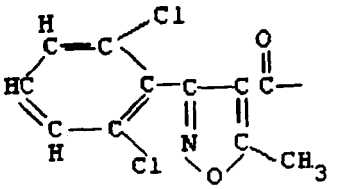
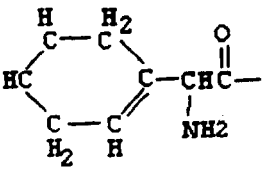
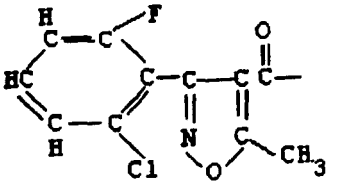
PENICILLINS

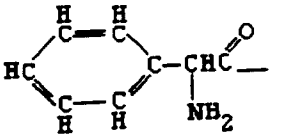
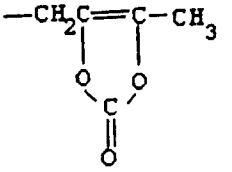
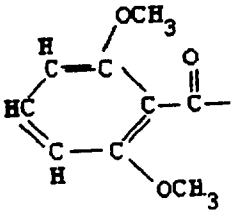
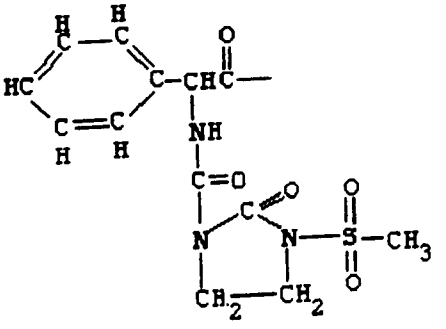
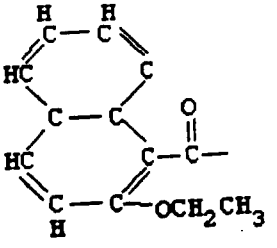
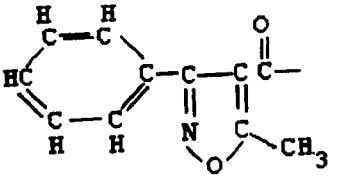
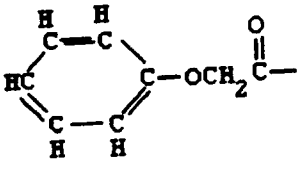
Common Nucleus

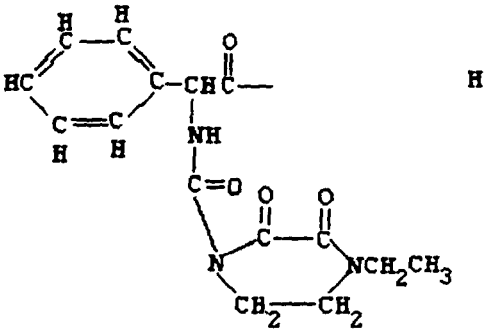
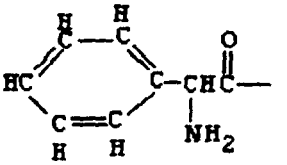
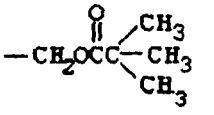
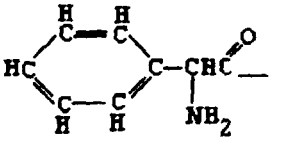
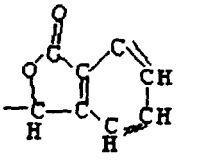
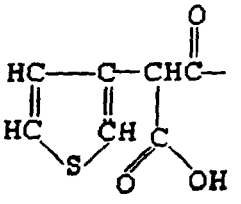
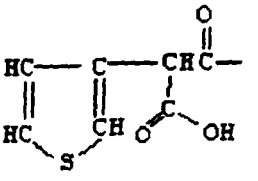


Name	R1	R3	R4
1-Amdinocillin (Mecillinam)		H	H
2-Amdinocillin-pivoxil (Pivmecillinam)		H	
3-Amoxycillin		H	H
4-Ampicillin		H	H
5-Apalcillin		H	H

Name	R1	R3	R4
6-Azidocillin		H	H
7-Azlocillin		H	H
8-Bacampicillin		H	
9-Benzylpenicillin		H	H
10-Carbenicillin		H	H
11-Cardinacillin		H	H

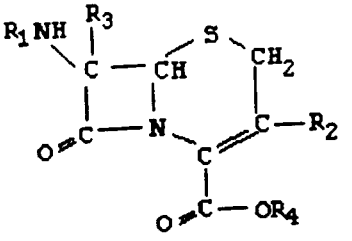
Name	R1	R3	R4
12-Carfecillin		H	H
13-Cyclacillin		H	H
14-Cloxacillin		H	H
15-Dicloxacillin		H	H
16-Epicillin		H	H
17-Flucloxacillin		H	H

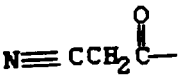
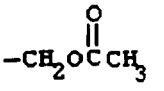
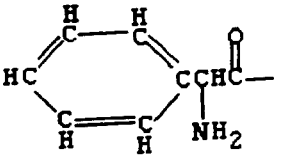
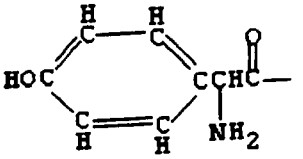
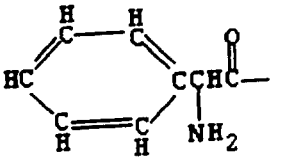
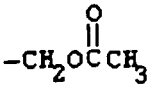
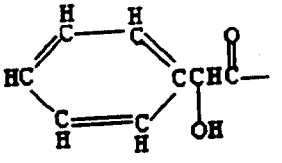
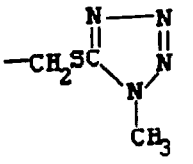
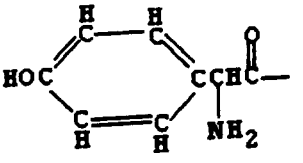
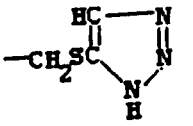
Name	R1	R3	R4
18-Lenampicillin		H	
19-Methicillin		H	H
20-Mezlocillin		H	H
21-Nafcillin		H	H
22-Oxacillin		H	H
23-Phenoxymethyl Penicillin		H	H

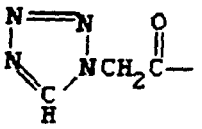
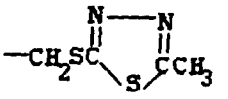
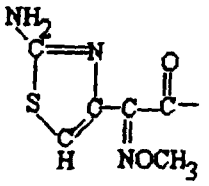
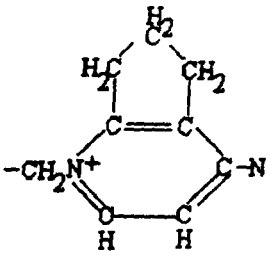
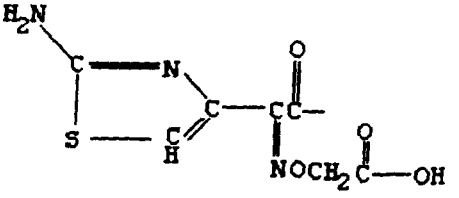
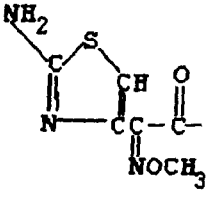
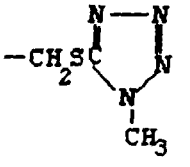
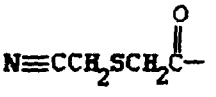
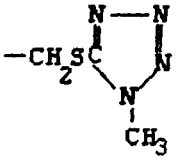
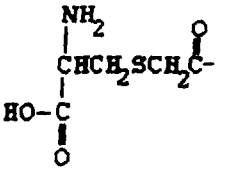
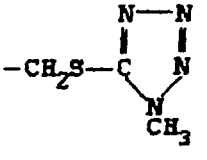
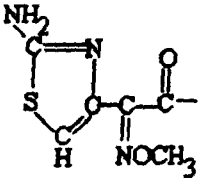
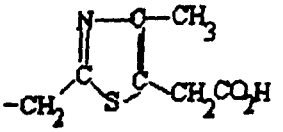
Name	R1	R3	R4
24-Piperacillin		H	H
25-Pivampicillin		H	
26-Talampicillin		H	
27-Temocillin		$-OCH_3$	H
28-Ticacillin		H	H

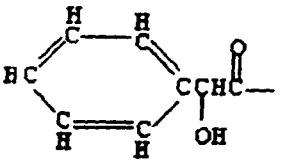
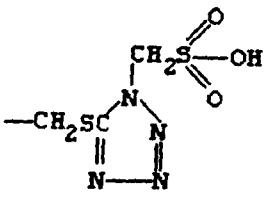
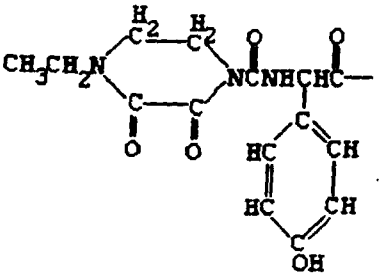
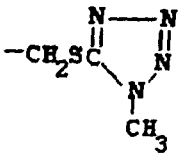
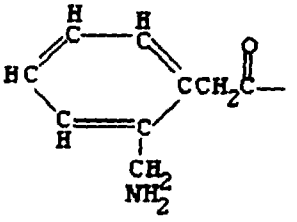
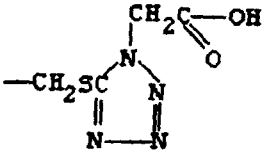
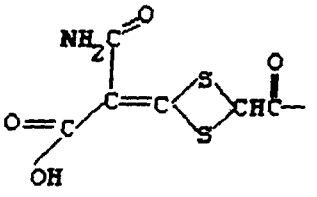
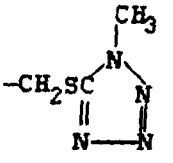
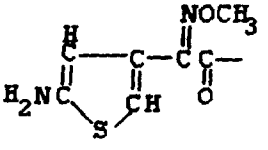
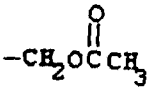
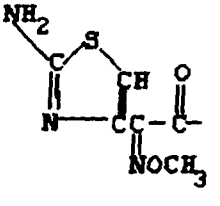
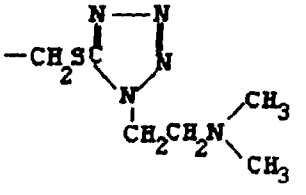
CEPHALOSPORINS

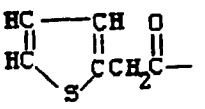
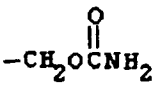
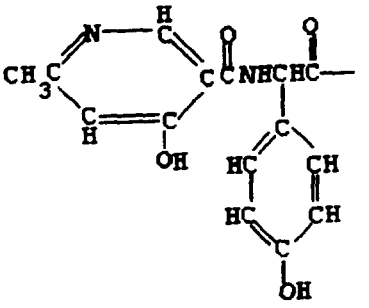
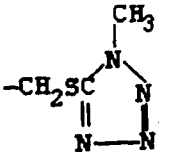
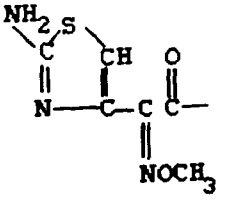
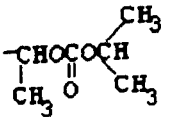
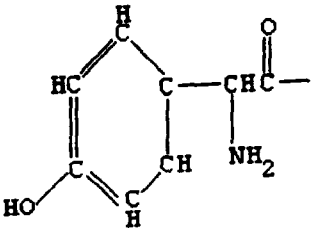
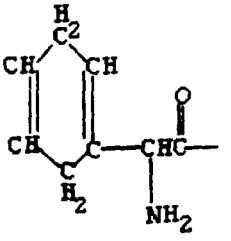
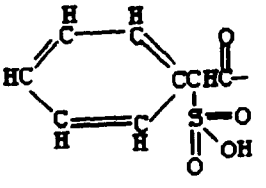
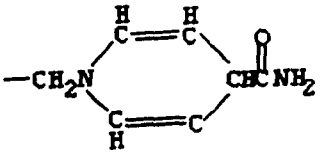
Common Nucleus

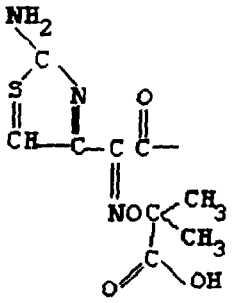
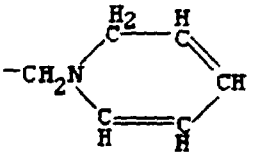
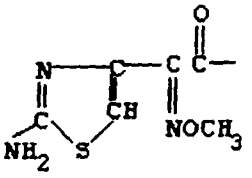
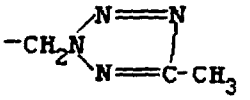
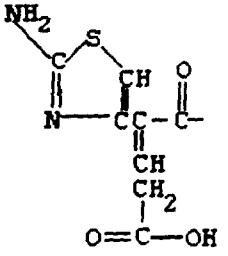
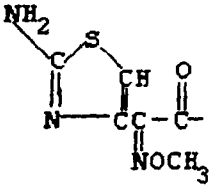
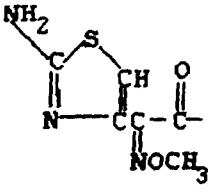
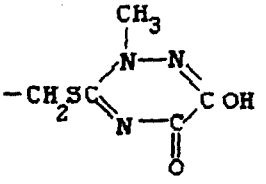
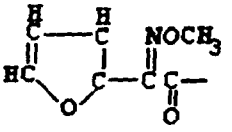
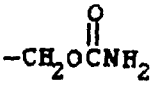


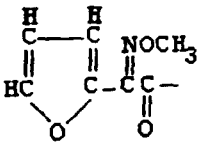
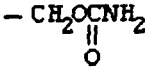
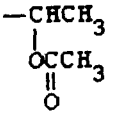
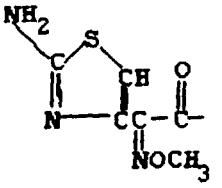
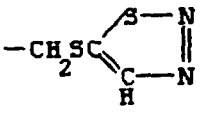
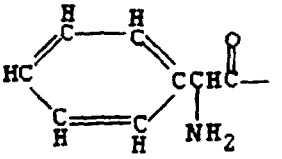
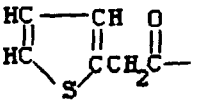
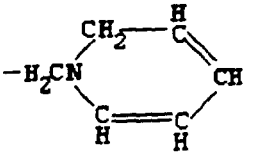
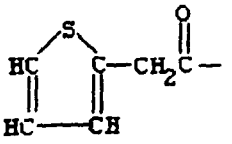
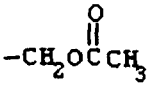
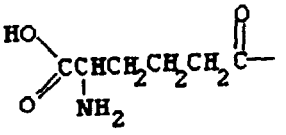
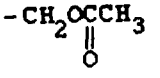
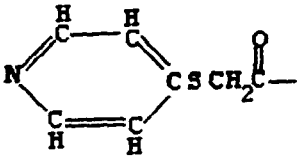
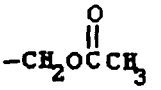
Name	R_1	R_2	R_3	R_4
29-Cefacetrile			H	H
30-Cefaclor		—Cl	H	H
31-Cefadroxil		$-CH_3$	H	H
32-Cefaloglycin			H	H
33-Cefamandole			H	H
34-Cefatrizine			H	H

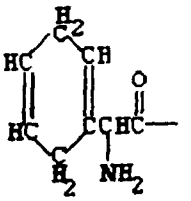
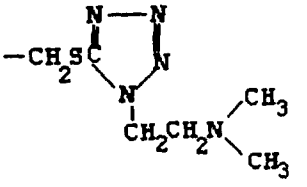
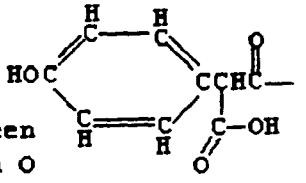
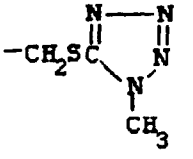
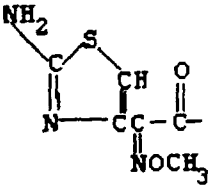
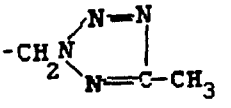
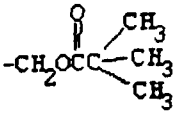
Name	R_1	R_2	R_3	R_4
35-Cefazolin			H	H
36-Cefepirome			H	H
37-Cefixime			H	H	—CH=CH$_2$
38-Cefmenoxime			H	H
39-Cefmetazole			—OCH$_3$	H
40-Cefminox			—OCH$_3$	H
41-Cefodizime			H	H

Name	R_1	R_2	R_3	R_4
42-Cefonicid			H	H
43-Cefoperazone			H	H
44-Ceforanide			H	H
45-Cefotetan			H	H
46-Cefotaxime			H	H
47-Cefotiam			H	H

Name	R_1	R_2	R_3	R_4
48-Cefoxitin			$-OCH_3$	H
49-Cefpiramide			H	H
50-Cefpodoxime-proxetil		$-CH_2OCH_3$	H	
51-Cefprozil		H	H	$-CH=CHCH_3$
52-Cefroxadine		$-OCH_3$	H	H
53-Cefsulodin			H	H

Name	R_1	R_2	R_3	R_4
54-Ceftazidime			H	H
55-Cefteram			H	H
56-Ceftibuten		H	H	H
57-Ceftizoxime		H	H	H
58-Ceftriaxone			H	H
59-Cefuroxime			H	H

Name	R_1	R_2	R_3	R_4
60-Cefuroxime-Axetil			H	
61-Cefuzonom			H	H
62-Cephalexin		$-CH_3$	H	H
63-Cephaloridine			H	H
64-Cephalothin			H	H
65-Cephamycin			$-OCH_3$	H
66-Cephapirin			H	H

Name	R_1	R_2	R_3	R_4
67-Cephradine		$-CH_3$	H	H
68-Flomoxef NOTE; S of the B ring has been replaced with an O.			$-OCH_3$	H
69-Latamoxef NOTE; S in the B ring has been replaced with O in this compound.			$-OCH_3$	H
70-T-2588			H	

APPENDIX 2 SMILES Codes of 70 β-Lactam Antibiotics

PENICILLINS

Amdinocillin (Mecillinam) CC3(C)SC2C(N=CN1CCCCCC1)C(=O)N2C3C(O)=O

Amdinocillin-pivoxil (Pivmecillinam)
CC(C)(C)OC(=O)COC(=O)C1N3C(SC1(C)C)C(N=CN2CCCCCC2)C3=O

Amoxycillin CC3(C)SC2C(NC(=O)C(N)c1ccc(O)cc1)C(=O)N2C3C(O)=O

Ampicillin CC3(C)SC2C(NC(=O)C(N)c1ccccc1)C(=O)N2C3C(O)=O

Apalcillin
CC5(C)SC4(NC(=O)C(NC(=O)c2cnc1ccccnc1c2O)c3ccccc3)C(=O)N4C5C(O)=O

Azidocillin CC3(C)SC2C(NC(=O)C(N=N=N)c1ccccc1)C(=O)N2C3C(O)=O

Azlocillin
CC4(C)SC3C(NC(=O)C(NC(=O)N1CCNC1=O)c2ccccc2)C(=O)N3C4C(O)=O

Bacampicillin CCOC(=O)OC(C)0C(=O)C1N3C(SC1(C)C)C(NC(=O)C(N)c2ccccc2)C3=O

Benzylpenicillin CC3(C)SC2C(NC(=O)Cc1ccccc1)C(=O)N2C3C(O)=O

Carbenicillin CC3(C)SC2C(NC(=O)C(C(O)=O)c1ccccc1)C(=O)N2C3C(O)=O

Cardinacillin
CC5(C)SC4C(NC(=O)C(C(=O)Oc2ccc1CCCc1c2)c3ccccc3)C(=O)N4C5C(O)=O

Carfecillin CC4(C)SC3C(NC(=O)C(C(=O)Oc1ccccc1)c2ccccc2)C(=O)N3C4C(O)=O

Ciclacillin CC3(C)SC2C(NC(=O)C1(N)CCCCC1)C(=O)N2C3C(O)=O

Cloxacillin Cc2onc(c1ccccc1Cl)c2C(=O)NC4C3SC(C)(C)C(N3C4=O)C(O)=O

Dicloxacillin Cc1onc(c1C(=O)NC3C2SC(C)(C)C(N2C3=O)C(O)=O)c4c(Cl)cccc4Cl

Epicillin CC3(C)SC2C(NC(=O)C(N)C1=CCC=CC1)C(=O)N2C3C(O)=O

Flucloxacillin Cc1onc(c1C(=O)NC3C2SC(C)(C)C(N2C3=O)C(O)=O)c4c(F)cccc4Cl

Lenampicillin
c1ccccc1C(N)C(=O)NC2C(=O)N3C(C(=O)OCC4=C(C)OC(=O)O4)C(C)(C)SC32

Methicillin COc1cccc(OC)c1C(=O)NC3C2SC(C)(C)C(N2C3=O)C(O)=O

Mezlocillin
CC4(C)SC3C(NC(=O)C(NC(=O)N1CCN(C1=O)S(C)(=O)=O)c2ccccc2)C(=O)N3C4C(O)=O

Nafcillin CCOc1ccc2ccccc2c1(C(=O)NC3C(=O)N4C(C(=O)O)C(C)(C)SC43)

Oxacillin CC1=C(C(=O)NC2C(=O)N3C(C(=O)O)C(C)(C)SC32)C(c4ccccc4)=NO1

Phenoxymethylpen. CC3(C)SC2C(NC(=O)COc1ccccc1)C(=O)N2C3C(O)=O

Piperacillin
CCN1C(=O)C(=O)N(C(=O)NC(c2ccccc2)C(=O)NC3C(=O)N4C(C(=O)O)C(C)(C)SC43)CC1

Pivampicillin CC(C)(C)C(=O)OCOC(=O)C1N3C(SC1(C)C)C(NC(=O)C(N)c2ccccc2)C3=O

Talampicillin
CC3(C)SC2C(NC(=O)C(N)c1ccccc1)C(=O)N2C3C(=O)OC4OC(=O)c5ccccc45

Temocillin COC3(NC(=O)C(C(O)=O)c1ccsc1)C2SC(C)(C)C(N2C3=O)C(O)=O

Ticarcillin CC3(C)SC2C(NC(=O)C(C(O)=O)C1=CSC=C1)C(=O)N2C3C(O)=O

CEPHALOSPORINS

Cefacetrile CC(=O)OCC1=C(N2C(SC1)C(NC(=O)CC#N)C2=O)C(O)=O

Cefaclor NC(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)Cl)c3ccccc3

Cefadroxil NC(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)C)c3ccc(O)cc3

Cefaloglycin NC(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)COC(=O)C)c3ccccc3

Cefamandole CN1N=NN=C1(SCC2=C(C(=O)O)N3C(=O)C(NC(=O)C(O)c4ccccc4)C3SC2)

Cefatrizine NC(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)CSC3=CN=NN3)c4ccc(O)cc4

Cefazolin CC1=NN=C(SCC2=C(C(=O)O)N3C(=O)C(NC(=O)CN4N=NN=C4)C3SC2)S1

Cefepirome
CON=C(C1=CSC(N)=N1)C(=O)NC2C(=O)N3C(C(=O)O)=C(Cn4c5CCCc5c(N)cc4)CSC32

Cefixime NC1=NC(=CS1)C(=NOCC(O)=O)C(=O)NC3C2SCC(=C(N2C3=O)C(=O)OC=C)

Cefmenoxime
CON=C(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)CSC3=NN=NN3C)C4=CSC(N)=N4

Cefmetazole N#CCSCC(=O)NC1C(=O)N2C(C(=O)O)=C(CSC3=NN=NN3(C))CSC21

Cefminox
CN1N=NN=C1(SCC3=C(C(=O)O)N4C(=O)C(OC)(NC(=O)CSCC(N)C(=O)O)C4SC3)

Cefodizime
CON=C(C1=CSC(N)=C1)C(=O)NC2C(=O)N3C(C(=O)O)=C(CC4=NC(C)=C(CC(=O)O)S4)
CSC32

Cefonicid
OS(=O)(=O)CN1N=NN=C1(SCC2=C(C(=O)O)N3C(=O)C(NC(=O)C(O)c4ccccc4)C3SC2)

Cefoperazone
CN1N=NN=C1(SCC2=C(C(=O)O)N3C(=O)C(NC(=O)C(c4ccc(O)cc4)NC(=O)N5C(=O)C
(=O)N(CC)CC5)C3SC2)

Ceforanide
NCc1ccccc1CC(=O)NC3C2SCC(=C(N2C3=O)C(O)=O)CSC4=NN=NN4CC(O)=O

Cefotetan
CN1N=NN=C1(SCC2=C(C(=O)O)N3C(=O)C(OC)(NC(=O)C4SC(=C(C(=O)N)C(=O)O)S4)
C3SC2)

Cefotaxime
CON=C(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)COC(C)=O)C3=CS=C(N)N3

Cefotiam
CN(C)CCN1N=NN=C1(SCC2=C(C(=O)O)N3C(=O)C(NC(=O)CC4=CSC(N)=N4)C3SC2)

Cefoxitin NC(=O)OCC1=C(C(=O)O)N2C(=O)C(OC)(NC(=O)CC3=CC=CS3)C2SC1

Cefpiramide
CN1N=NN=C1(SCC2=C(C(=O)O)N3C(=O)C(NC(=O)C(c4ccc(O)cc4)NC(=O)C5=C
N=C(C)C=C5(O))C3SC2)

Cefpodoxime-proxetil
COCC1=C(C(=O)OC(C)OC(=O)OCC(C))N2C(=O)C(NC(=O)C(=NOC)C3=CSC
(N)=N3)C2SC1

Cefprozil CC=COC(=O)C1=CCSC2C(NC(=O)C(N)c3ccc(O)cc3)C(=O)N21

Cefroxadine COC1=C(C(=O)O)N2C(=O)C(NC(=O)C(N)C3=CCC=CC3)C2SC1

Cefsulodin
OS(=O)(=O)C(c1ccccc1)C(=O)NC2C(=O)N3C(C(=O)O)C(Cn4ccc(C(=O)N)cc4)CSC32

Ceftazidime
OC(=O)C(C)(C)ON=C(C1=CSC(N)=N1)C(=O)NC2C(=O)N3C(C(=O)O)=C(CN4=CC=CC=C
4)CSC32

Cefteram
CON=C(C1=CSC(N)=N1)C(=O)NC2C(=O)N3C(C(=O)O)=C(CN4N=NC(C)=N4)
CSC32

Ceftibuten NC1=NC(=CS1)C(=CCC(O)=O)C(=O)NC3C2SCC=C(N2C3=O)C(O)=O

Ceftizoxime CON=C(C(=O)NC2C1SCC=C(N1C2=O)C(O)=O)C3=CSC(N)=N3

Ceftriaxone
CON=C(C1=CSC(N)=N1)C(=O)NC2C(=O)N3C(C(=O)O)=C(CSC4=NC(=O)C(O)NN4(C))
CSC32

Cefuroxime CON=C(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)COC(N)=O)C3=CC=CO3

Cefuroxime-axetil
CON=C(C(=O)NC2C1SCC(=C(N1C2=O)C(=O)OC(C)=O)COC(N)=O)C3=CC=CO3

Cefuzonam
CON=C(C(=O)NC2C1SCC(=C(N1C2=O)C(O)=O)CSC3=CN=NS3)C4=CSC(N)=N4

Cephalexin CC1=C(N3C(SC1)C(NC(=O)C(N)c2ccccc2)C3=O)C(O)=O

Cephaloridine OC(=O)C1=C(CN2=CC=CC=C2)CSC3C(NC(=O)CC4=CC=CS4)C(=O)N31

Cephalothin CC(=O)OCC1=C(N3C(SC1)C(NC(=O)CC2=CC=CS2)C3=O)C(O)=O

Cephamycin COC2(NC(=O)CCCC(N)C(O)=O)C1SCC(=C(N1C2=O)C(O)=O)COC(C)=O

Cephapirin CC(=O)OCC1=C(N3C(SC1)C(NC(=O)CSC2=CC=NC=C2)C3=O)C(O)=O

Cephradine CC1=C(N3C(SC1)C(NC(=O)C(N)C2=CCC=CC2)C3=O)C(O)=O

Flomoxef COC2(NC(=O)CSC(F)F)C1OCC(=C(N1C2=O)C(O)=O)CSC3=NN=NN3CCO

Latamoxef
COC3(NC(=O)C(C(O)=O)c1ccc(O)cc1)C2OCC(=C(N2C3=O)C(O)=O)CSC4=NN=NN4C

T-2588 CON=C(C(=O)NC2C1SCC(=C(N1C2=O)C(=O)OC(C)(C)C)CN3N=NC(C)=N3)
C4=CSC(N)=N4

APPENDIX 3 Physico-Chemical and Structural Parameters Generated

- ClogP
- Total Surface Area
- Accessible Surface Area of whole molecule
- Electrostatic potential of whole molecule
- Whole molecule Ionisization potential
- Whole molecule Superdelocalizability parameters
- Pka
- Log water solubility of whole molecule
- Topological indices of Randic, Weiner & Bonchev of whole molecule
- Whole molecule Molecular volume
- Whole molecule Flexiblility
- Whole molecule Hydrogen Acceptor ability (Yangs)
- Whole molecule Total Dipole moment
- Whole molecule Dipole Vectors (X,Y,Z)
- Whole molecule Kier Connectivity indices
- Number of Carbon atoms in whole molecule
- Number of Nitrogen atoms in whole molecule
- Number of Oxygen atoms in whole molecule
- Number of Sulphur atoms in whole molecule
- Whole molecule Number methyl groups
- Whole molecule Number hydroxyl groups
- Whole molecule Homo energy
- Whole molecule Homo-Lumo Energy
- Flexibility of the whole molecule
- Total Lipole of whole molecule
- Whole molecule Hydrophilicity
- Verloop Parameters for whole molecule measured from the first methyl group attached to the Thiazoline or Dihydrothiazone ring of molecule
- Verloop Parameters for whole molecule measured from the carbonyl bond of the β-ring
- Whole molecule Carbo shape similarity index of compounds with benzylpenicillin
- Whole molecule Carbo indices of Lipophilicity similarity to benzylpenicillin
- Whole molecule Carbo indices of Refractivity similarity to benzylpenicillin
- Whole molecule Carbo indices of shape dissimilarity to benzylpenicillin
- Whole molecule Carbo indices of shape similarity to cefadroxil
- Whole molecule Carbo indices of shape dissimilarity to cefadroxil

- Whole molecule Carbo indices of shape similarity to cefmetazole
- Whole molecule Carbo indices of shape dissimilarity to cefmetazole
- Whole molecule Carbo indices of shape similarity to cefacetrile
- Whole molecule Carbo indices of shape dissimilarity to cefacetrile
- Whole molecule Carbo indices of shape similarity to cefsulodin
- Whole molecule Carbo indices of shape dissimilarity to cefsulodin
- Whole molecule Carbo indices of shape similarity to cefepirome
- Whole molecule Carbo indices of shape dissimilarity to cefepirome
- Whole molecule Carbo indices of shape similarity to cefuzonam
- Whole molecule Carbo indices of shape dissimilarity to cefuzonam
- Whole molecule Carbo indices of shape similarity to cefroxadine
- Whole molecule Carbo indices of shape dissimilarity to cefroxadine
- Whole molecule Carbo indices of shape similarity to ceftibuten
- Whole molecule Carbo indices of shape dissimilarity to ceftibuten
- Whole molecule Carbo indices of shape similarity to cefatrizine
- Whole molecule Carbo indices of shape dissimilarity to cefatrizine
- Whole molecule Carbo indices of shape similarity to piperacillin
- Whole molecule Carbo indices of shape dissimilarity to piperacillin
- Whole molecule Carbo indices of shape similarity to methicillin
- Whole molecule Carbo indices of shape dissimilarity to methicillin
- Charge on Nitrogen of β-lactam ring
- Charge on Carbonyl carbon of β-lactam ring
- Charge on Carbonyl oxygen of β-lactam ring
- Charge on first Nitrogen of R1 substituent
- Charge on first Carbonyl carbon of R1 substituent
- Charge on first Carbonyl oxygen of R1 substituent
- Charge on first Carbon of R2 substituent
- Sum of charges on cabonyl oxygens in whole molecule
- Number of carbonyl oxygens in whole molecule
- Surface Area of R1 substituent
- Hydrogen Acceptor ability of R1 substituent (Yangs)
- Hydrogen Doanting ability of R1 substituent (Yangs)
- Flexibility of R1 substituent
- Kier Connectivity Indices of R1 substituent
- Number of Carbon atoms in the R1 substituent
- Number of Nitrogen atoms in the R1 substituent

- Number of Oxygen atoms in the R1 substituent
- Number of Sulphur atoms in the R1 substituent
- Number of Methyl groups in the R1 substituent
- Number of Hydroxyl groups in the R1 substituent
- Number of Amino groups in the R1 substituent
- Surface Area of R2 substituent
- Molar Volume of R2 substituent
- Verloop parameters of R2 substituent
- Flexibility of R2 substituent
- Kier Connectivity Indices of R2 substituent
- Number of Carbon atoms in the R2 substituent
- Number of Nitrogen atoms in the R2 substituent
- Number of Oxygen atoms in the R2 substituent
- Number of Sulphur atoms in the R2 substituent
- Number of Methyl groups in the R2 substituent
- Number of Hydroxyl groups in the R2 substituent
- Number of Amino groups in the R2 substituent
- R3 indicator variable
- R4 indicator variable
- Penicillin or Cephalosporin indicator variable
- Oral or parenteral indicator variable

APPENDIX 4 Compounds, Descriptor Values, Actual-Predicted & Residual Values, Correlation Matrices and Validity Plots for QSARs Generated in 9.3

ALL ARS

Model 9.1 All β-Lactams

	Flex R_1	# Amino R_1	R_3I	R_4I	#O	SS Cefmet	SS Fluc	SS Cephap	Actual Log_{10} AR	Predicted Log_{10} AR	Residual
1	6.370	0	1	1	3	0.745	0.776	0.757	0.815	1.006	-0.191
2	6.370	0	1	2	5	0.768	0.672	0.640	0.788	0.686	0.092
3	5.876	1	1	1	5	0.678	0.706	0.605	1.196	0.832	0.364
4	5.511	1	1	1	4	0.682	0.714	0.637	1.228	0.828	0.400
5	9.446	0	1	1	6	0.606	0.652	0.616	0.998	0.915	0.083
7	9.007	0	1	1	6	0.724	0.739	0.697	1.243	0.966	0.277
8	5.511	1	1	2	7	0.711	0.639	0.609	0.951	0.894	0.257
9	5.173	0	1	1	4	0.675	0.686	0.608	0.826	0.979	-0.153
10	6.588	0	1	1	6	0.692	0.661	0.623	1.269	1.001	0.268
11	8.950	0	1	1	6	0.549	0.675	0.610	1.114	1.024	0.090
12	9.116	0	1	1	6	0.620	0.744	0.690	0.703	1.079	-0.375
13	4.905	1	1	1	4	0.717	0.688	0.733	1.001	0.915	0.087
14	6.426	0	1	1	5	0.802	0.889	0.739	1.568	1.134	0.435
15	7.320	0	1	1	5	0.752	0.682	0.745	0.840	0.980	-0.140
16	5.969	1	1	1	4	0.678	0.751	0.625	0.872	0.821	0.050
17	6.801	0	1	1	5	0.767	1.000	0.777	1.564	1.286	0.279
18	5.511	1	1	2	7	0.727	0.685	0.717	0.590	0.838	-0.248
19	7.101	0	1	1	6	0.762	0.655	0.750	1.214	1.030	0.184
20	10.78	0	1	1	8	0.751	0.646	0.713	0.897	0.901	-0.005
21	6.679	0	1	1	5	0.771	0.734	0.756	1.089	1.051	0.038
22	5.552	0	1	1	5	0.698	0.717	0.642	1.305	1.058	0.247
23	6.405	0	1	1	5	0.741	0.725	0.689	0.430	1.017	-0.588
24	11.69	0	1	1	7	0.750	0.773	0.757	0.761	0.936	-0.175
25	5.511	1	1	2	6	0.701	0.721	0.709	1.050	0.823	0.227
26	5.511	1	1	2	6	0.757	0.682	0.816	1.316	0.851	0.465
27	6.426	0	2	1	7	0.663	0.763	0.708	0.538	0.855	-0.348
28	6.426	0	1	1	6	0.706	0.733	0.637	1.130	1.068	0.062

Continued over page

Model 9.1 Continued

	Flex R_1	# Amino R_1	R_3I	R_4I	# O	SS Cefmet	SS Fluc	SS Cephap	Actual Log10 AR	Predicted Log10 AR	Residual
29	5.106	0	1	1	6	0.708	0.699	0.687	1.125	1.176	-0.051
30	5.511	0	1	1	4	0.676	0.712	0.639	0.489	0.836	-0.348
31	5.876	1	1	1	5	0.685	0.705	0.634	0.867	0.857	0.010
33	5.511	1	1	1	5	0.598	0.661	0.571	0.930	1.052	-0.121
34	5.876	0	1	1	5	0.619	0.655	0.621	1.274	0.880	0.395
35	3.976	1	1	1	4	0.877	0.733	0.743	0.769	1.007	-0.239
36	7.486	0	1	1	5	0.751	0.729	0.740	1.176	0.825	0.351
37	9.799	1	1	2	7	0.782	0.839	0.746	0.384	0.672	-0.288
38	7.486	1	1	1	5	0.777	0.688	0.736	0.493	0.757	-0.264
39	9.765	1	2	1	5	1.000	0.750	0.742	0.041	0.186	-0.145
40	11.22	0	2	1	7	0.658	0.734	0.685	0.324	0.375	-0.051
41	7.486	1	1	1	7	0.789	0.692	0.640	0.556	0.763	-0.207
42	5.511	1	1	1	8	0.652	0.698	0.573	1.114	1.123	-0.100
43	11.69	0	1	1	7	0.707	0.626	0.621	1.187	0.711	0.477
44	6.773	0	1	1	6	0.609	0.686	0.568	0.305	0.865	-0.560
45	7.734	1	2	1	8	0.663	0.709	0.680	0.678	0.617	0.061
46	7.617	1	1	1	7	0.733	0.700	0.667	0.667	0.859	-0.193
47	5.226	1	1	1	4	0.638	0.696	0.698	0.605	0.954	-0.349
48	5.005	1	2	1	7	0.776	0.751	0.727	0.792	0.850	-0.058
49	10.22	0	1	1	7	0.718	0.622	0.616	0.587	0.780	-0.194
50	7.486	0	1	2	9	0.694	0.642	0.732	0.994	0.870	0.124
51	5.876	1	1	2	5	0.789	0.784	0.713	0.274	0.688	-0.414
52	5.969	1	1	1	5	0.678	0.734	0.655	0.886	0.907	-0.021
53	6.767	1	1	1	8	0.660	0.662	0.605	1.342	1.137	0.205
54	9.793	0	1	0	7	0.691	0.705	0.674	0.890	0.992	-0.102
55	7.486	1	1	1	5	0.808	0.714	0.692	0.505	0.689	-0.184
56	8.697	1	1	1	6	0.714	0.685	0.711	0.924	0.792	0.133
57	7.486	1	1	1	5	0.790	0.851	0.718	1.036	0.851	0.185
58	7.486	1	1	1	7	0.669	0.722	0.633	0.733	0.921	-0.188
60	6.569	0	1	2	10	0.647	0.703	0.648	0.952	1.175	-0.223
61	7.486	1	1	1	5	0.621	0.742	0.691	0893	0.932	-0.039

Continued over page

Model 9.1 continued

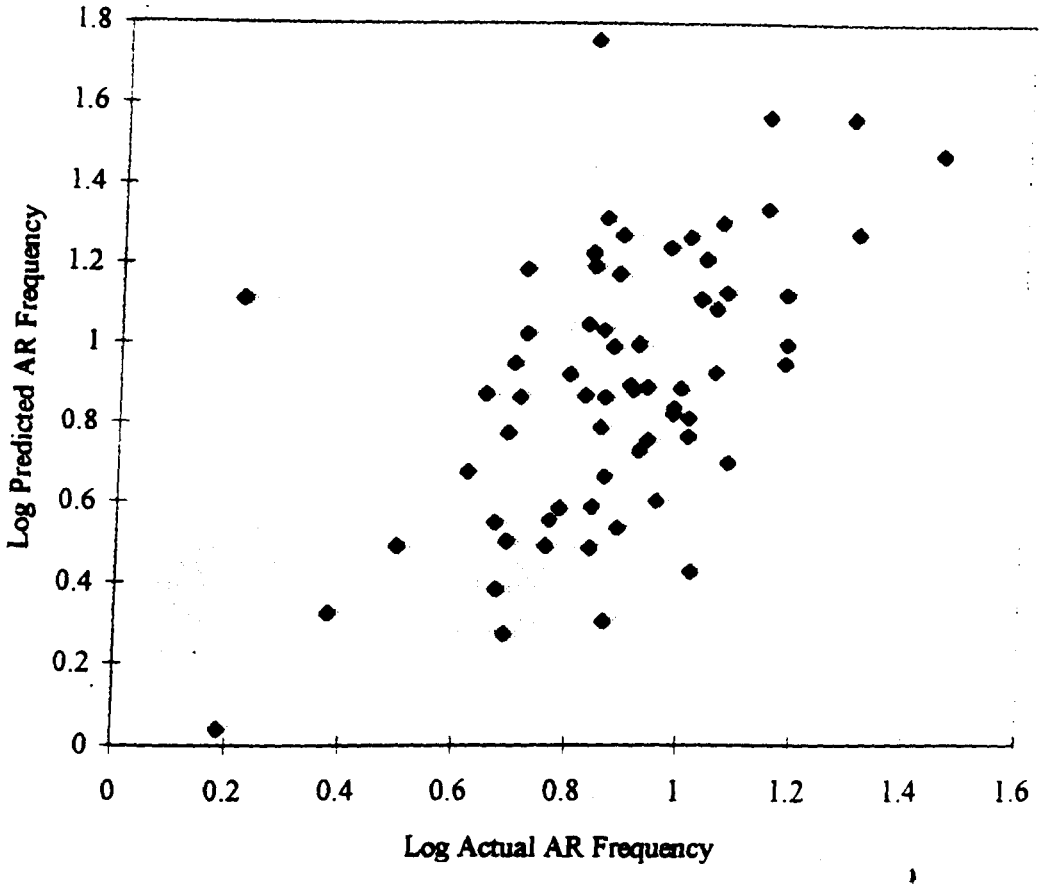
	Flex R_1	# Amino R_1	R_3I	R_4I	# O	SS Cefmet	SS Fluc	SS Cephap	Actual Log10 AR	Predicted Log10 AR	Residual
62	5.511	1	1	1	4	0.738	0.715	0.734	1.176	0.876	0.300
63	5.005	0	1	0	4	0.797	0.780	0.761	1.279	1.300	-0.022
64	5.005	0	1	1	6	0.754	0.721	0.715	1.000	1.178	-0.178
65	9.869	0	2	1	9	0.800	0.768	0.704	0.875	0.645	0.230
66	7.038	0	1	1	6	0.742	0.777	1.000	1.475	1.447	0.028
67	5.969	1	1	1	4	0.662	0.730	0.524	0.867	0.706	0.161
68	9.759	0	2	1	6	0.688	0.710	0.661	0.494	0.494	4.94^{-4}
69	6.953	0	2	1	8	0.716	0.713	0.622	1.025	0.715	0.310
70	7.486	1	1	2	7	0.651	0.652	0.618	0.511	0.667	-0.115

Correlation Matrix Model 9.1

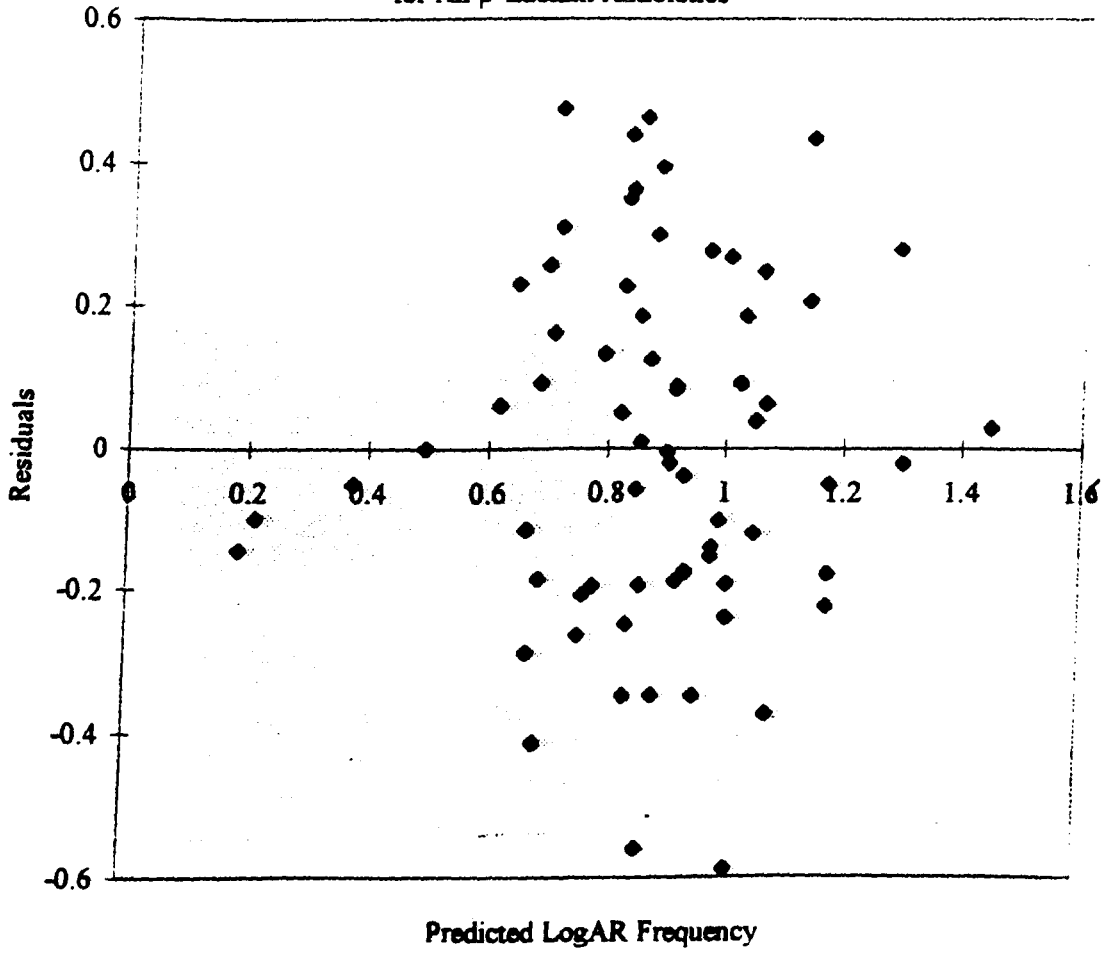
	Flex R_1	# Amino R_1	R_3I	R_4I	# Ox	SS Cefmet	SS Fluc	SS Cephap	Log_{10} AR
Flex R_1	1.0	-0.132	0.254	-0.121	0.438	0.030	-0.043	0.039	-0.251
# Amino R_1		1.0	-0.168	0.234	-0.130	-0.150	-0.082	-0.160	-0.260
R_3I			1.0	-0.108	0.323	0.160	0.117	0.043	-0.338
R_4I				1.0	0.284	0.009	-0.127	0.026	-0.167
# Ox					1.0	-0.136	-0.230	-0.090	-0.053
SS Cefmet						1.0	0.368	0.543	-0.136
SS Fluc							1.0	0.435	0.124
SS Cephap								1.0	0.149
Log_{10} AR									1.0

Model 9.1 Validity Plots

Plot of Actual Verses Predicted AR Frequencies for All β-Lactam Antibiotics



Predicted LogAR Values Against Residual Values for All β-Lactam Antibiotics



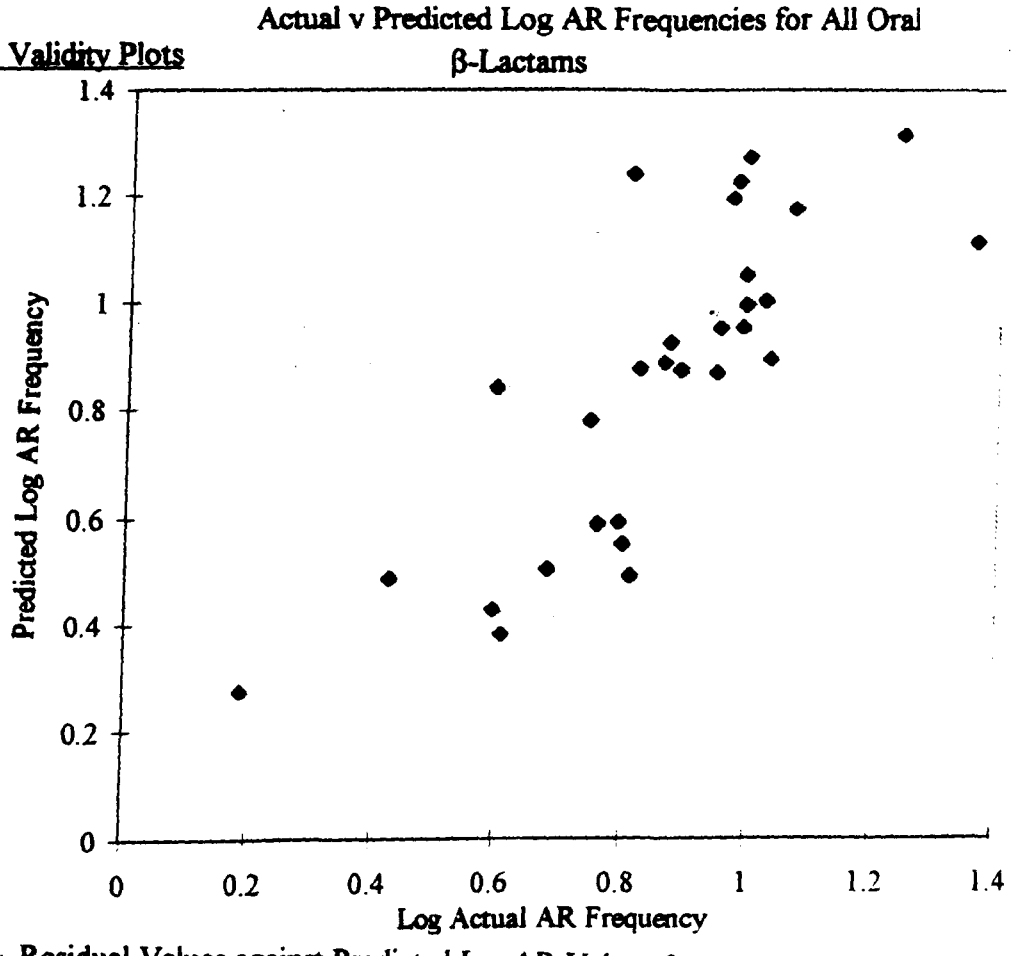
Model 9.2 Oral β-Lactams

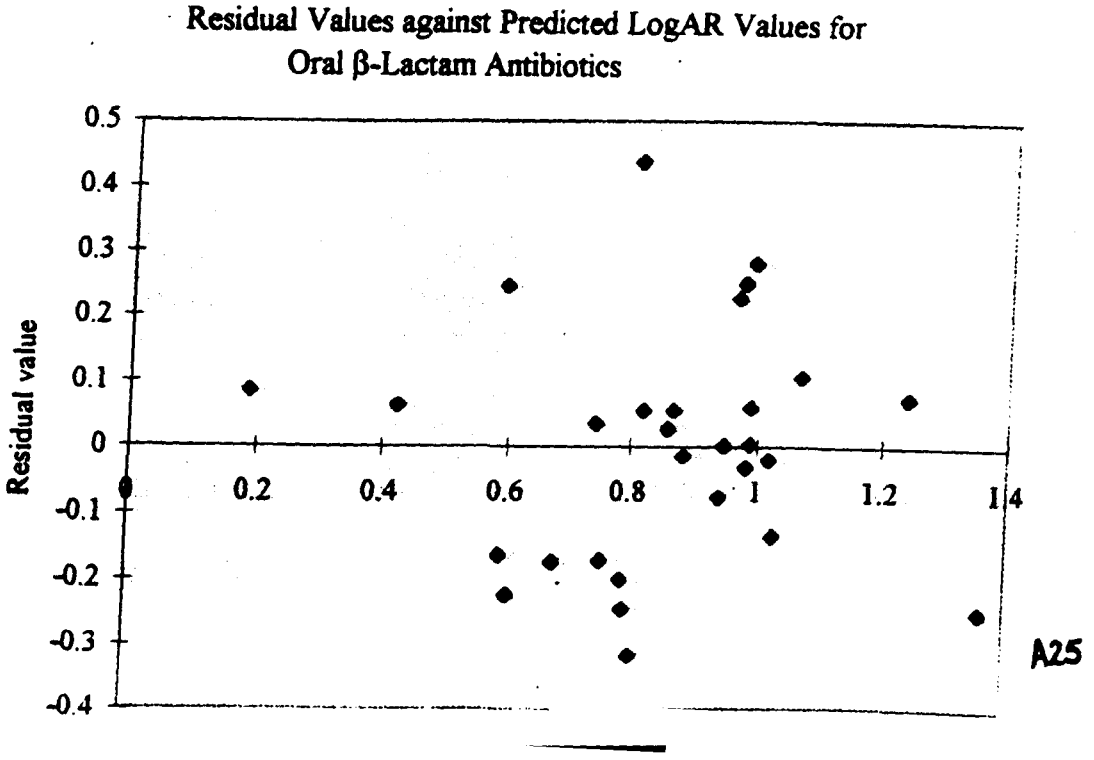
	CLogP	DVZ	SS Cefmet	SS Cephap	Actual Log_{10} AR	Predicted Log_{10} AR	Residual
2	-1.988	0.43	0.768	0.640	0.788	0.743	0.035
3	-1.936	0.83	0.678	0.605	1.196	0.966	0.230
4	-1.269	1.35	0.682	0.637	1.228	0.975	0.253
7	0.600	1.54	0.724	0.697	1.243	0.805	0.438
8	-2.038	4.3	0.711	0.609	0.951	0.949	0.002
11	-1.901	-0.96	0.549	0.610	1.114	1.363	-0.249
13	-1.123	-0.64	0.717	0.733	1.001	1.021	-0.020
15	2.269	-2.03	0.752	0.745	0.840	0.593	0.247
16	-1.356	-2.56	0.678	0.625	0.872	0.885	-0.014
18	-0.274	-4.51	0.727	0.717	0.590	0.791	-0.201
23	1.879	0.56	0.741	0.689	0.430	0.593	-0.164
25	-0.316	1.36	0.701	0.709	1.050	0.989	0.061
26	-2.874	-0.40	0.757	0.816	1.316	1.241	0.075
30	4.115	-2.59	0.676	0.639	0.489	0.424	0.064
31	-1.350	0.31	0.685	0.634	0.867	0.944	-0.077
34	0.000	-0.56	0.619	0.621	1.274	0.991	0.283
37	1.311	-0.85	0.782	0.746	0.384	0.608	-0.224
38	0.979	7.44	0.777	0.736	0.493	0.810	-0.317
49	-2.082	-4.77	0.718	0.616	0.587	0.758	-0.172
50	-0.374	-2.33	0.694	0.732	0.994	0.990	0.004
51	3.120	-0.18	0.789	0.713	0.274	0.188	0.086
52	-1.791	-0.83	0.678	0.655	0.886	0.859	0.027
55	-1.933	-1.62	0.808	0.692	0.505	0.679	-0.174
56	0.000	-1.12	0.714	0.711	0.924	0.868	0.056
60	0.251	1.69	0.647	0.648	0.952	0.985	-0.032
61	2.544	5.01	0.621	0.691	0.893	1.030	-0.17
62	-1.673	2.35	0.738	0.734	1.176	1.068	0.108
65	-2.054	1.91	0.800	0.704	0.875	0.819	0.056
70	0.133	-3.77	0.651	0.618	0.551	0.797	-0.246

Correlation Matrix Model 9.2

	CLogP	DVZ	SS Cefmet	SS Cephap	Log_{10} AR
CLogP	1.0	-0.049	0.007	0.174	-0.501
DVZ		1.0	-0.002	0.078	0.314
SS Cefmet			1.0	0.584	-0.428
SS Cephap				1.0	-0.050
Log_{10} AR					1.0

Model 9.2 Validity Plots





Model 9.3 Parenteral β-Lactams

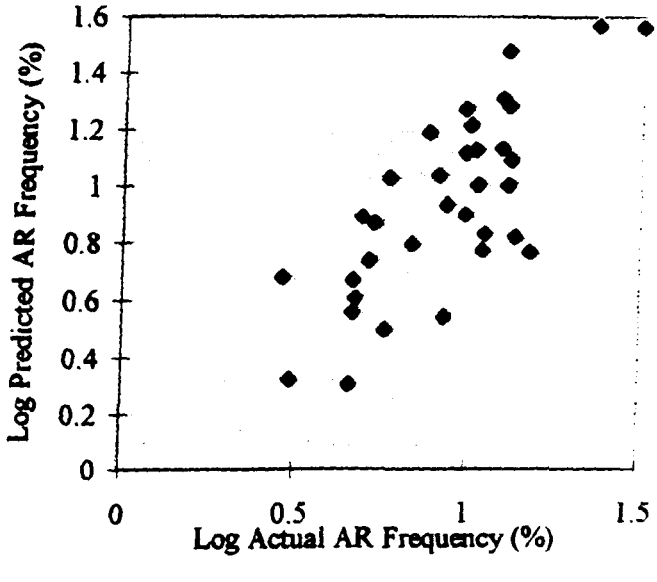
	# R1 Amino Groups	β-ring carbonyl C Charge	SS Fluc	Actual Log_{10} AR	Predicted Log_{10} AR	Residual
1	0	0.281	0.776	0.815	1.238	-0.423
5	0	0.276	0.652	0.998	1.115	-0.117
9	0	0.285	0.686	0.826	1.050	-0.224
10	0	0.287	0.661	1.269	0.986	0.283
14	0	0.284	0.889	1.568	1.371	0.198
17	0	0.287	1.000	1.564	1.500	0.064
19	0	0.285	0.665	1.214	1.003	0.211
20	0	0.285	0.646	0.897	0.989	-0.093
21	0	0.285	0.734	1.089	1.123	-0.033
22	0	0.285	0.717	1.305	1.097	0.209
24	0	0.285	0.773	0.761	1.182	-0.421
27	1	0.303	0.763	0.538	0.933	-0.395
28	0	0.287	0.733	1.130	1.095	0.035
29	0	0.289	0.699	1.125	1.018	0.107
33	1	0.291	0.661	0.930	0.934	-0.004
35	1	0.291	0.733	0.769	1.043	-0.275
40	0	0.310	0.734	0.324	0.487	-0.162
41	1	0.291	0.692	0.556	0.669	-0.113
42	1	0.291	0.698	1.114	0.990	0.123
43	0	0.291	0.626	1.187	0.881	0.303
44	0	0.291	0.686	0.305	0.660	-0.355
45	1	0.309	0.709	0.678	0.462	0.216
46	1	0.292	0.700	0.667	0.668	-0.002
47	1	0.291	0.696	0.605	0.675	-0.070
48	1	0.309	0.751	0.792	0.837	-0.046
54	0	0.291	0.705	0.890	0.689	0.201
57	1	0.291	0.851	1.036	0.910	0.126
58	1	0.291	0.722	0.733	0.715	0.019
63	0	0.291	0.780	1.279	1.115	0.164
64	0	0.291	0.721	1.000	1.025	-0.025
66	0	0.291	0.777	1.475	1.110	0.365
67	1	0.291	0.730	0.867	0.717	0.140
68	0	0.310	0.710	0.494	0.762	-0.268
69	0	0.310	0.713	1.025	0.767	0.258

Model 9.3 Correlation Matrix

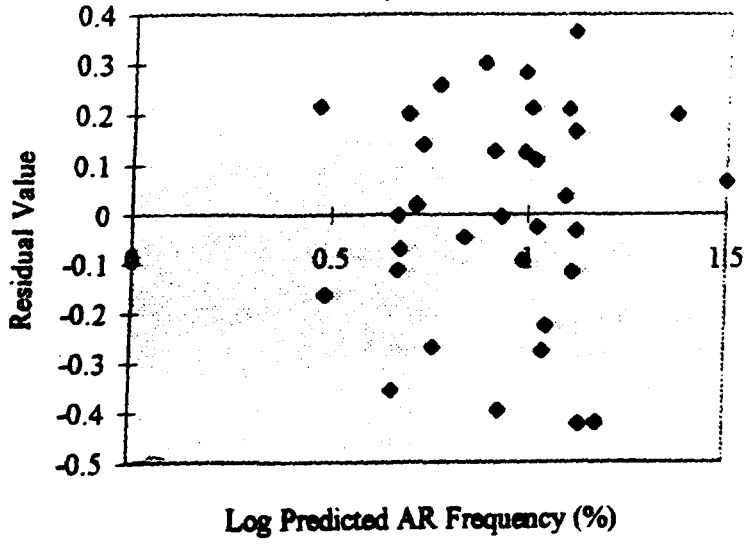
	# R1 Aminos	β-ring C carbonyl Charge	SS Fluc	$Log_{10}AR$
# R1 Amino Groups	1.0	0.233	-0.057	-0.549
β-ring C carbonyl Charge		1.0	-0.018	-0.461
SS Fluc			1.0	0.373
$Log_{10}AR$				1.0

Model 9.3 Validity Plots

Plot Predicted Log AR frequency against Actual Log AR Frequency for All Parenteral β-Lactam Antibiotics



Plot Predicted Log AR Frequency against Residual Values for All Parenteral β-Lactam Antibiotics



Model 9.4 All Penicillins

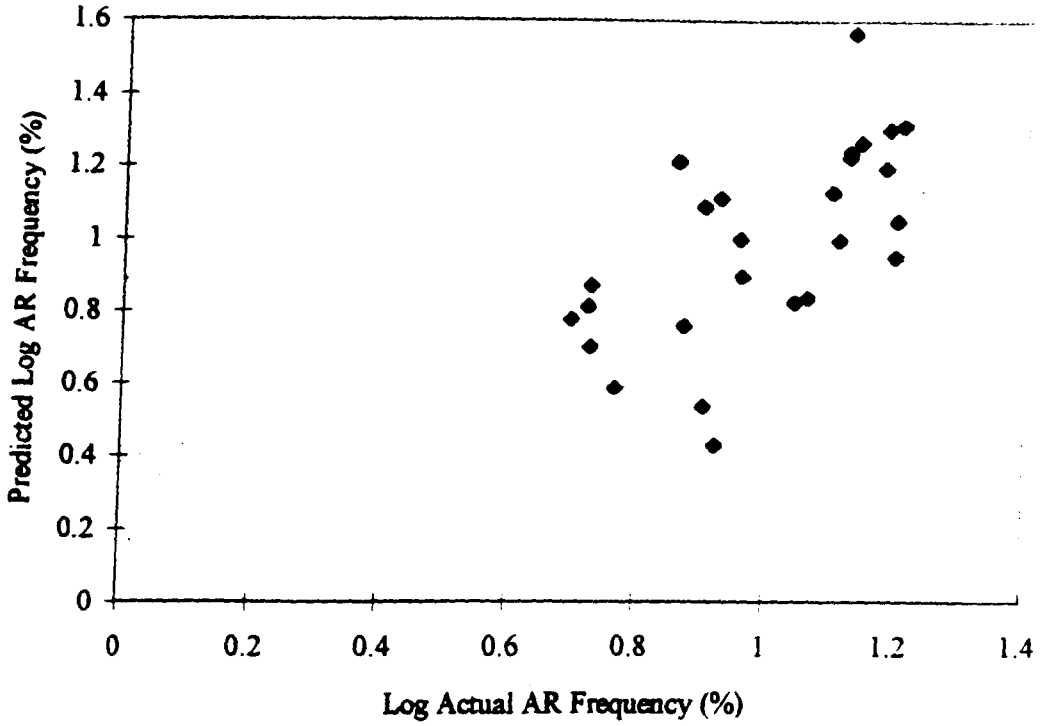
	R_1 HBD Ability	R1 Flex	SLipo BP	Actual $Log_{10}AR$	Predicted $Log_{10}AR$	Residual
1	-1	6.370	0.939	0.815	0.722	0.093
2	-1	6.370	0.875	0.778	0.696	0.083
3	2	5.876	0.451	1.196	1.180	0.016
4	1	5.511	0.752	1.228	1.123	0.105
5	2	9.446	0.917	0.998	1.110	-0.112
7	3	9.007	0.366	1.243	1.123	0.121
8	1	5.511	0.932	0.951	1.197	-0.246
9	0	5.173	1.00	0.826	1.042	-0.216
10	1	6.588	0.988	1.269	1.141	0.128
11	1	8.950	0.881	1.114	0.924	0.190
12	0	9.116	0.935	0.703	0.727	-0.023
13	1	4.905	0.240	1.001	0.956	0.045
14	1	6.426	0.925	1.568	1.127	0.441
15	1	7.320	0.925	0.840	1.062	-0.222
16	1	5.969	-0.127	0.872	0.727	0.145
18	1	5.511	-0.111	0.590	0.767	-0.177
19	0	7.101	0.894	1.214	0.857	0.357
20	3	10.78	0.287	0.897	0.960	-0.063
21	0	6.679	0.919	1.089	0.899	0.191
22	1	5.552	0.908	1.305	1.184	0.121
23	0	6.405	0.928	0.430	0.922	-0.493
24	3	11.69	0.232	0.761	0.871	-0.109
25	1	5.511	0.939	1.050	1.200	-0.150
26	1	5.511	0.953	1.316	1.206	0.110
27	0	6.426	0.886	0.538	0.903	-0.366
28	1	6.426	0.854	1.130	1.098	0.033

Model 9.4 Correlation Matrix

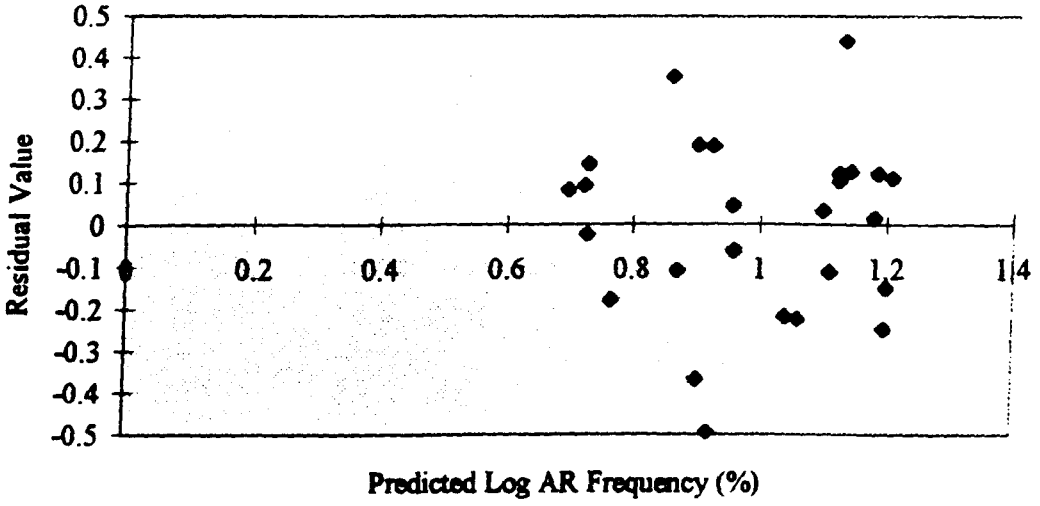
	R1HBD	R1 Flex	SLipo BP	$Log_{10}AR$
R1HBD	1.0	0.545	-0.503	0.278
R1 Flex		1.0	-0.163	-0.130
SLipo BP			1.0	0.201
$Log_{10}AR$				1.0

Model 9.4 Validity Plots

Plot Predicted Log AR Frequency against Actual Log AR Frequency for All Penicillin Antibiotics



Plot Residual Values against Predicted Log AR Frequency for All Penicillin Antibotics



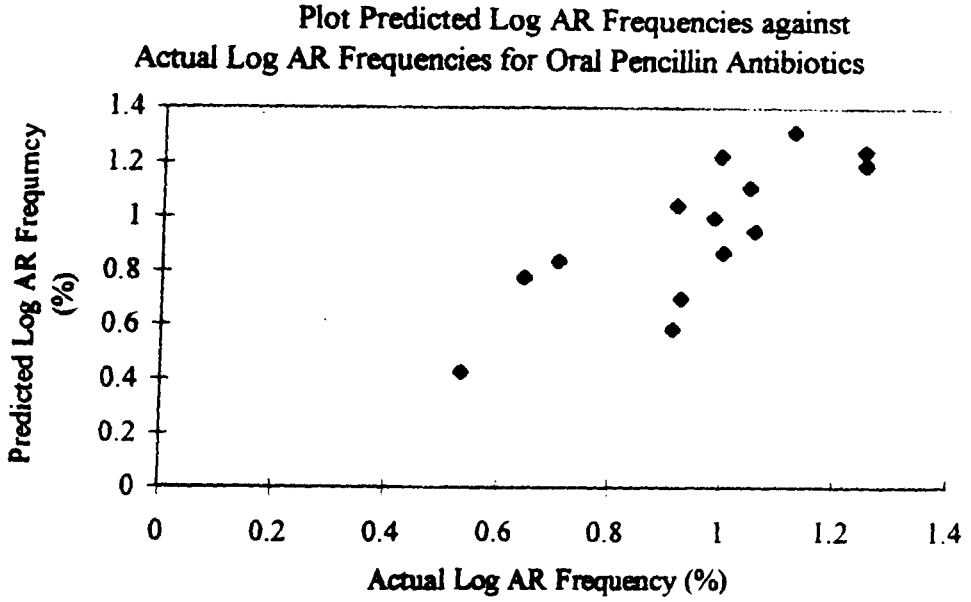
Model 9.5 Oral Penicillins

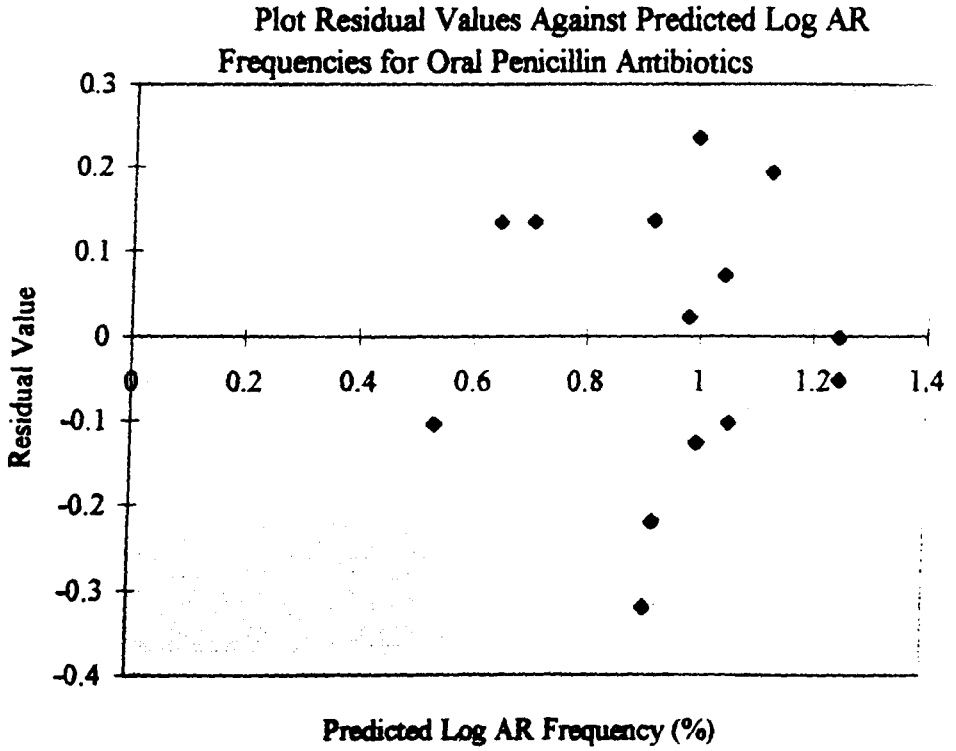
	R_1 HBD Ability	CLogP	Actual $Log_{10}AR$	Predicted $Log_{10}AR$	Residual
2	-1	-1.988	0.778	0.644	0.134
3	2	-1.936	1.196	1.248	-0.052
4	1	-1.269	1.228	0.991	0.237
7	3	0.600	1.243	1.246	-0.002
8	1	-2.038	0.951	1.054	-0.103
11	1	-1.901	1.114	1.042	0.071
12	0	-2.942	0.703	0.924	-0.221
13	1	-1.123	1.001	0.980	0.022
15	1	2.269	0.840	0.705	0.135
16	1	-1.356	0.872	0.998	-0.127
18	1	-0.274	0.590	0.911	-0.321
23	0	1.879	0.430	0.534	-0.104
25	1	-0.316	1.050	0.914	0.136
26	1	-2.874	1.316	1.121	0.195

Model 9.5 Correlation Matrix

	R1HBD	CLogP	$Log_{10}AR$
R1HBD	1.0	0.175	-0.371
CLogP		1.0	0.618
$Log_{10}AR$			1.0

Model 9.5 Validity Plots





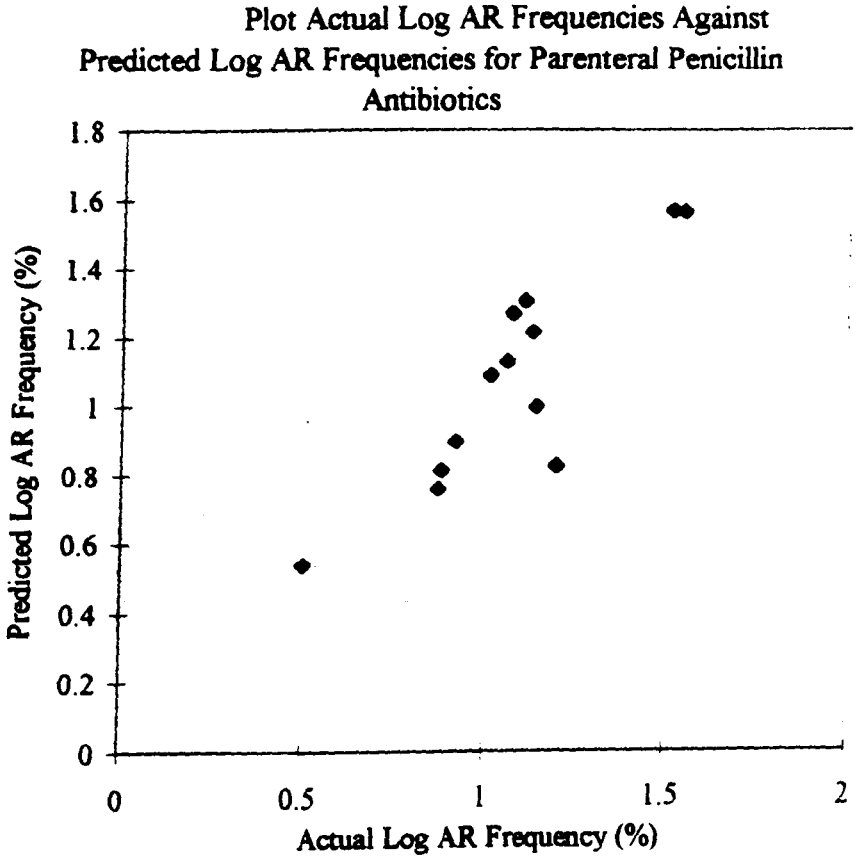
Model 9.6 Parenteral Penicillins

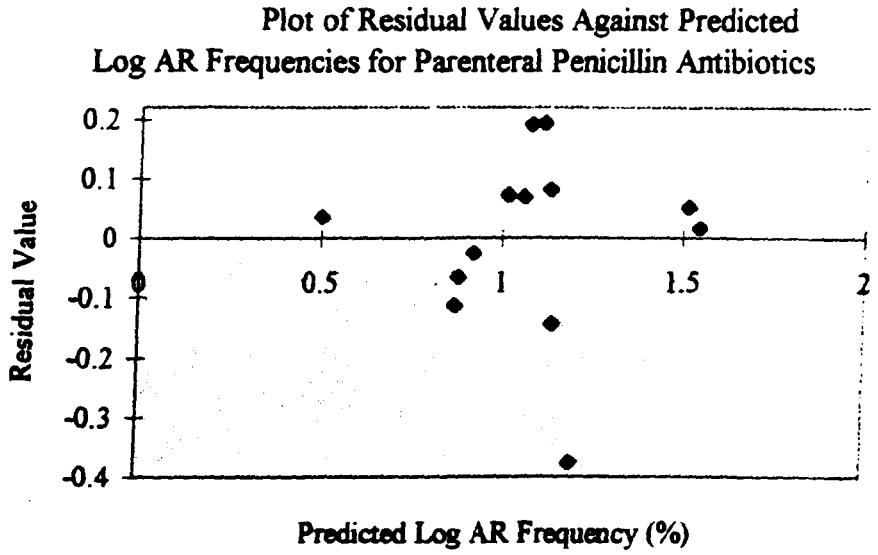
	CLogP	Sterimol B2 (CH_3)	Actual $Log_{10}AR$	Predicted $Log_{10}AR$	Residual
1	-0.519	0.408	0.815	0.881	0.066
5	3.911	0.503	0.998	1.142	0.144
9	1.683	0.389	0.826	1.201	-0.375
10	1.572	0.428	1.269	1.075	0.193
14	2.469	0.313	1.568	1.515	0.053
17	2.734	0.313	1.564	1.546	0.018
19	1.710	0.415	1.214	1.131	0.083
20	0.000	0.416	0.897	0.921	-0.025
21	2.214	0.476	1.089	1.015	0.074
22	1.802	0.426	1.305	1.110	0.195
24	0.550	0.455	0.761	0.874	-0.113
27	-0.174	0.554	0.538	0.502	0.036
28	1.218	0.419	1.130	1.060	0.070

Model 9.4 Correlation Matrix

	CLogP	Sterimol B2 (CH_3)	$Log_{10}AR$
CLogP	1.0	-0.224	0.630
Sterimol B2(CH_3)		1.0	-0.728
$Log_{10}AR$			1.0

Model 9.6 Validity Plots





Model 9.7 All Cephalosporins

	w.mol HA	Total Dipole 10	R1 # Amino	Sterimol B3 R2	# N R2	# O R2	SS Cefmet	SS Cephap	Actual Log_{10} AR	Pred. Log AR	Residual
29	6	0.159	0	1.915	0	2	0.708	0.687	1.125	1.015	0.110
30	6	0.721	1	1.800	0	0	0.676	0.639	0.489	0.761	-0.272
31	9	0.207	1	1.948	0	0	0.685	0.634	0.867	1.097	-0.230
33	5	0.494	0	5.556	4	0	0.598	0.571	0.930	1.030	-0.100
34	5	0.100	1	4.989	3	0	0.619	0.621	1.274	1.048	0.226
35	5	0.942	0	3.583	2	0	0.877	0.743	0.769	0.502	0.266
36	10	0.758	1	4.447	2	0	0.751	0.740	1.760	1.044	0.132
37	7	0.590	1	1.000	0	0	0.782	0.746	0.384	0.593	-0.210
38	11	0.884	1	3.585	4	0	0.777	0.736	0.493	0.412	0.081
39	10	0.601	0	3.585	4	0	1.000	0.742	0.041	0.216	-0.174
40	10	0.788	1	3.586	4	0	0.658	0.685	0.324	0.581	-0.257
41	6	0.750	1	4.398	1	2	0.789	0.640	0.556	0.685	-0.129
42	10	0.706	0	5.669	4	2	0.652	0.573	1.114	0.899	0.215
43	6	0.469	0	5.050	4	0	0.707	0.621	1.187	0.819	0.369
44	5	0.700	1	4.987	4	2	0.609	0.568	0.305	0.446	-0.141
45	9	0.962	1	4.922	4	0	0.663	0.680	0.678	0.726	-0.048
46	6	0.654	1	2.007	0	2	0.733	0.667	0.667	0.580	0.086
47	8	0.877	1	5.966	5	0	0.638	0.698	0.605	0.781	-0.175
48	9	0.596	0	3.437	1	2	0.776	0.727	0.792	1.035	-0.244
49	8	0.750	0	5.408	4	0	0.718	0.616	0.587	0.878	-0.291
50	10	0.604	1	2.081	0	1	0.694	0.732	0.994	1.053	-0.060
51	6	0.959	1	1.000	0	0	0.789	0.713	0.274	0.367	-0.093
52	6	1.014	1	2.371	0	1	0.678	0.655	0.886	0.713	0.173
54	8	0.157	1	2.667	1	0	0.691	0.674	0.890	1.036	-0.146
55	10	0.475	1	3.535	4	0	0.808	0.692	0.505	0.373	0.132
56	8	0.489	1	1.000	0	0	0.714	0.711	0.924	0.781	0.143
57	11	0.393	1	1.000	0	0	0.790	0.718	1.036	0.823	0.213
58	11	1.026	1	4.268	3	2	0.669	0.633	0.733	0.659	0.074
60	9	0.263	0	1.944	1	2	0.3647	0.648	0.952	1.022	-0.070

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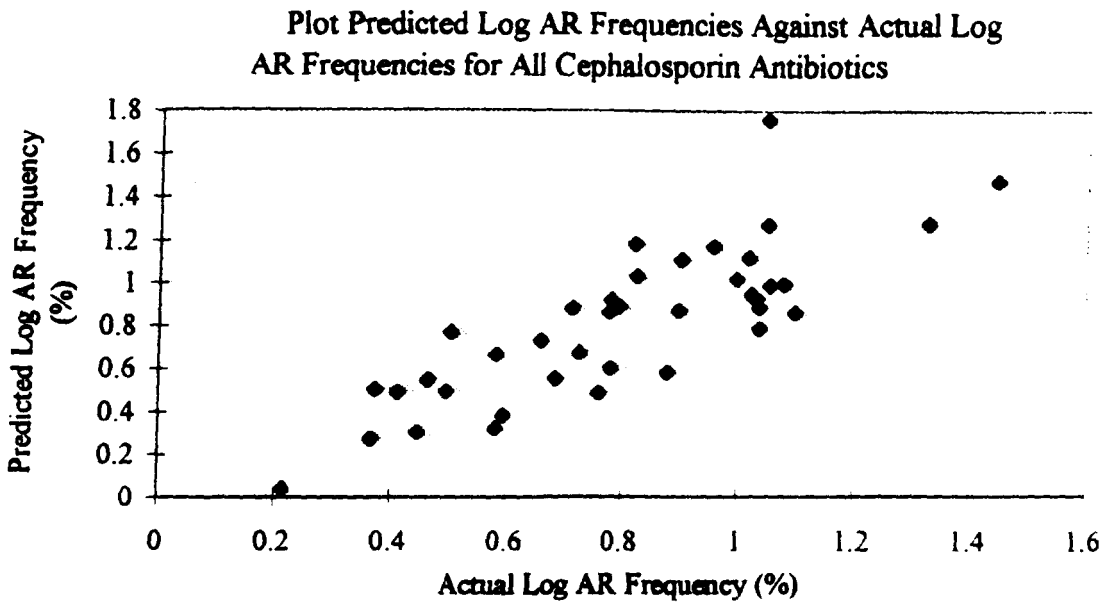
Model 9.7 continued

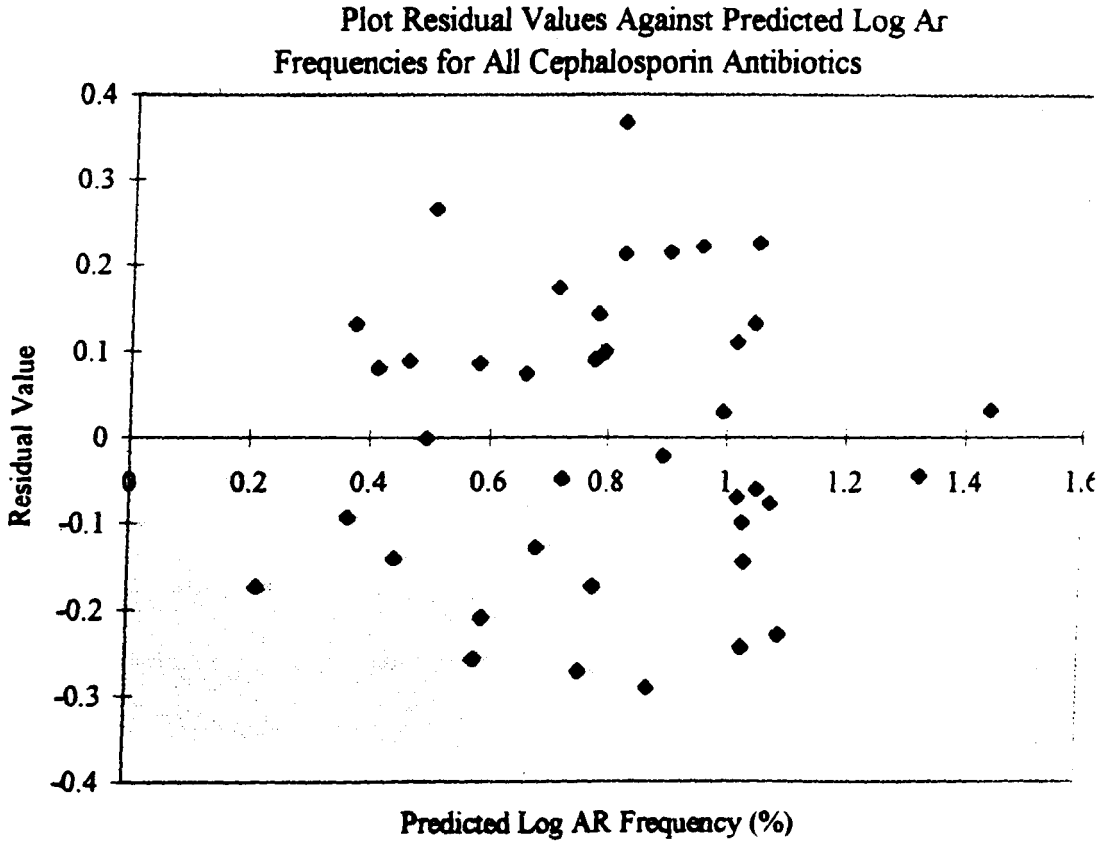
	w.mol HA	Total Dipole 10	R1 # Amino	Sterimol B3 R2	# N R2	# O R2	SS Cefmet	SS Cephap	Actual Log AR	Pred. Log AR	Residual
61	7	0.824	1	3.052	2	0	0.621	0.691	0.893	0.794	0.099
62	10	0.788	1	1.941	0	0	0.738	0.734	1.176	0.954	0.222
63	12	0.626	0	3.376	1	0	0.797	0.761	1.279	1.324	-0.045
64	5	0.558	0	3.448	0	2	0.754	0.715	1.000	1.077	-0.077
65	9	0.526	0	1.998	0	2	0.800	0.704	0.875	0.896	-0.021
66	6	0.634	0	3.458	0	2	0.742	1.000	1.475	1.443	0.032
67	8	0.793	1	1.954	0	0	0.662	0.524	0.867	0.777	0.090
68	10	0.649	0	2.705	4	1	0.688	0.661	0.494	0.495	-0.001
69	10	0.621	0	5.216	4	0	0.716	0.622	1.025	0.995	0.030
70	10	0.603	1	2.993	4	0	0.651	0.618	0.551	0.463	0.089

Model 9.7 Correlation Matrix

	HA	TD 10	R1 Aminos	B3R2	#N R2	#O R2	SS Cefmet	SS Cephap	Log10 AR
HA	1.0	0.078	0.049	-0.054	0.209	-0.149	0.184	0.090	-0.022
TD		1.0	0.205	0.186	0.189	-0.041	0.074	0.063	-0.348
R1 Amino			1.0	-0.263	-0.132	-0.335	-0.259	-0.127	-0.273
B3R2				1.0	0.391	0.050	-0.238	-0.255	0.061
#N R2					1.0	-0.200	-0.184	-0.326	-0.315
# OR2						1.0	-0.003	0.018	0.161
SSCefmet							1.0	0.504	-0.230
SS Cephap								1.0	0.206
$Log_{10}AR$									1.0

Model 9.7 Validity Plots





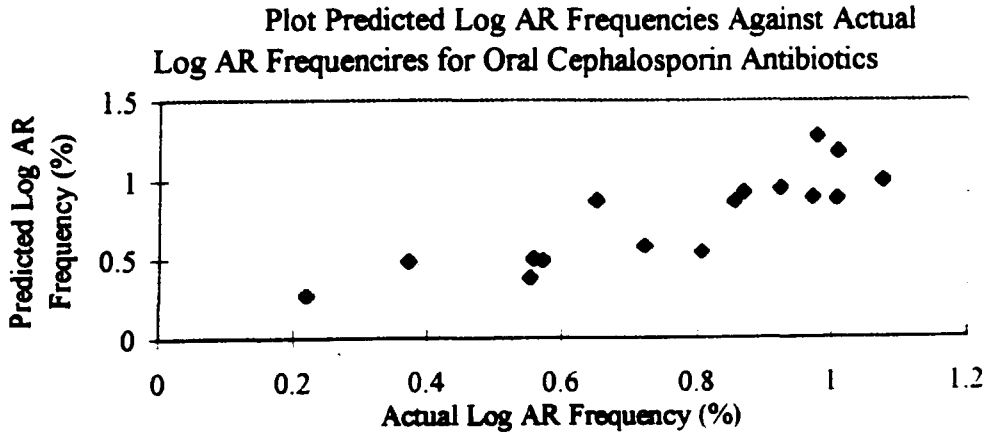
Model 9.8 Oral Cephalosporins

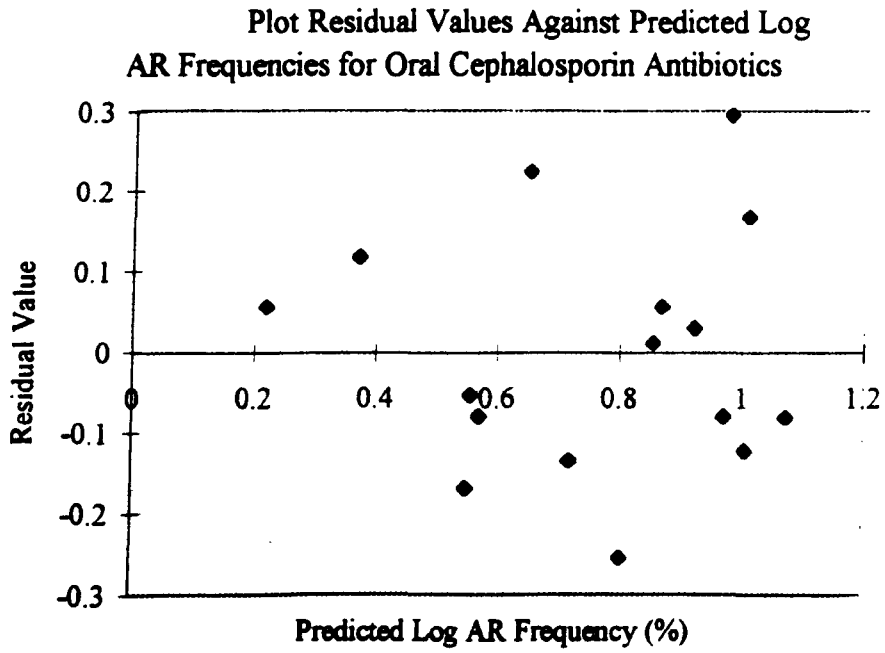
	CLogP	SS Cefmet	SS Cephap	Actual $Log_{10}AR$	Predicted $Log_{10}AR$	Residual
30	4.115	0.676	0.639	0.489	0.371	0.118
31	-1.350	0.685	0.634	0.867	0.854	0.012
34	0.000	0.619	0.621	1.274	0.977	0.297
37	1.311	0.782	0.746	0.384	0.551	-0.168
38	0.979	0.777	0.736	0.493	0.571	-0.078
49	-2.082	0.718	0.616	0.587	0.720	-0.133
50	-0.374	0.694	0.732	0.994	1.074	-0.080
51	3.120	0.789	0.713	0.274	0.219	0.056
52	-1.791	0.678	0.655	0.886	1.007	-0.121
55	-1.933	0.808	0.692	0.505	0.557	-0.052
56	0.000	0.714	0.711	0.924	0.868	0.056
60	0.251	0.647	0.648	0.952	0.922	0.030
61	2.544	0.621	0.691	0.893	0.971	-0.078
62	-1.673	0.738	0.734	1.176	1.008	0.169
65	-2.054	0.800	0.704	0.875	0.650	0.226
70	0.133	0.651	0.618	0.551	0.805	-0.254

Model 9.8 Correlation Matrix

	CLogP	SS Cefmet	SS Cephap	$Log_{10}AR$
CLogP	1.0	-0.177	0.102	-0.421
SS Cefmet		1.0	0.632	-0.525
SS Cephap			1.0	-0.122
Log_{10} AR				1.0

Model 9.8 Validity Plots





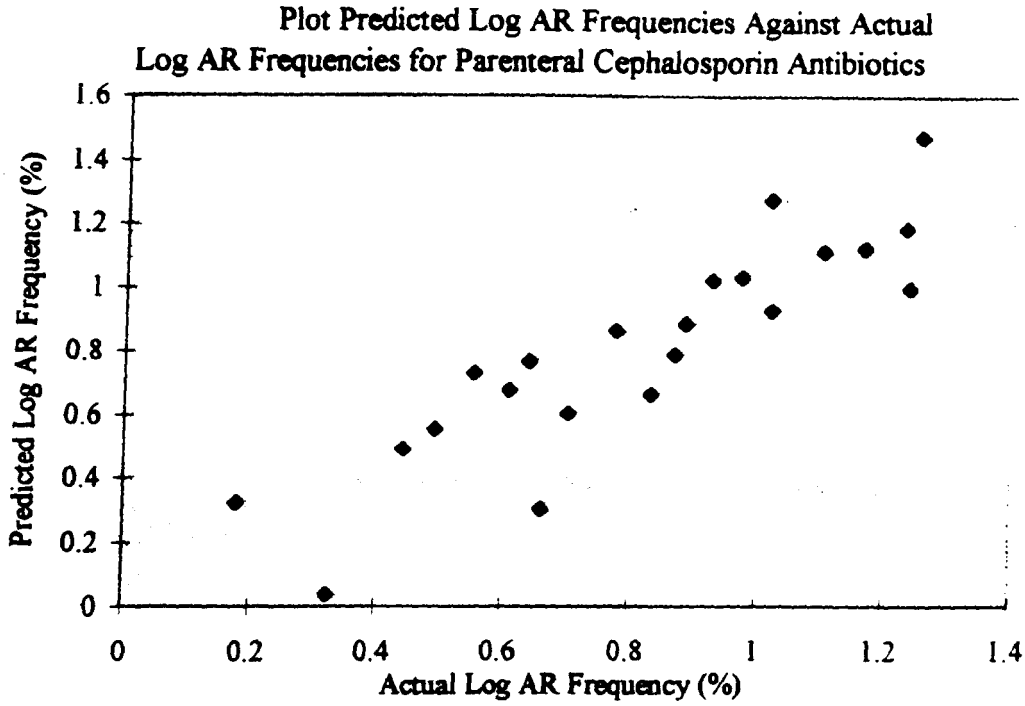
Model 9.9 Parenteral Cephalosporins

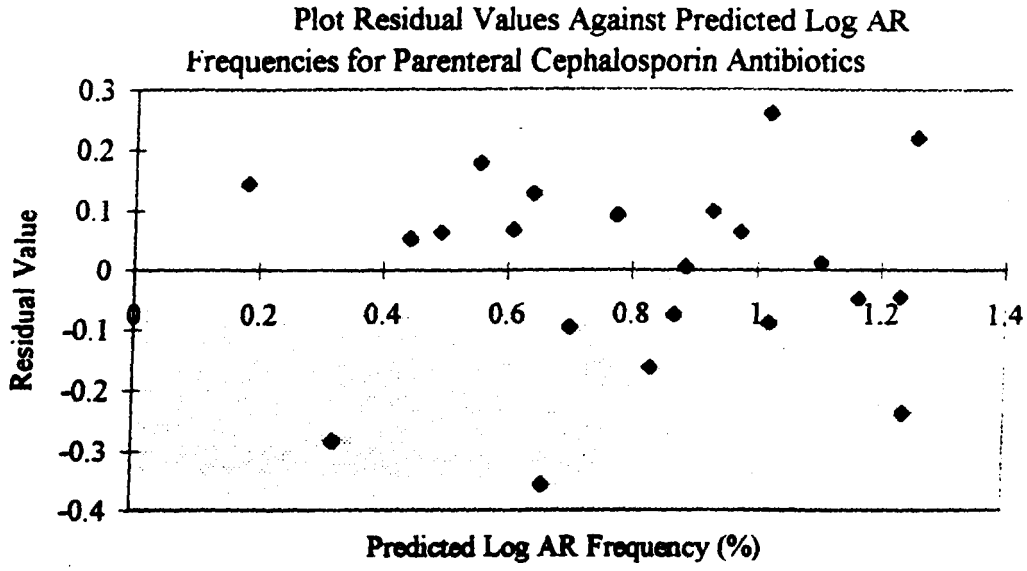
	R1 # Amino	Sterimol L R2	# O R2	β-ring carbonyl C Charge	Actual $Log_{10}AR$	Predicted $Log_{10}AR$	Residual
29	0	0.586	2	0.289	1.125	1.165	-0.048
33	1	0.565	0	0.291	0.930	1.020	-0.090
35	0	0.882	0	0.291	0.769	0.640	0.129
39	0	0.740	0	0.310	0.041	0.325	-0.284
40	1	0.740	0	0.310	0.324	0.179	0.145
41	1	1.004	2	0.291	0.556	0.493	0.063
42	0	0.836	2	0.291	1.114	1.102	0.012
43	0	0.564	0	0.291	1.187	0.232	-0.045
44	1	0.688	2	0.291	0.305	0.662	-0.357
45	0	0.399	0	0.309	0.678	0.609	0.068
46	1	0.581	2	0.292	0.667	0.832	-0.165
47	1	0.399	0	0.291	0.605	0.702	-0.097
48	0	0.390	2	0.309	0.792	0.868	-0.076
54	1	0.552	0	0.291	0.890	0.885	0.005
57	1	0.209	0	0.291	1.036	0.972	0.064
58	1	0.935	2	0.291	0.733	0.553	0.180
63	0	0.448	0	0.291	1.279	1.016	0.262
64	0	0.434	2	0.291	1.000	1.239	-0.239
66	0	0.417	2	0.291	1.475	1.254	0.221
67	1	0.316	0	0.291	0.867	0.774	0.093
68	0	0.727	1	0.310	0.494	0.442	0.052
69	0	0.412	0	0.310	1.025	0.926	0.099

Model 9.9 Correlation Matrix

	# R_1 Amino	Sterimol L R_2	# O R2	β-ring Carbonyl C Charge	Log_{10} AR
# R1 Amino	1.0	-0.003	0.076	-0.136	-0.399
Sterimol L R2		1.0	0.216	-0.034	-0.422
# O R2			1.0	0.344	0.011
β-ring Carbonyl C Charge				1.0	-0.479
Log10AR					1.0

Model 9.9 Validity Plots





Cutaneous Rash

Model 9.10 All -β-lactams

	Sterimol B2 (CH_3)	SS BP	# C R1	Log H_20 sol.	SS Meth	SS Cefep	SS Ceftib	SS Cefatriz	Actual $\sqrt{}$Rash	Predicted $\sqrt{}$Rash	Residual
1	0.408	0.837	7	-1.75	0.837	0.716	0.747	0.704	2.002	1.865	0.137
2	0.667	0.721	7	-2.07	0.725	0.630	0.692	0.621	1.000	1.337	-0.337
3	0.403	0.987	8	-1.28	0.766	0.653	0.689	0.792	1.709	1.792	-0.083
4	0.327	0.991	8	-1.11	0.773	0.652	0.675	0.817	2.173	1.794	0.379
5	0.503	0.756	17	-3.57	0.691	0.615	0.683	0.753	2.415	1.985	0.430
6	0.453	0.925	8	-1.52	0.796	0.760	0.741	0.765	1.937	1.674	0.263
7	0.317	0.876	12	-2.18	0.733	0.612	0.739	0.706	1.712	1.711	8.99^{-4}
8	0.316	0.890	8	-1.83	0.695	0.645	0.643	0.697	1.646	1.179	0.467
9	0.389	1.000	8	-1.66	0.753	0.674	0.684	0.828	1.643	1.852	-0.209
10	0.428	0.848	9	-1.79	0.776	0.615	0.685	0.775	2.561	1.889	0.662
11	0.486	0.674	18	-4.67	0.678	0.701	0.710	0.702	0.894	1.850	-0.956
12	0.503	0.815	15	-3.77	0.710	0.671	0.634	0.694	1.581	2.336	-0.755
13	0.405	0.843	7	-0.55	0.824	0.766	0.753	0.701	1.319	1.438	-0.119
14	0.313	0.668	11	-2.91	0.790	0.728	0.793	0.684	1.225	1.668	-0.443
15	0.313	0.693	11	-3.69	0.740	0.654	0.867	0.663	0.893	1.214	-0.321
17	0.313	0.688	11	-3.20	0.749	0.685	0.859	0.682	1.825	1.247	0.578
18	0.673	0.777	8	-0.07	0.736	0.704	0.755	0.742	0.889	1.306	-0.417
19	0.415	0.725	9	-1.76	1.00	0.633	0.854	0.734	3.299	2.370	0.929
20	0.416	0.831	13	-1.98	0.631	0.739	0.691	0.636	1.652	0.957	0.695
21	0.476	0.724	13	-3.36	0.805	0.661	0.836	0.736	2.961	2.071	0.890
22	0.426	0.827	11	-1.56	0.720	0.663	0.739	0.778	1.581	1.436	0.145
23	0.343	0.865	8	-1.56	0.807	0.754	0.715	0.753	1.449	1.721	-0.272
24	0.455	0.573	15	-1.41	0.705	0.590	0.637	0.677	1.127	1.650	-0.523
25	0.659	0.871	8	-2.81	0.679	0.702	0.625	0.690	1.792	1.666	0.126
26	0.529	0.846	8	-2.66	0.700	0.650	0.656	0.700	1.477	1.777	-0.297
27	0.554	0.770	7	-1.31	0.787	0.684	0.784	0.678	1.581	1.229	0.282
28	0.419	0.964	7	-0.56	0.780	0.677	0.750	0.767	1.049	1.526	-0.477

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Model 9.10 continued

	Sterimol B2 (CH_3)	SS BP	# C R1	Log H_20 sol.	SS Meth	SS Cefep	SS Ceftib	SS Cefatriz	Actual $\sqrt{}$Rash	Predicted $\sqrt{}$Rash	Residual
29	0.267	0.792	3	1.68	0.740	0.650	0.646	0.700	0.100	0.536	-0.436
30	0.549	0.870	8	-0.17	0.795	0.642	0.801	0.760	1.082	1.374	-0.292
31	0.403	0.864	8	-0.68	0.743	0.751	0.767	0.708	0.693	1.004	-0.311
33	0.577	0.806	8	-2.69	0.711	0.610	0.702	0.823	1.304	0.622	-0.318
34	0.569	0.817	8	-0.94	0.741	0.774	0.690	1.000	2.437	0.882	0.555
35	0.637	0.663	3	-1.59	0.720	1.00	0.715	0.589	1.253	0.189	0.064
36	0.533	0.646	6	-1.26	0.704	0.648	0.694	0.559	2.236	0.639	0.597
37	0.542	0.700	7	-0.42	0.718	0.566	0.865	0.655	0.387	0.390	-0.003
38	0.638	0.641	6	-2.13	0.734	0.719	0.693	0.560	0.917	0.994	-0.027
39	0.638	0.665	4	-1.27	0.762	0.614	0.742	0.557	0.762	1.108	-0.346
40	0.635	0.755	5	-1.23	0.720	0.672	0.702	0.518	1.072	0.804	0.268
41	0.628	0.536	6	0.01	0.699	0.605	0.684	0.552	0.950	0.535	0.424
42	0.580	0.779	8	-2.05	0.714	0.705	0.696	0.816	1.400	1.555	-0.155
43	0.482	0.574	15	-2.09	0.651	0.649	0.696	0.579	1.594	1.042	0.552
44	0.506	0.735	9	-1.89	0.669	0.739	0.660	0.780	1.285	1.362	-0.077
45	0.557	0.756	6	-1.99	0.721	0.760	0.694	0.724	1.136	1.618	-0.482
46	0.633	0.695	7	1.27	0.774	0.592	0.699	0.618	1.349	1.217	0.132
47	0.386	0.723	5	-1.01	0.714	0.727	0.683	0.659	0.100	0.647	-0.547
48	0.573	0.702	6	0.53	0.703	0.676	0.736	0.660	1.565	0.751	0.814
49	0.552	0.583	15	-2.66	0.658	0.556	0.721	0.594	1.136	1.152	-0.016
50	0.624	0.624	6	-0.70	0.693	0.743	0.649	0.588	0.714	0.596	0.118
51	0.505	0.693	8	-1.18	0.767	0.663	0.824	0.592	0.949	1.036	-0.087
53	0.584	0.760	8	-0.97	0.748	0.727	0.573	0.814	2.345	2.033	.0312
54	0.599	0.667	9	-1.52	0.681	0.738	0.678	0.641	1.613	1.292	0.321
55	0.512	0.647	6	-0.61	0.715	0.663	0.686	0.583	0.100	0.932	-0.832
56	0.657	0.704	8	0.76	0.854	0.685	1.000	0.690	0.100	0.802	-0.702
57	0.529	0.730	6	-0.24	0.771	0.693	0.801	0.677	0.959	0.958	0.001

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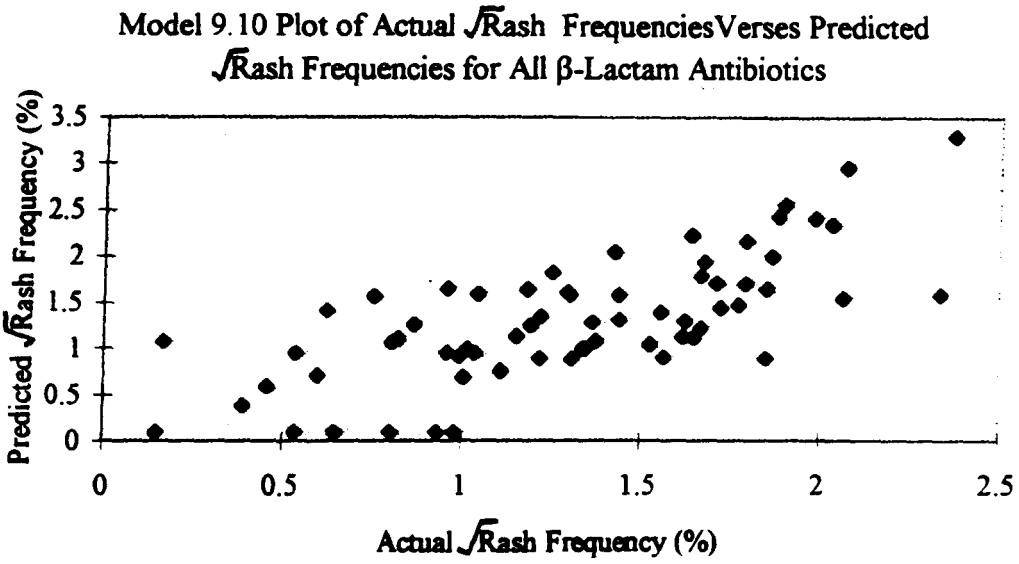
Model 9.10 continued

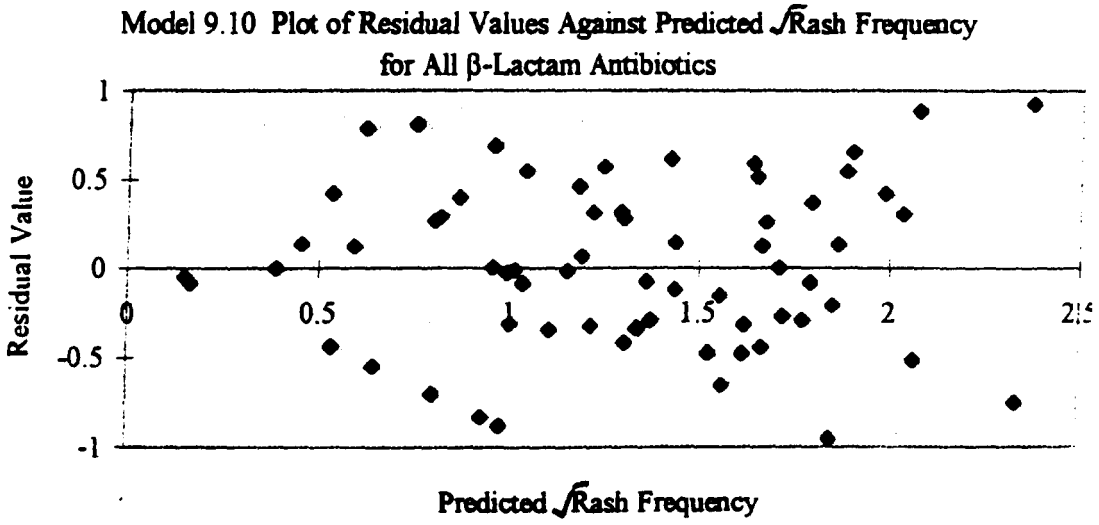
	Sterimol B2 (CH_3)	SS BP	# C R1	Log H_20 sol.	SS Meth	SS Cefep	SS Ceftib	SS Cefatriz	Actual √Rash	Predicted √Rash	Residual
58	0.589	0.534	6	-1.13	0.685	0.653	0.608	0.558	1.114	0.821	-0.293
59	0.521	0.676	7	0.69	0.864	0.704	0.733	0.723	0.911	1.565	-0.654
60	0.622	0.735	7	1.17	0.700	0.658	0.551	0.665	1.007	1.165	-0.088
62	0.497	0.790	8	-0.51	0.780	0.714	0.775	0.697	1.000	1.342	-0.342
63	0.638	0.638	6	-0.31	0.713	0.703	0.699	0.657	1.000	1.014	-0.014
64	0.621	0.697	6	1.40	0.768	0.653	0.796	0.673	1.414	0.621	0.793
65	0.620	0.672	6	0.94	0.755	0.716	0.737	0.685	0.100	0.981	-0.881
66	0.512	0.649	7	1.23	0.717	0.648	0.736	0.523	0.100	0.150	-0.050
67	0.551	0.831	8	0.01	0.788	0.670	0.773	0.770	2.047	1.422	0.625
68	0.637	0.690	4	-1.85	0.690	0.605	0.695	0.638	1.269	0.866	0.403
69	0.537	0.716	9	-3.17	0.777	0.726	0.732	0.735	1.549	20.66	-0.517
70	0.536	0.592	6	-0.60	0.682	0.573	0.658	0.623	0.592	0.457	0.135

Correlation Matrix Model 9.10

	Sterimol B2 (CH_3)	SS BP	# C R1	Log H_20 sol.	SS Meth	SS Cefep	SS Ceftib	SS Cefatriz	√Rash
Sterimol B2	1.0	-0.433	-0.306	0.252	-0.204	-0.129	-0.122	-0.290	-0.183
SS BP		1.0	0.0	-0.078	0.249	-0.192	-0.021	0.663	0.330
Log H_20 sol.			1.0	-0.617	-0.175	0.043	0.050	0.192	0.344
# C R1				1.0	0.204	0.020	0.037	-0.132	-0.416
SS Meth					1.0	0.191	0.563	0.289	0.249
SS Cefep						1.0	0.228	-0.201	0.166
SS Ceftib							1.0	0.006	-0.068
SS Cefatriz								1.0	0.451
√Rash									1.0

Model 9.10 Validity Plots





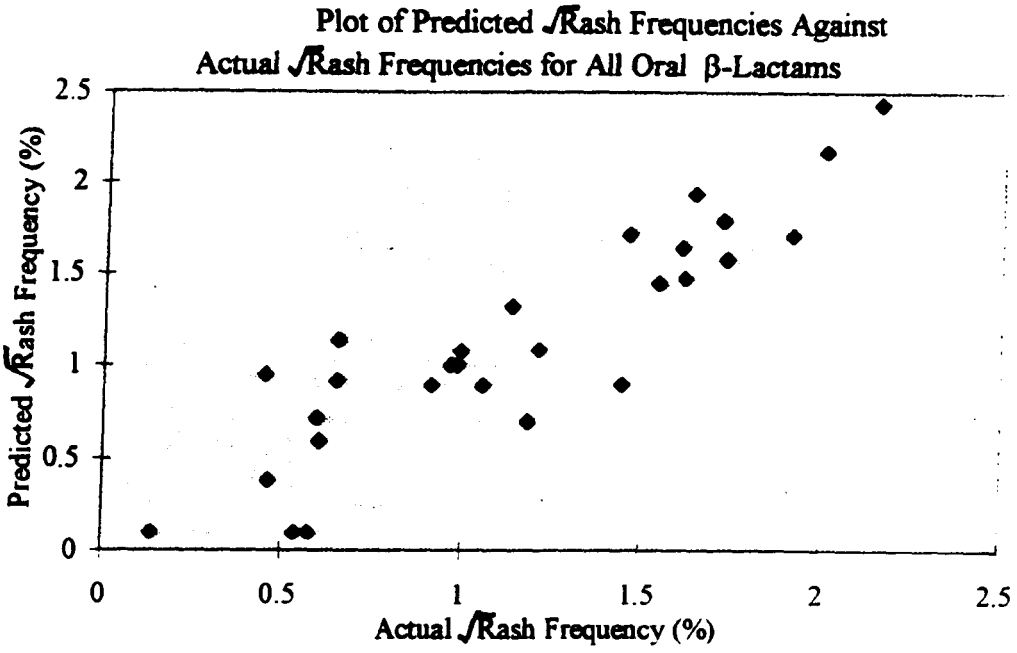
Model 9.11 Oral β-Lactams

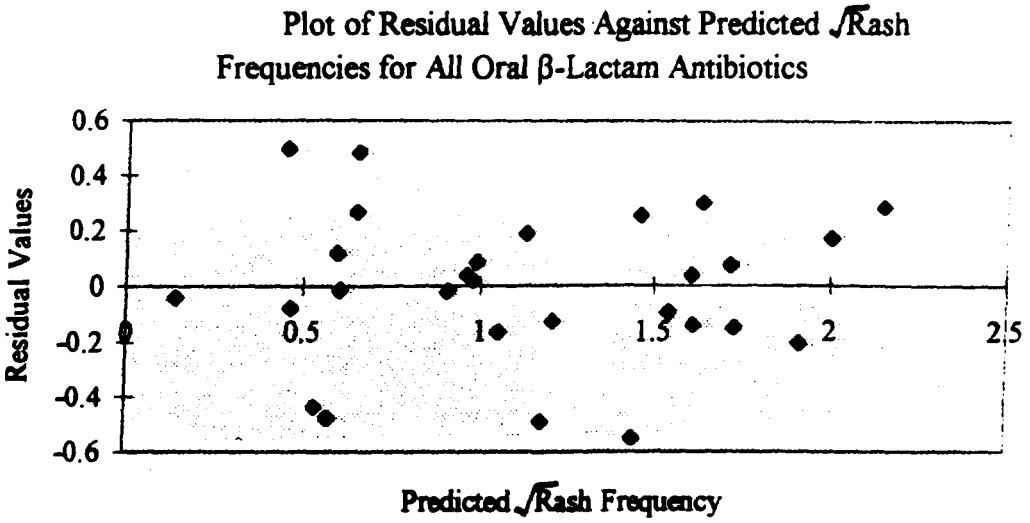
	SS BP	Log H_20 sol.	SS Ceftib	SS Cefatriz	Actual √Rash	Predicted √Rash	Residual
2	0.721	-2.07	0.692	0.621	1.000	0.981	0.019
3	0.987	-1.28	0.689	0.792	1.709	1.914	-0.205
4	0.991	-1.11	0.675	0.817	2.173	2.002	0.171
6	0.925	-1.52	0.741	0.765	1.937	1.637	0.300
7	0.876	-2.18	0.739	0.706	1.712	1.458	0.254
8	0.890	-1.83	0.643	0.697	1.646	1.608	0.038
11	0.674	-4.67	0.710	0.702	0.894	1.444	-0.550
12	0.815	-3.77	0.634	0.694	1.581	1.732	-0.151
13	0.843	-0.55	0.753	0.701	1.319	1.129	0.190
15	0.693	-3.69	0.867	0.663	0.893	0.911	-0.018
18	0.777	-0.07	0.755	0.742	0.889	1.053	-0.164
23	0.865	-1.56	0.715	0.753	1.449	1.542	-0.093
25	0.871	-2.81	0.625	0.690	1.792	1.719	0.073
26	0.846	-2.66	0.656	0.700	1.477	1.616	-0.139
30	0.870	-0.17	0.801	0.760	1.082	1.209	-0.127
31	0.864	-0.68	0.767	0.708	0.693	1.180	-0.487
34	0.817	-0.94	0.690	1.000	2.437	2.152	0.285
37	0.700	-0.42	0.865	0.655	0.387	0464	-0.077
38	0.641	-2.13	0.693	0.560	0.917	0.648	0.269
49	0.583	-2.66	0.721	0.594	1.136	0.649	0.487
50	0.624	-0.70	0.649	0.588	0.714	0.595	0.119
51	0.693	-1.18	0.824	0.592	0.949	0.449	0.500
55	0.647	-0.61	0.686	0.583	0.100	0.994	0.006
56	0.704	0.76	1.000	0.690	0.100	0.537	-0.437
60	0.735	1.17	0.551	0.665	1.007	0.140	-0.033
62	0.790	-0.51	0.775	0.697	1.000	0.991	0.086
65	0.672	0.94	0.737	0.685	0.100	0.096	0.014
70	0.592	-0.60	0.658	0.623	0.592	0.557	-0.045

Correlation Matrix Model 9.11

	SS BP	Log H_20 sol.	SS Ceftib	SS Cefatriz	$\sqrt{}$Rash
SS BP	1.0	-0.040	-0.136	0.655	0.720
Log H_20 sol.		1.0	0.205	0.139	-0.346
SS Ceftibut			1.0	-0.001	-0.423
SS cefatriz				1.0	0.643
$\sqrt{}$Rash					1.0

Model 9.11 Validity Plots





Model 9.12 Parenteral -β-lactams

	Sterimol B2 (CH_3)	# C R1	Log H_20 sol.	SS Meth	Actual √Rash	Predicted √Rash	Residual
1	0.408	7	-1.75	0.837	2.002	1.750	-0.252
5	0.503	17	-3.57	0.691	2.415	2.433	-0.018
9	0.389	8	-1.66	0.753	1.643	1.308	0.335
10	0.428	9	-1.79	0.776	2.561	1.648	0.913
14	0.313	11	-2.91	0.790	1.225	1.894	-0.669
17	0.313	11	-3.20	0.749	1.825	1.701	0.124
19	0.415	9	-1.76	1.00	3.299	2.934	0.365
20	0.416	13	-1.98	0.631	1.652	1.222	0.430
21	0.476	13	-3.36	0.805	2.961	2.594	0.367
22	0.426	11	-1.56	0.720	1.581	1.486	0.095
24	0.455	15	-1.41	0.705	1.127	1.853	-0.736
27	0.554	7	-1.31	0.787	1.581	1.677	-0.096
28	0.419	7	-0.56	0.780	1.049	1.240	-0.191
29	0.267	3	1.68	0.740	0.100	-0.088	0.188
33	0.577	8	-2.69	0.711	1.304	1.608	-0.304
35	0.637	3	-1.59	0.720	1.253	1.073	0.180
39	0.638	4	-1.27	0.762	0.762	1.374	-0.612
40	0.635	5	-1.23	0.720	1.072	1.221	-0.149
41	0.628	6	0.01	0.699	0.950	0.984	-0.025
42	0.580	8	-2.05	0.714	1.400	1.526	-0.126
43	0.482	15	-2.09	0.651	1.594	1.701	-0.107
44	0.506	9	-1.89	0.669	1.285	1.192	0.093
45	0.557	6	-1.99	0.721	1.136	1.301	-0.165
46	0.633	7	1.27	0.774	1.349	1.333	0.016
47	0.386	5	-1.01	0.714	0.100	0.650	-0.550

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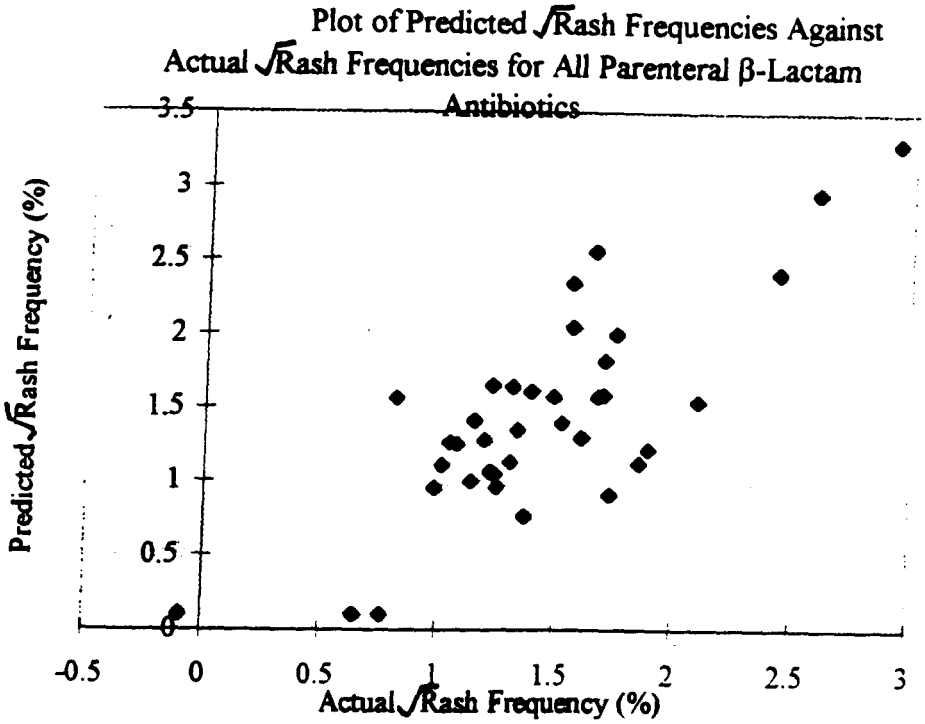
Model 9.12 continued

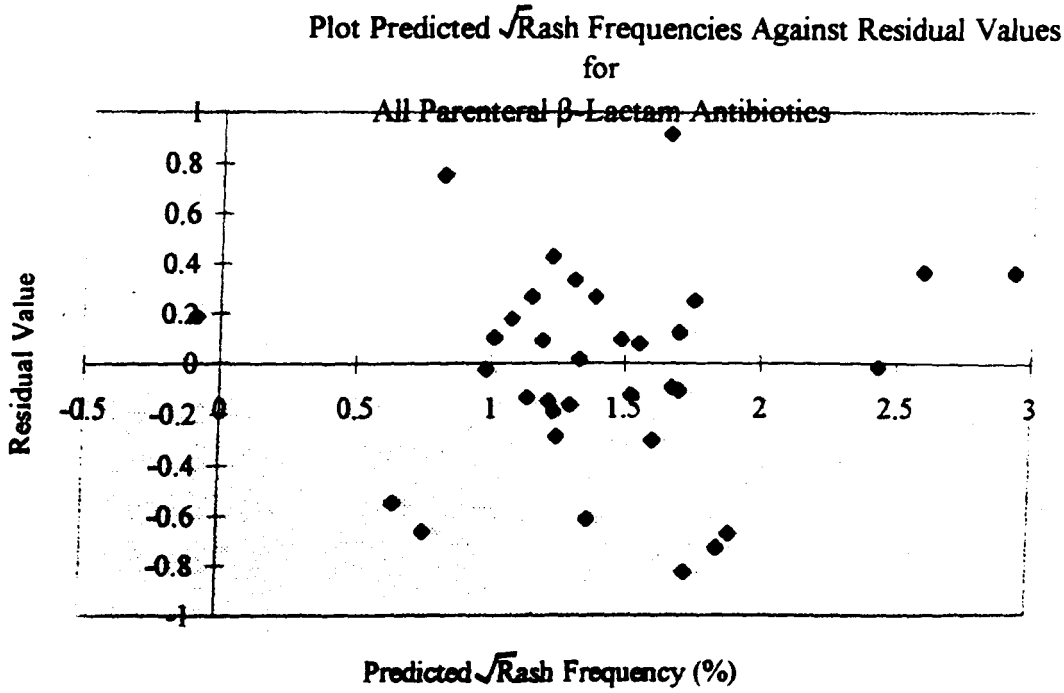
	Sterimol B2 (CH_3)	# C R1	Log H_20 sol.	SS Meth	Actual √Rash	Predicted √Rash	Residual
48	0.573	6	0.53	0.703	1.565	0.813	0.752
53	0.584	8	-0.97	0.748	2.345	1.555	0.790
54	0.599	9	-1.52	0.681	1.613	1.387	0.226
57	0.529	6	-0.24	0.771	0.959	1.251	-0.292
58	0.589	6	-1.13	0.685	1.114	1.012	0.102
59	0.521	7	0.69	0.864	0.911	1.734	-0.823
63	0.638	6	-0.31	0.713	1.000	1.138	-0.138
64	0.621	6	1.40	0.768	1.414	1.147	0.267
66	0.512	7	1.23	0.717	0.100	0.761	-0.661
67	0.551	8	0.01	0.788	2.047	1.563	0.484
68	0.637	4	-1.85	0.690	1.269	1.046	0.223
69	0.537	9	-3.17	0.777	1.549	2.100	-0.551

Correlation Matrix Model 9.12

	Sterimol B2 (CH_3)	# C R1	Log H_20 sol.	SS Meth	√Rash
Sterimol B2 (CH_3)	1.0	-0.353	0.205	-0.226	-0.071
# C R1		1.0	-0.553	-0.121	0.508
Log H_20 sol.			1.0	0.074	-0.499
SS Meth				1.0	0.407
√Rash					1.0

Model 9.12 Validity Plots





Model 9.13 All Penicillins, Model 9.14 Oral Penicillins and Model 9.15 Parenteral Penicillins

	Sterimol B3 (C=O)	SS BP	SS Meth	Admin ID	Actual√Rash	All Pens Predicted√Rash	All Pens Residual	Oral Pens Predicted√Rash	Oral Pens Residual	Par. Pens Predicted√Rash	Par. Pens Residual
1	6.839	0.837	0.837	4	2.002	2.477	-0.475	-	-	2.334	-0.332
2	8.745	0.721	0.725	3	1.000	1.642	-0.642	1.003	-0.003	-	-
3	6.587	0.987	0.766	3	1.709	1.751	-0.042	2.020	-0.311	-	-
4	6.608	0.991	0.773	3	2.173	1.806	0.367	2.036	0.137	-	-
5	7.943	0.756	0.691	4	2.415	1.894	0.521	-	-	1.998	0.417
6	5.972	0.925	0.796	3	1.937	1.530	0.407	1.783	0.154	-	-
7	8.082	0.876	0.733	3	1.712	1.837	-0.125	1.596	0.116	-	-
8	7.893	0.890	0.695	3	1.646	1.601	0.045	1.650	-0.004	-	-
9	5.910	1.000	0.753	4	1.643	2.104	-0.461	-	-	1.397	0.246
10	7.491	0.848	0.776	4	2.561	2.417	0.144	-	-	2.285	0.276
11	6.845	0.674	0.678	3	0.894	0.580	0.314	0.824	-0.070	-	-
12	7.491	0.815	0.710	3	1.581	1.344	0.237	1.363	0.218	-	-
13	7.033	0.843	0.824	3	1.319	1.857	-0.538	1.470	-0.151	-	-
14	5.904	0.668	0.790	4	1.225	1.457	-0.232	-	-	1.612	-0.387

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Models 9.13, 9.14 and 9.15 continued

	Sterimol B3 (C=O)	SS BP	SS Meth	Admin ID	Actual √Rash	All Pens Predicted √Rash	All Pens Residual	Oral Pens Predicted √Rash	Oral Pens Residual	Par. Pens Predicted √Rash	Par. Pens Residual
15	6.760	0.693	0.740	3	0.893	0.929	-0.036	0.896	-0.003	-	-
17	6.658	0.688	0.749	4	1.825	1.563	0.262	-	-	1.729	0.096
18	6.312	0.777	0.736	3	0.889	0.957	-0.068	1.217	-0.328	-	-
19	6.686	0.725	1.00	4	3.299	3.008	0.291	-	-	3.223	0.076
20	8.026	0.831	0.631	4	1.652	1.794	-0.142	-	-	1.684	-0.032
21	8.096	0.724	0.805	4	2.961	2.447	0.484	-	-	2.743	0.218
22	6.017	0.827	0.720	4	1.581	1.528	0.053	-	-	1.253	0.328
23	6.162	0.865	0.807	3	1.449	1.505	-0.056	1.554	-0.105	-	-
24	7.728	0.573	0.705	4	1.127	1.425	-0.298	-	-	1.978	-0.851
25	8.129	0.871	0.679	3	1.792	1.553	0.239	1.557	0.215	-	-
26	8.102	0.846	0.700	3	1.477	1.592	-0.115	1.481	-0.004	-	-
27	5.988	0.770	0.787	4	1.581	1.730	-0.149	-	-	1.634	-0.053

Correlation Matrix Model 9.13

	SS BP	Sterimol B3 (C=O)	Admin ID	SS Meth	√Rash
SS BP	1.0	-0.200	-0.353	-0.009	0.146
Sterimol B3 (C=O)		1.0	-0.089	-0.366	0.169
Admin ID			1.0	0.229	0.466
SS Meth				1.0	0.539
√Rash					1.0

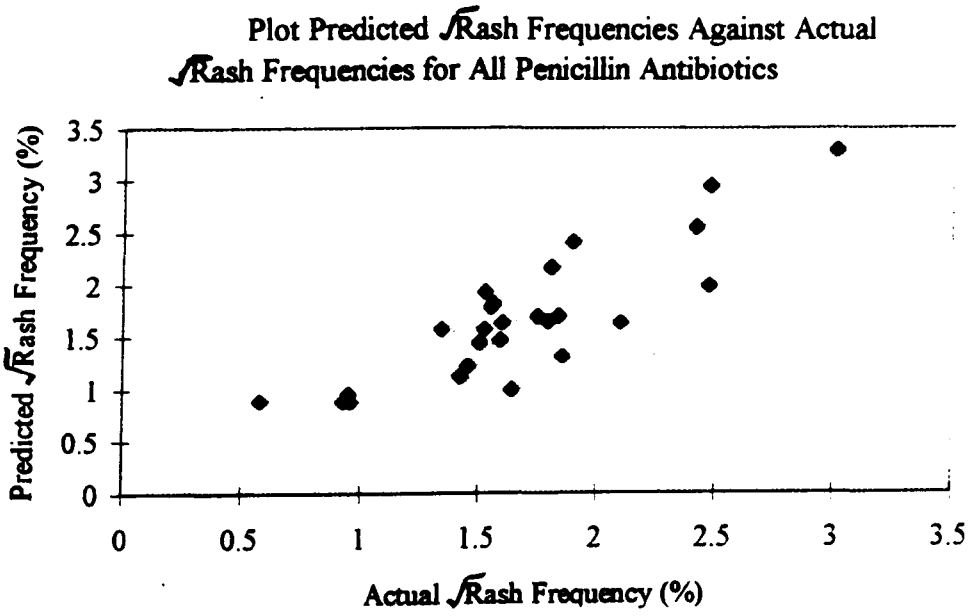
Correlation Matrix Model 9.14

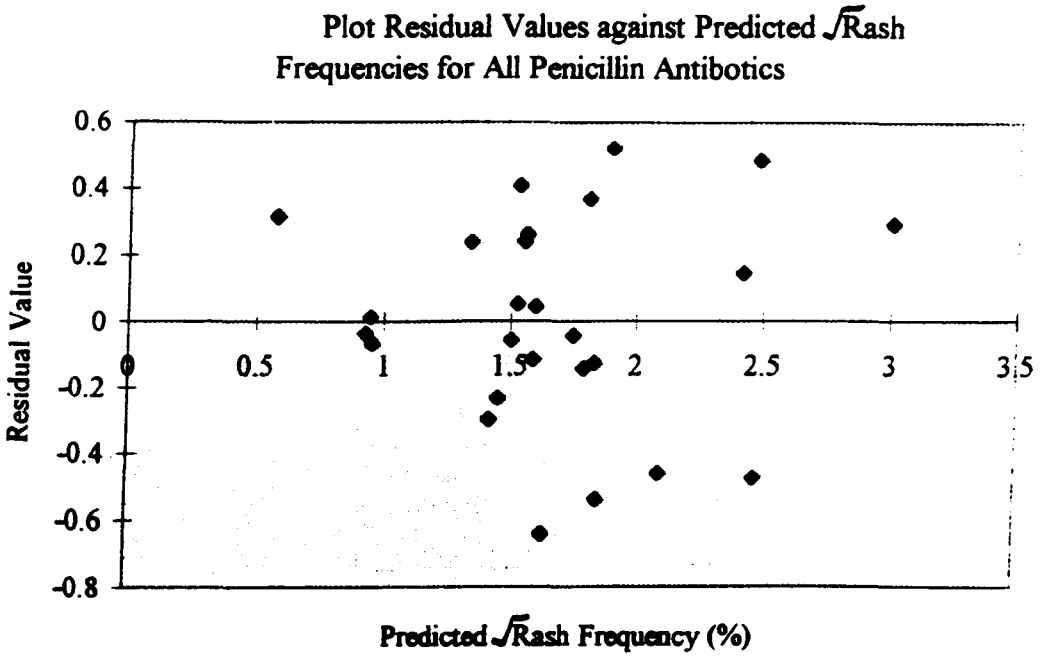
	SS BP	√Rash
SS BP	1.0	0.907
√Rash		1.0

Correlation Matrix Model 9.15

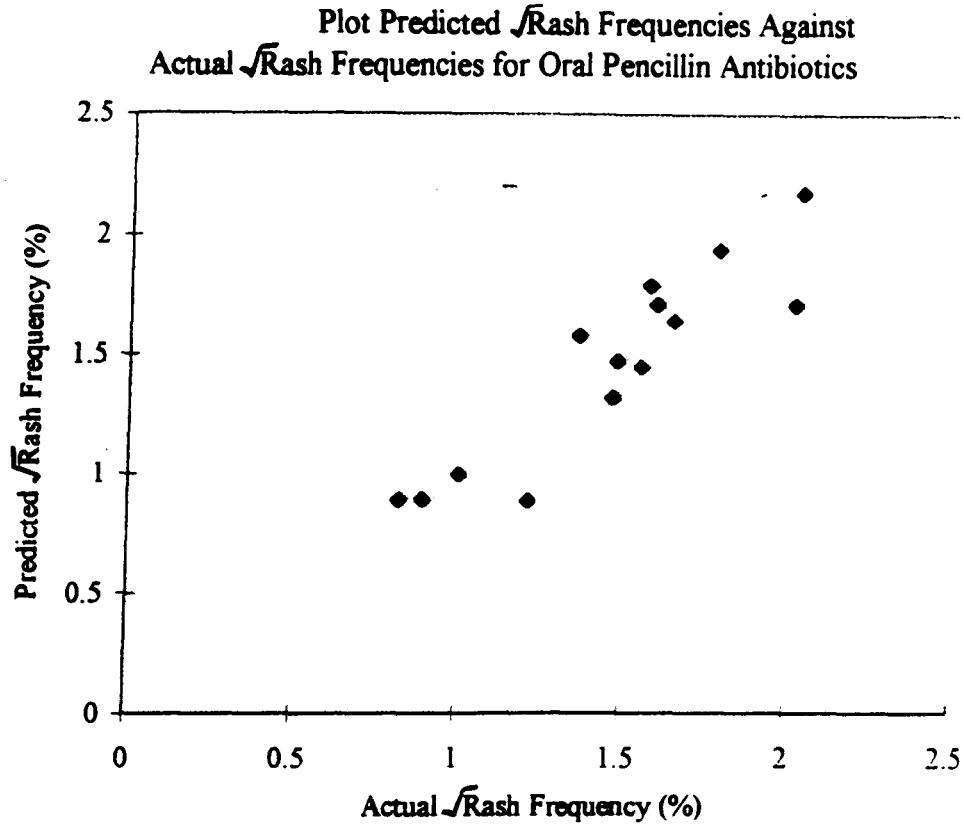
	Sterimol B3 (C=O)	SS Meth	√Rash
Sterimol B3 (C=O)	1.0	-0.305	0.372
SS Meth		1.0	0.607
√Rash			1.0

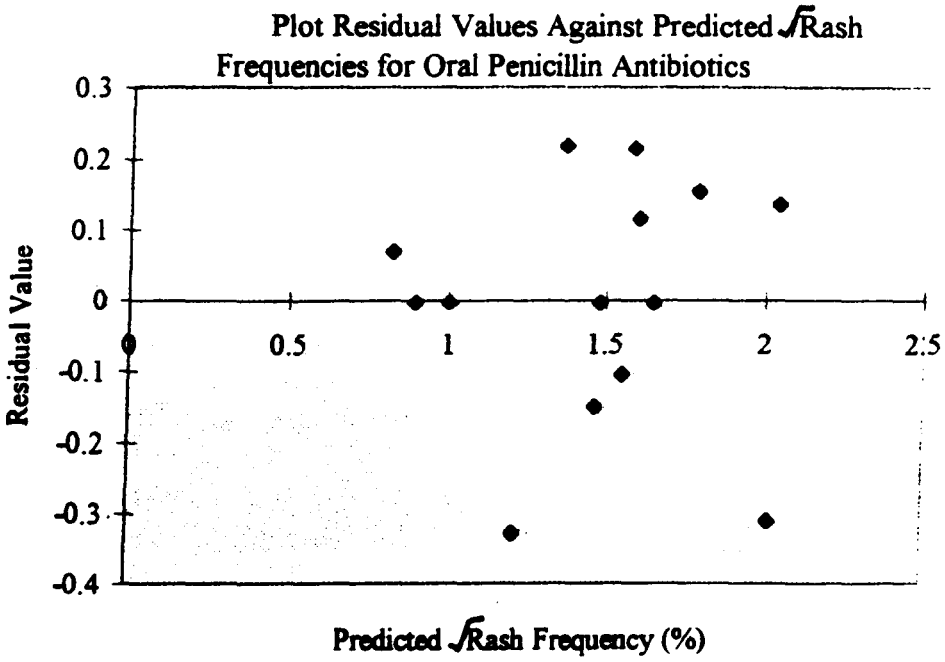
Model 9.13 Validity Plots



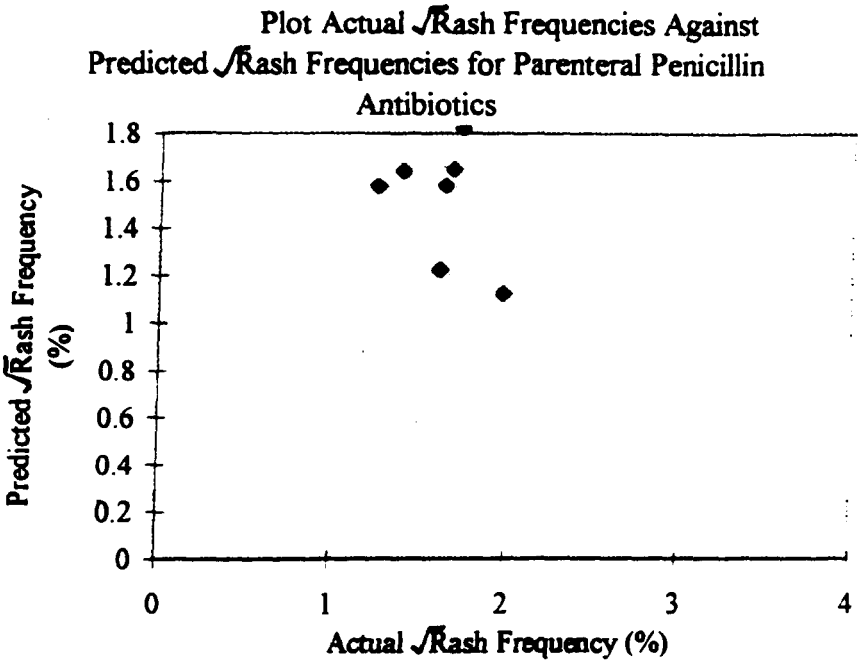


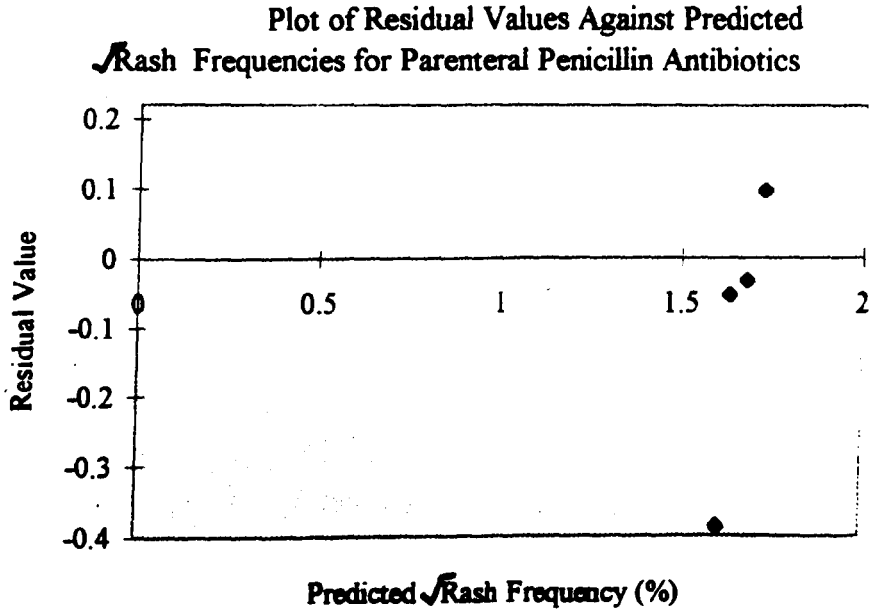
Model 9.14 Validity Plots





Model 9.15 Validity Plots





Model 9.16 All Cephalosporins, Model 9.17 Oral Cephalosporins and Model 9.18 Parenteral Cephalosporins

	Admin ID	Log H_20 sol.	SS Cefac	SS Cefep	DVZ	SS Cefatriz	Actual $\sqrt{}$Rash	All Cephs Predicted $\sqrt{}$Rash	All Cephs Residual	Oral Cephs Predicted $\sqrt{}$Rash	Oral Cephs Residual	Par. Cephs Predicted $\sqrt{}$Rash	Par. Cephs Residual
29	4	1.68	1.000	0.650	0.58	0.700	0.100	0.040	0.060	-	-	0.190	-0.090
30	3	-0.17	0.655	0.642	-2.59	0.760	1.082	1.403	-0.321	0.959	0.123	-	-
31	3	-0.68	0.794	0.751	0.31	0.708	0.693	0.602	0.091	0.948	-0.255	-	-
33	4	-2.69	0.699	0.610	-1.40	0.823	1.304	1.443	-0.139	-	-	1.051	0.253
34	3	-0.94	0.646	0.774	-0.56	1.000	2.437	2.207	0.230	2.403	0.034	-	-
35	4	-1.59	0.726	1.00	-5.88	0.589	1.253	1.087	0.166	-	-	1.785	-.0532
36	4	-1.26	0.663	0.648	-0.70	0.559	2.236	1.991	0.245	-	-	2.202	0.034
37	3	-0.42	0.658	0.566	-0.85	0.655	0.387	0.990	-0.603	0.589	-0.202	-	-
38	3	-2.13	0.639	0.719	7.44	0.560	0.917	0.439	0.478	0.918	-0.001	-	-
39	4	-1.27	0.708	0.614	5.58	0.557	0.762	0.859	-0.097	-	-	0.871	-0.109
40	4	-1.23	0.633	0.672	3.64	0.518	1.072	0.674	0.398	-	-	0.975	0.097
41	4	0.01	0.597	0.605	6.83	0.552	0.950	1.145	-0.186	-	-	1.228	-0.269
42	4	-2.05	0.653	0.705	-4.73	0.816	1.400	1.679	-0.279	-	-	1.493	-0.093
43	4	-2.09	0.608	0.649	2.48	0.579	1.594	1.311	0.283	-	-	1.377	0.217
44	4	-1.89	0.648	0.739	-6.45	0.780	1.285	1.711	-0.426	-	-	1.748	-0.463

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Models 9.16, 9.17, 9.18 continued

	Admin ID	Log H_20 sol.	SS Cefac	SS Cefep	DVZ	SS Cefatriz	Actual $\sqrt{}$Rash	All Cephs Predicted $\sqrt{}$Rash	All Cephs Residual	Oral Cephs Predicted $\sqrt{}$Rash	Oral Cephs Residual	Par. Cephs Predicted $\sqrt{}$Rash	Par. Cephs Residual
45	4	-1.99	0.721	0.760	6.82	0.724	1.136	1.488	-0.352	-	-	0.784	-0.352
46	4	1.27	0.689	0.592	3.99	0.618	1.349	1.303	0.046	-	-	1.153	-0.196
47	4	-1.01	0.738	0.727	4.79	0.659	0.100	0.693	-0.593	-	-	0.550	-0.400
48	4	0.53	0.714	0.676	-1.28	0.660	1.565	1.247	0.318	-	-	1.368	0.197
49	3	-2.66	0.626	0.556	-4.77	0.594	1.136	0.987	0.149	1.311	-0.175	-	-
50	3	-0.70	0.706	0.743	-2.33	0.588	0.714	0.232	0.482	0.407	0.307	-	-
51	3	-1.18	0.673	0.663	-0.18	0.592	0.949	1.008	-0.059	0.640	0.309	-	-
53	4	-0.97	0.679	0.727	-9.71	0.814	2.345	1.740	0.605	-	-	1.908	0.437
54	4	-1.52	0.653	0.738	-1.21	0.641	1.613	1.429	0.184	-	-	1.557	0.056
55	3	-0.61	0.712	0.663	-1.62	0.583	0.100	0.795	-0.695	0.334	-0.244	-	-
56	3	0.76	0.670	0.685	-1.12	0.690	0.100	-	-	0.222	-0.122	-	-
57	4	-0.24	0.720	0.693	-1.00	0.677	0.959	1.172	-0.213	-	-	1.240	-0.281
58	4	-1.13	0.584	0.653	0.52	0.558	1.114	1.158	-0.044	-	-	1.459	-0.345
59	4	0.69	0.706	0.704	-1.32	0.723	0.911	1.440	-0.529	-	-	1.336	-0.425
60	3	1.17	0.731	0.658	1.69	0.665	1.007	0.757	0.320	-	-	-	-
62	3	-0.51	0.778	0.714	2.35	0.697	1.000	0.868	0.132	0.822	0.178	-	-

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Models 9.16, 9.17, 9.18 continued

	Admin ID	Log H_20 sol.	SS Cefac	SS Cefep	DVZ	SS Cefatriz	Actual $\sqrt{R}$ash	All Cephs Predicted $\sqrt{R}$ash	All Cephs Residual	Oral Cephs Predicted $\sqrt{R}$ash	Oral Cephs Residual	Par. Cephs Predicted $\sqrt{R}$ash	Par. Cephs Residual
63	4	-0.31	0.730	0.703	2.88	0.657	1.000	1.089	-0.009	-	-	0.954	0.046
64	4	1.40	0.685	0.653	0.24	0.673	1.414	1.169	0.245	-	-	1.157	0.257
65	3	0.94	0.694	0.716	1.91	0.685	0.100	-	-	0.119	-0.019	-	-
66	4	1.23	0.666	0.648	-3.13	0.523	0.100	0.669	-0.569	-	-	-	-
67	4	0.01	0.726	0.670	-7.25	0.770	2.047	1.419	0.628	-	-	1.612	0.435
68	4	-1.85	0.656	0.605	2.00	0.638	1.269	0.998	0.271	-	-	0.997	0.272
69	4	-3.17	0.724	0.726	-2.08	0.735	1.549	1.483	0.066	-	-	1.391	0.158
70	3	-0.60	0.636	0.573	-3.77	0.623	0.592	0.711	-0.119	0.523	0.069	-	-

Model 9.16 Correlation Matrix

	Admin ID	SS Cefac	SS Cefep	SS Cefatriz	√Rash
Admin ID	1.0	0.008	0.254	-0.079	0.232
SS Cefac		1.0	0.096	0.209	-0.322
SS Cefep			1.0	-0.237	0.289
SS Cefatriz				1.0	0.444
√Rash					1.0

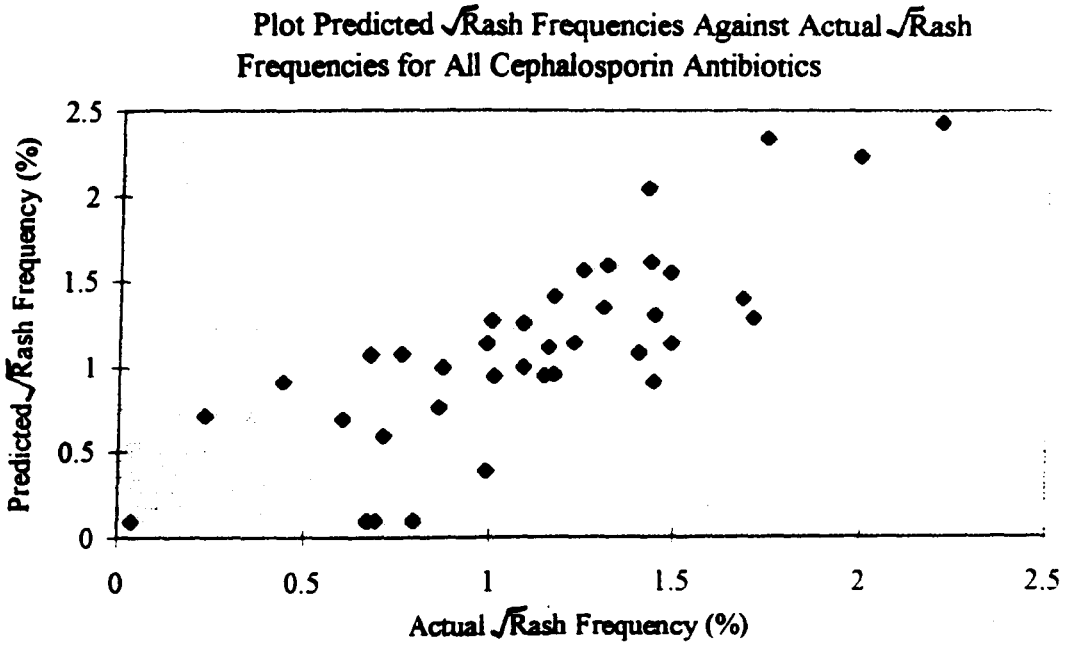
Model 9.17 Correlation Matrix

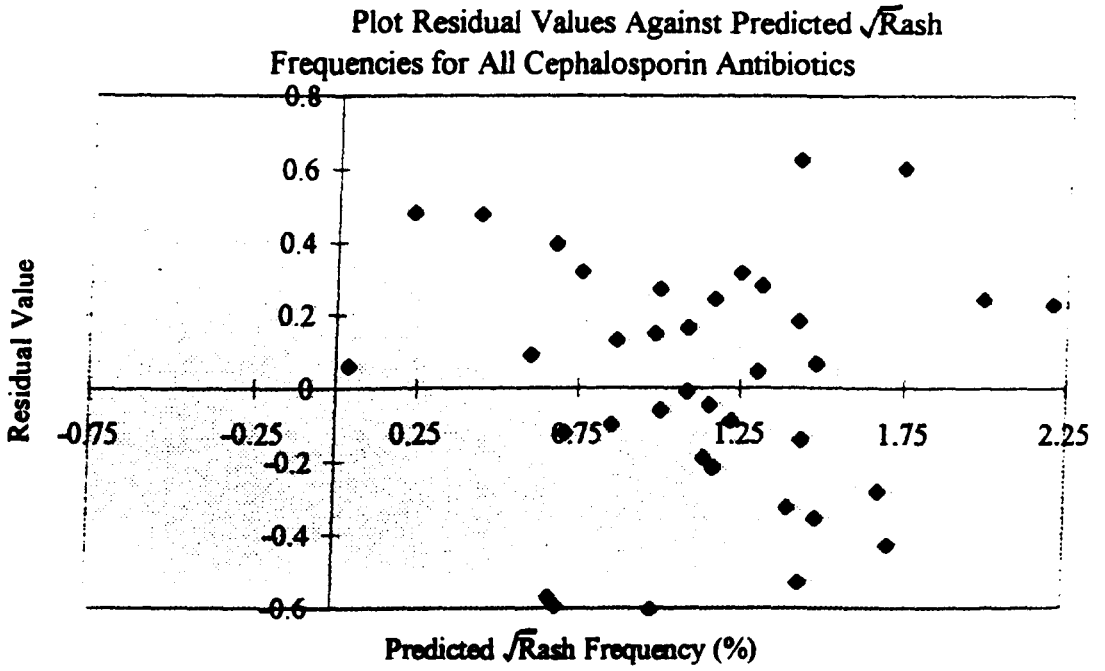
	Log H2O sol	SS Cefatriz	√Rash
Log H2O sol	1.0	0.262	-0.477
SS Cefatriz		1.0	0.668
√Rash			1.0

Model 9.18 Correlation Matrix

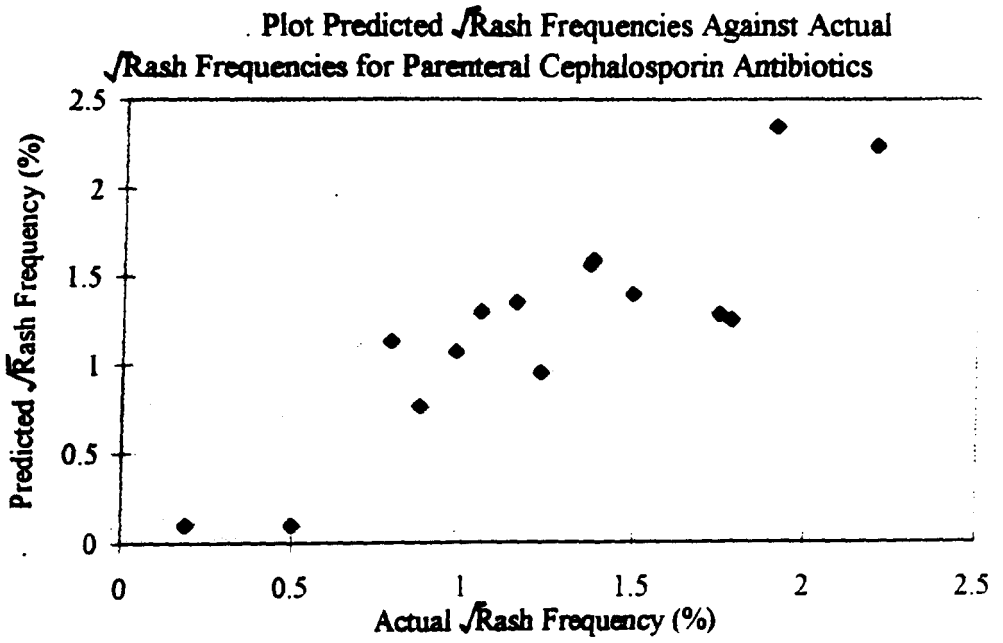
	DVZ	SS Cefac	SS Cefep	√Rash
DVZ	1.0	-0.007	0.023	-0.564
SS Cefac		1.0	0.030	-0.453
SS Cefep			1.0	0.384
√Rash				1.0

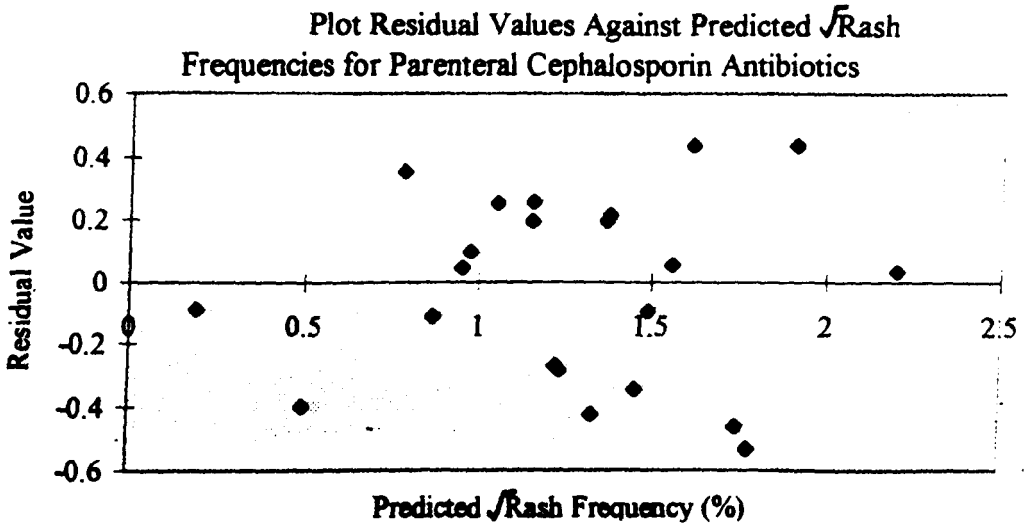
Model 9.16 Validity Plots





Model 9.18 Validity Plots





GI Disruption

Model 9.19 All β-Lactams

	R1 CH_3s	# S	Σ C=O* Charges	Ionist^n ptl.	Admin ID	SS Cephalor	Actual LogGI	Predicted LogGI	Residual
1	0	1	-0.787	8.99	4	0.781	-0.051	-0.210	0.159
2	0	1	-1.157	8.88	3	0.620	0.522	0.294	0.228
3	0	1	-1.204	9.00	3	0.649	0.407	0.508	-0.101
4	0	1	-1.204	9.18	3	0.669	0.724	0.496	0.228
5	0	1	-1.147	8.95	4	0.712	-0.377	-0.160	-0.217
6	0	1	-1.206	9.10	3	0.692	0.751	0.340	0.411
7	0	1	-2.106	8.94	3	0.749	0.241	0.363	-0.122
8	0	1	-1.580	9.03	3	0.604	0.193	0.487	-0.294
9	0	1	-1.207	9.52	4	0.651	0.398	0.398	-3.135^{-4}
10	0	1	-1.5028	8.99	4	0.664	-0.108	0.358	-0.466
11	0	1	-1.568	8.89	3	0.642	0.204	0.449	-0.245
12	0	1	-1.576	8.97	3	0.652	0.236	0.431	-0.195
13	0	1	-1.205	8.97	3	0.717	0.667	0.377	0.290
14	1	1	-1.211	8.70	4	0.770	0.398	0.185	0.213
15	1	1	-1.170	9.12	3	0.731	0.568	0.527	0.041
16	0	1	-1.135	9.05	3	0.671	0.782	0.446	0.336
18	0	1	-1.587	9.09	3	0.695	0.104	0.335	-0.231
19	2	1	-1.210	8.48	4	0.683	1.086	0.764	0.322
20	1	2	-2.631	9.03	4	0.713	0.297	0.591	-0.294
21	1	1	-1.209	9.08	4	0.738	0.786	0.319	0.467
22	1	1	-1.215	9.09	4	0.654	0.301	0.613	-0.312
23	0	1	-1.203	9.32	3	0.712	0.403	0.327	0.076
24	1	1	-2.026	8.99	4	0.746	0.396	0.363	0.033
25	0	1	-1.574	8.92	3	0.731	0.241	0.095	0.146
26	0	1	-1.576	9.19	3	0.717	0.173	0.315	-0.142
27	0	2	-1.573	7.39	4	0.692	-0.284	0.017	-0.301
28	0	2	-1.529	8.97	4	0.697	0.230	0.185	0.045

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Model 9.19 Continued

	R1 CH_3s	# S	Σ C=O* Charges	Ionist^n ptl.	Admin ID	SS Cephalor	Actual LogGI	Predicted LogGI	Residual
29	0	1	-1.565	9.18	4	0.643	0.223	0.394	-0.171
30	0	1	-1.170	9.03	3	0.690	0.435	0.365	0.070
31	0	1	-1.163	8.72	3	0.681	-0.319	0.322	-0.641
33	0	2	-1.157	8.87	4	0.614	-0.097	0.080	-0.177
34	0	2	-1.161	10.70	3	0.657	0.695	0.357	0.338
35	0	2	-1.162	8.45	4	0.792	-0.444	-0.621	0.177
36	1	2	-1.120	8.38	4	0.740	0.522	-0.122	0.644
37	0	2	-1.492	8.83	3	0.773	-0.347	-0.201	-0.146
38	1	3	-1.126	8.84	3	0.663	-0.119	0.244	-0.363
39	0	3	-1.156	9.10	4	0.663	-0.569	-0.147	-0.422
40	0	3	-1.528	9.22	4	0.742	-0.319	0.348	0.029
41	1	3	-1.126	9.00	4	0.603	0.173	-0.021	0.194
42	0	3	-1.661	8.78	4	0.666	0.322	-0.200	0.522
43	1	2	-2.363	9.18	4	0.652	0.507	-0.471	0.036
44	0	2	-1.535	9.09	4	0.625	0.00	0.033	-0.033
45	0	4	-1.975	8.71	4	0.668	0.428	-0.063	0.491
46	1	2	-1.497	8.80	4	0.736	0.045	0.076	-0.310
47	0	3	-1.162	8.88	4	0.641	-0.409	-0.293	-0.116
48	0	2	-1.587	9.28	4	0.872	-0.032	-0.441	0.409
49	1	2	-1.563	8.82	3	0.653	0.021	0.299	-0.278
50	1	2	-1.489	8.74	3	0.669	0.631	0.17	0.484
51	0	1	-1.154	8.51	3	0.701	0.161	0.109	0.052
52	0	1	-1.183	8.87	3	0.668	0.342	0.389	-0.470
53	0	2	-1.831	8.94	4	0.600	0.919	0.218	0.701
54	2	2	-1.560	8.98	4	0.599	0.270	0.692	-0.422
55	1	2	-1.121	9.34	3	0.771	0.558	0.110	0.448
56	0	2	-1.529	9.21	3	0.699	0.740	0.235	0.505
57	1	2	-1.121	8.82	4	0.683	-0.108	0.296	-0.404

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Model 9.19 Continued

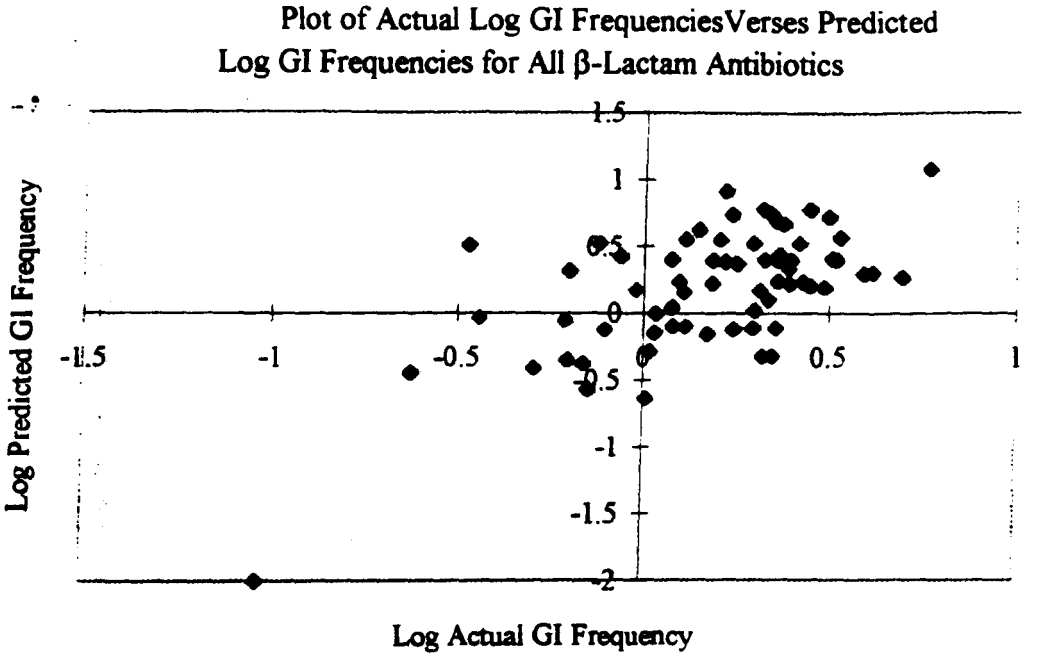
	R1 CH_3s	# S	Σ C=O* Charges	Ionist^n ptl.	Admin ID	SS Cephalo r	Actual LogGI	Predicted LogGI	Residual
58	1	3	-1.560	9.09	4	0.689	0.405	0.074	-0.331
59	1	1	-1.552	9.16	4	0.818	-0.143	0.031	-0.174
60	1	1	-1.919	7.54	3	0.711	0.554	0.204	0.350
61	1	3	-1.126	9.01	3	0.712	-0.638	0.009	-0.647
62	0	1	-1.159	9.52	3	0.692	0.522	0.417	0.105
63	0	2	-1.162	9.23	4	1.000	-2.000	-1.031	-0.969
64	0	22	-1.535	9.17	4	0.658	-0.155	0.173	-0.328
65	0	1	-1.910	9.52	3	0.659	0.398	0.518	-0.128
66	0	2	-1.535	8.93	4	0.632	-0.097	0.112	-0.209
67	0	1	-1.161	9.18	4	0.679	0.389	0.219	0.170
68	0	2	-1.161	9.20	4	0.618	-0.119	-0.103	-0.016
69	0	1	-1.533	9.40	4	0.603	0.373	0.251	0.122
70	1	2	-1.487	9.00	3	0.531	0.086	0.623	-0.537

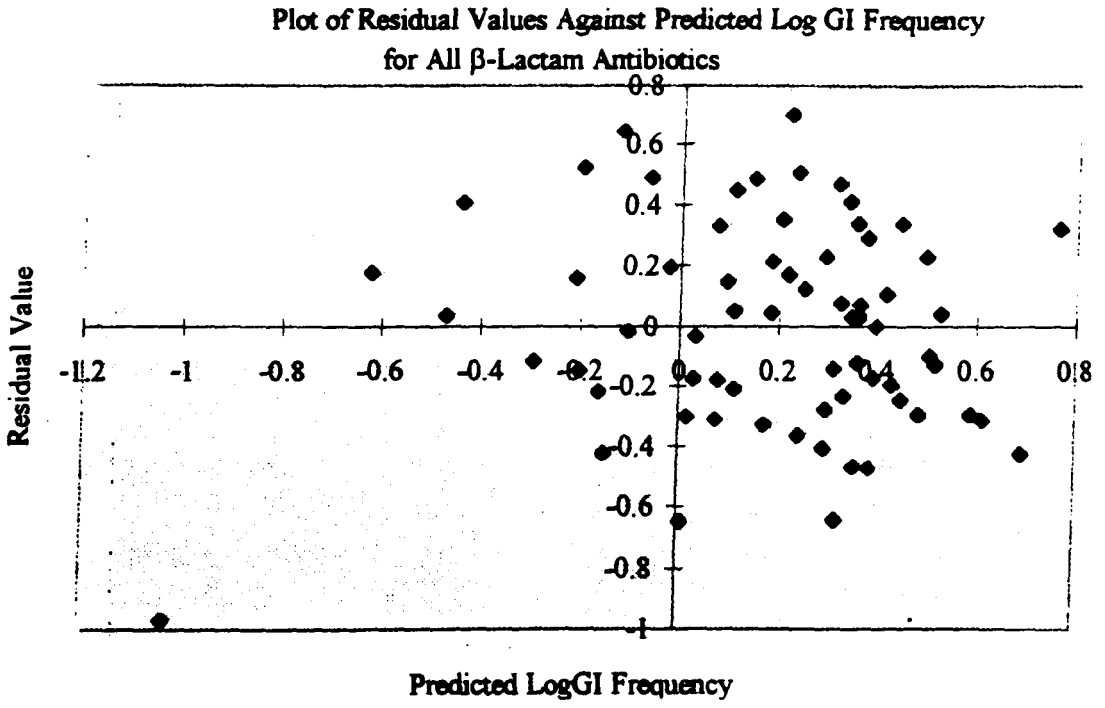
Values of K_3 were not recorded.

Correlation Matrix Model 9.19

	R1 CH_3s	# S	Σ C=O* Charges	K_3	Ionist^n ptl.	Admin ID	SS Cephalor	LogGI
R1 CH_3s	1.0	0.132	-0.098	0.306	-0.204	0.162	-0.005	0.200
# S		1.0	-0.043	0.462	-0.041	-0.103	0.090	-0.348
Σ C=O* Charges			1.0	-0.454	0.098	-0.103	0.074	-0.140
K_3				1.0	-0.087	0.016	-0.285	-0.093
Ionist^n ptl.					1.0	-0.093	-0.042	-0.092
Admin ID						1.0	0.090	-0.256
SS Cephalor							1.0	-0.396
Log_{10} GI								1.0

Model 9.19 Validity Plots





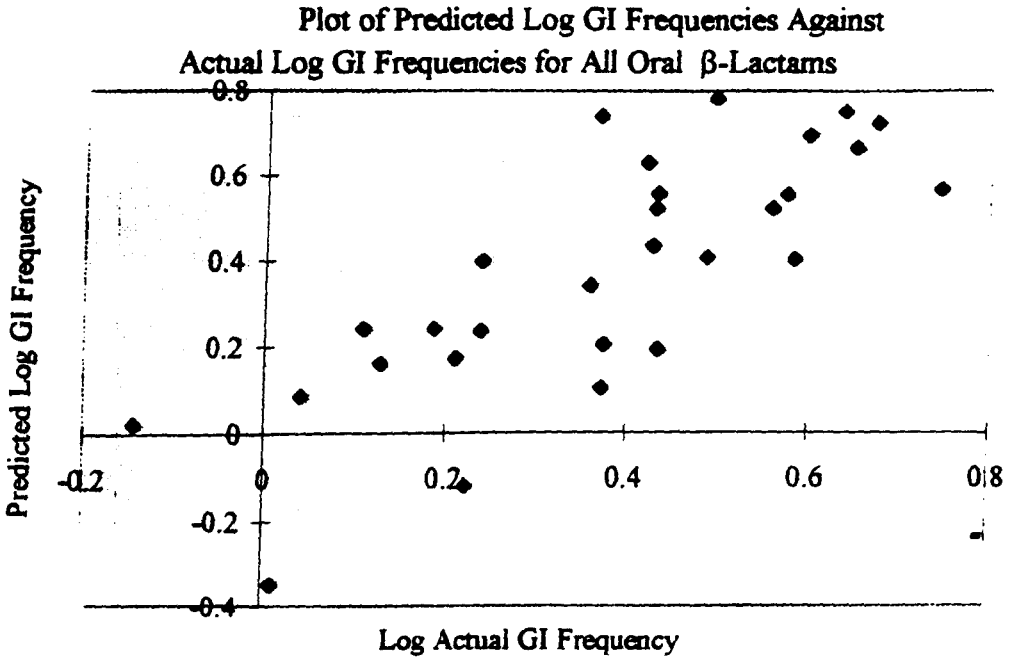
Model 9.20 Oral β-Lactams

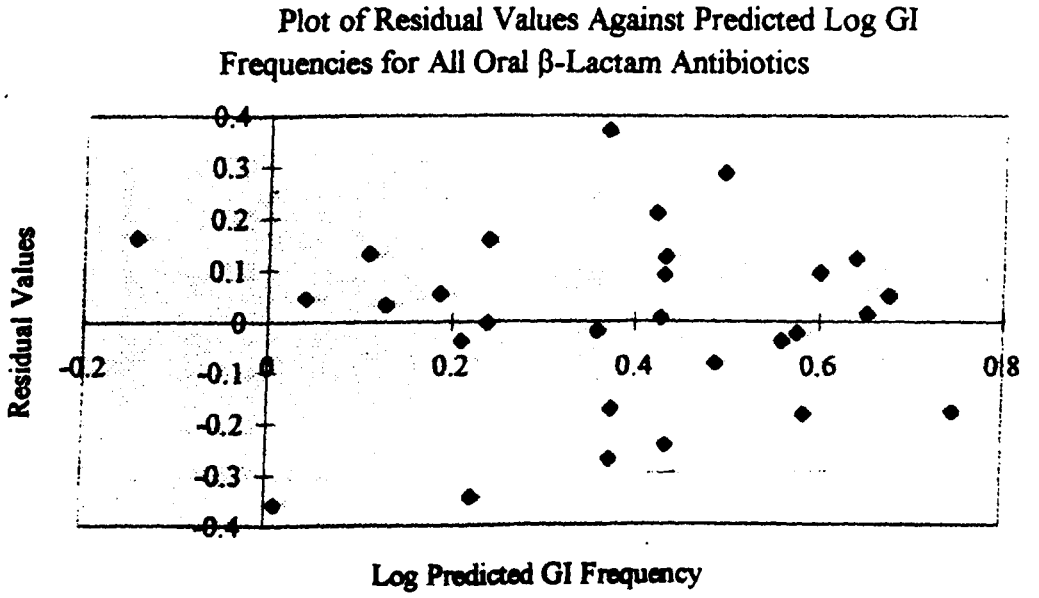
	R1 CH_3s	SA	Total Dipole 10	R1 HA	Actual Log GI	Predicted LogGI	Residual
2	0	30.64	0.593	1	0.522	0.560	-0.038
3	0	31.64	0.415	3	0.407	0.488	-0.081
4	0	28.70	0.359	2	0.724	0.675	0.049
6	0	29.69	0.497	1	0.751	0.639	0.112
7	0	37.42	0.412	6	0.241	0.109	0.132
8	0	33.94	0.506	2	0.193	0.435	-0.242
11	0	40.62	0.208	2	0.204	0.376	-0.172
12	0	36.80	0.711	2	0.236	0.239	-0.003
13	0	30.01	0.323	2	0.667	0.652	0.015
15	1	36.97	0.413	2	0.568	0.746	-0.178
16	0	30.36	0.607	2	0.782	0.496	0.286
18	0	36.52	0.466	2	0.104	0.374	-0.270
23	0	30.83	0.403	2	0.403	0.585	-0.182
25	0	40.93	0.558	2	0.241	0.187	0.054
26	0	38.25	0.676	2	0.173	0.211	-0.038
30	0	30.66	0.721	2	0.435	0.428	0.007
34	0	33.20	0.100	3	0.695	0.600	0.095
37	0	35.55	0.590	7	-0.347	0.011	-0.358
38	1	39.60	0.884	5	-0.119	0.224	-0.343
49	1	51.28	0.750	6	0.021	-0.143	0.164
50	1	37.86	0.604	5	0.631	0.422	0.209
51	0	34.21	0.959	3	0.161	0.128	0.033
52	0	30.15	1.014	1	0.342	0.360	-0.018
55	1	39.59	0.475	5	0.558	0.433	0.125
56	0	34.20	0.489	3	0.740	0.369	0.371
60	1	42.66	0.263	3	0.554	0.576	-0.022
62	0	29.45	0.788	3	0.522	0.432	0.090
65	0	37.68	0.526	5	0.398	0.240	0.158
70	1	49.90	0.603		0.086	0.041	0.045

Correlation Matrix Model 9.20

	SA	TD	R1 HA	R1 CH_3	LogGI
SA	1.0	0.023	0.584	0.674	-0.501
Total Dipole		1.0	0.060	0.059	-0.391
R1 HA			1.0	0.513	-0.496
R1 CH_3s				1.0	-0.096
Log_{10} GI					1.0

Model 9.20 Validity Plots





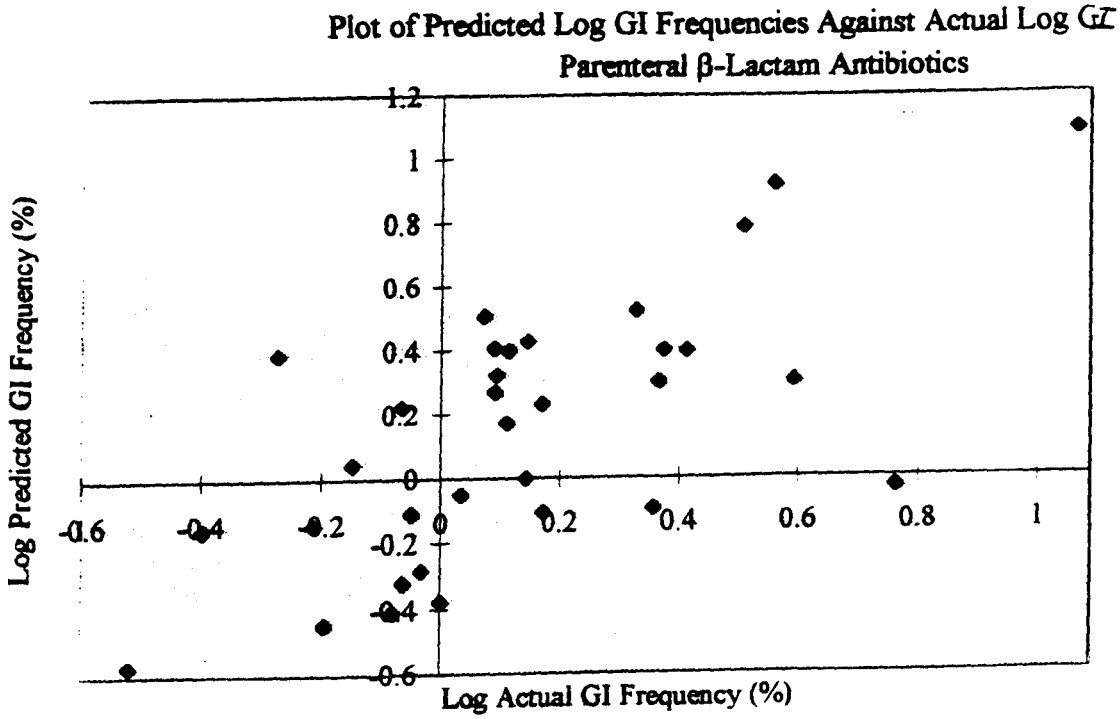
Model 9.21 Parenteral β-Lacatms

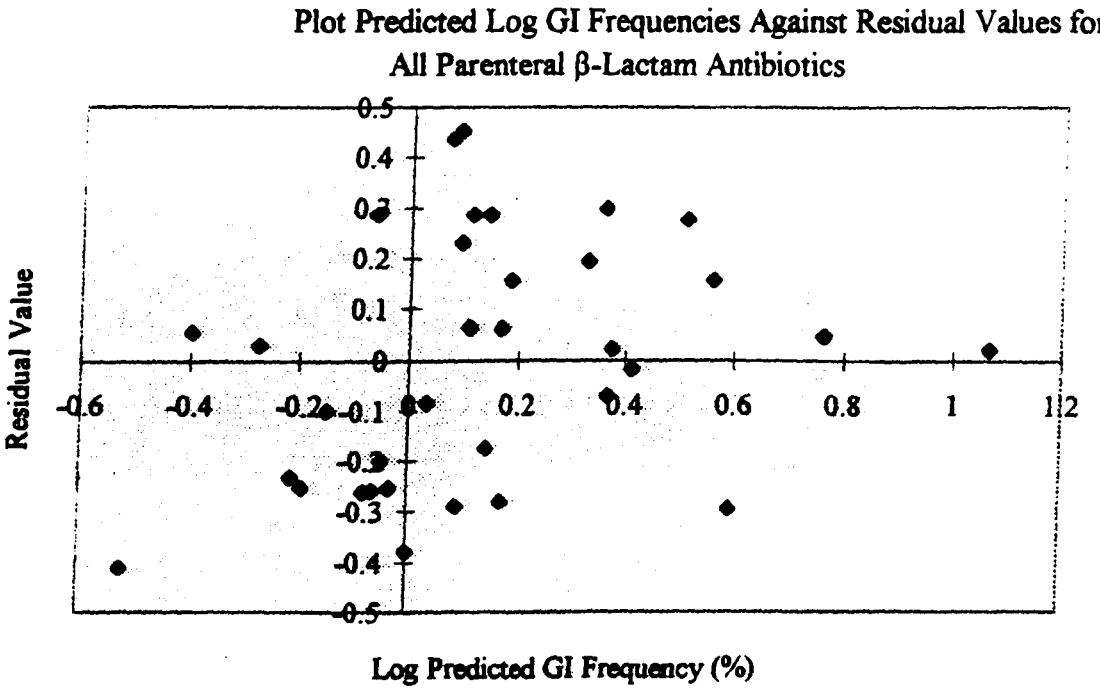
	R1 CH_3s	SS Cefsul	Actual LogGI	Predicted LogGI	Residual
1	0	0.731	-0.051	0.033	-0.084
5	0	0.719	-0.377	7.53^{-4}	-0.378
9	0	0.760	0.398	0.112	-0.286
10	0	0.782	-0.108	0.172	-0.280
14	1	0.675	0.398	0.375	0.023
19	2	0.748	1.086	1.066	0.020
20	1	0.672	0.297	0.367	-0.070
21	1	0.714	0.786	0.508	0.278
22	1	0.756	0.301	0.594	-0.293
24	1	0.689	0.396	0.413	-0.017
27	0	0.707	-0.284	-0.032	-0.252
28	0	0.781	0.230	0.169	0.061
29	0	0.695	0.223	-0.064	0.287
35	0	0.648	-0.444	-0.192	-0.252
36	1	0.658	0.522	0.329	0.193
39	0	0.660	-0.569	-0.159	-0.410
40	0	0.696	-0.319	-0.062	-0.257
41	1	0.577	0.173	0.109	0.064
42	0	0.753	0.322	0.093	0.229
43	1	0.763	0.507	0.071	0.436
44	0	0.783	0.00	0.174	-0.174
45	0	0.771	0.428	0.142	0.286
46	1	0.590	0.045	0.144	-0.099
47	0	0.664	-0.409	-0.148	-0.261
48	0	0.690	-0.032	-0.078	0.046
53	0	1.000	0.919	0.763	0.156
54	2	0.561	0.270	0.559	-0.289
57	1	0.570	-0.108	0.090	-0.198
58	1	0.519	0.405	-0.048	0.453
59	1	0.569	-0.143	0.088	-0.231
64	0	0.641	-0.155	-0.211	0.056
66	0	0.573	-0.097	-0.396	0.298
67	0	0.851	0.389	0.359	0.030
68	0	0.618	-0.119	-0.273	0.154
69	0	0.787	0.373	0.185	0.188

Correlation Matrix Model 9.21

	R1 CH_3s	SS Cefsul	LogGI
R1 CH_3s	1.0	-0.431	0.484
SS Cephalor		1.0	0.348
Log_{10} GI			1.0

Model 9.21 Validity Plots





Models 9.22 All Penicillins, 9.23 Oral Penicillins and 9.24 Parenteral Penicillins

	R1 CH_3	# CH_3	SA	Actual LogGI	All Pens Pred. LogGI	All Pens Resid.	Oral Pens Pred. LogGI	Oral Pens. Resid.	Par.Pens. Pred. LogGI	Parent. Pens. Resid.
1	0	2	30.23	-0.051	0.130	-0.181	-	-	0.081	-0.132
2	0	5	30.64	0.522	0.528	-0.005	0.563	-0.040	-	-
3	0	2	31.64	0.407	0.486	-0.080	0.516	-0.109	-	-
4	0	2	28.70	0.724	0.608	0.116	0.654	0.070	-	-
5	0	2	40.97	-0.377	-0.316	-0.061	-	-	-0.301	-0.076
6	0	2	29.69	0.751	0.568	0.183	0.608	0.143	-	-
7	0	2	37.42	0.241	0.246	-0.005	0.224	-0.003	-	-
8	0	4	33.94	0.193	0.391	-0.198	0.407	-0.214	-	-
9	0	2	27.34	0.398	0.250	0.148	-	-	0.184	0.214
10	0	2	30.99	-0.108	0.098	-0.206	-	-	0.054	-0.162
11	0	2	40.62	0.204	0.113	0.091	0.093	0.111	-	-
12	0	2	36.80	0.236	0.272	-0.036	0.273	-0.037	-	-
13	0	2	30.01	0.667	0.554	0.113	0.593	0.074	-	-
14	1	3	35.60	0.398	0.439	-0.041	-	-	0.460	-0.062
15	1	3	36.97	0.568	0.796	-0.228	0.568	-1.11^{-16}	-	-
16	0	2	30.36	0.782	0.540	0.242	0.576	0.206	-	-
18	0	3	36.52	0.104	0.283	-0.179	-	-	1.138	-0.052
19	2	4	32.56	1.086	1.097	-0.011	0.286	-0.182	-	-
20	1	3	42.11	0.297	0.168	0.129	-	-	0.229	0.068
21	1	3	33.14	0.786	0.541	0.245	-	-	0.548	0.238
22	1	3	32.46	0.301	0.570	-0.269	-	-	0.572	-0.271
23	0	2	30.83	0.403	0.520	-0.117	0.554	-0.151	-	-
24	1	3	41.11	0.396	0.210	0.186	-	-	0.264	0.132
25	0	5	40.93	0.241	0.100	0.141	0.078	0.163	-	-
26	0	2	38.25	0.173	0.211	-0.038	0.204	-0.031	-	-
27	0	3	39.74	-0.284	-0.265	-0.019	-	-	-0.257	-0.027
28	0	2	29.73	0.230	0.151	0.079	-	-	0.099	0.131

Correlation Matrix Model 9.22

	SA	# CH_3	Admin ID	LogGI
SA	1.0	0.175	0.049	-0.444
# CH_3		1.0	0.483	0.460
Admin ID			1.0	-0.240
Log_{10} GI				1.0

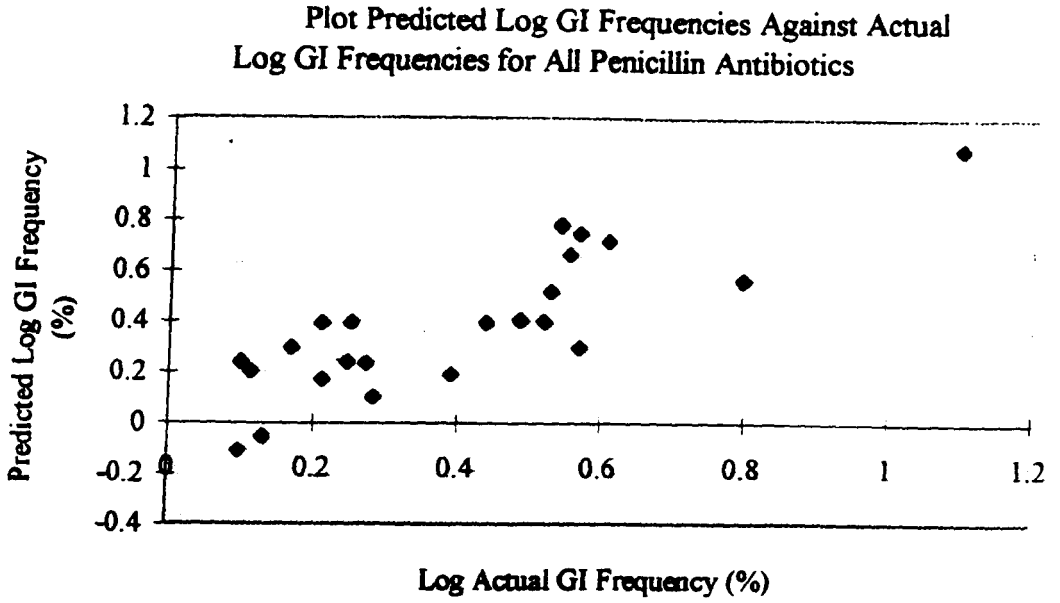
Correlation Matrix Model 9.23

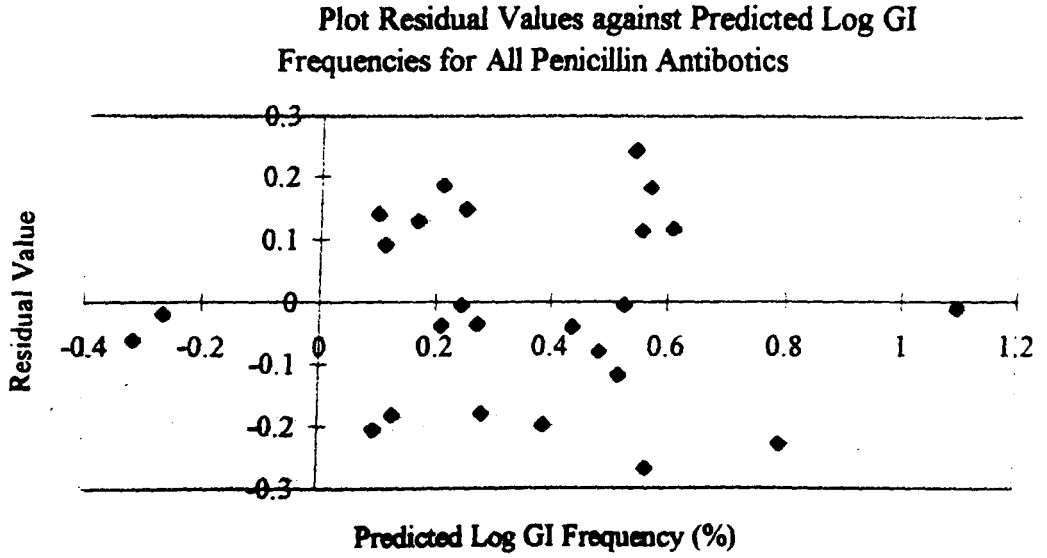
	SA	# CH_3	LogGI
SA	1.0	0.181	-0.775
# CH_3		1.0	0.180
Log_{10} GI			1.0

Correlation Matrix Model 9.24

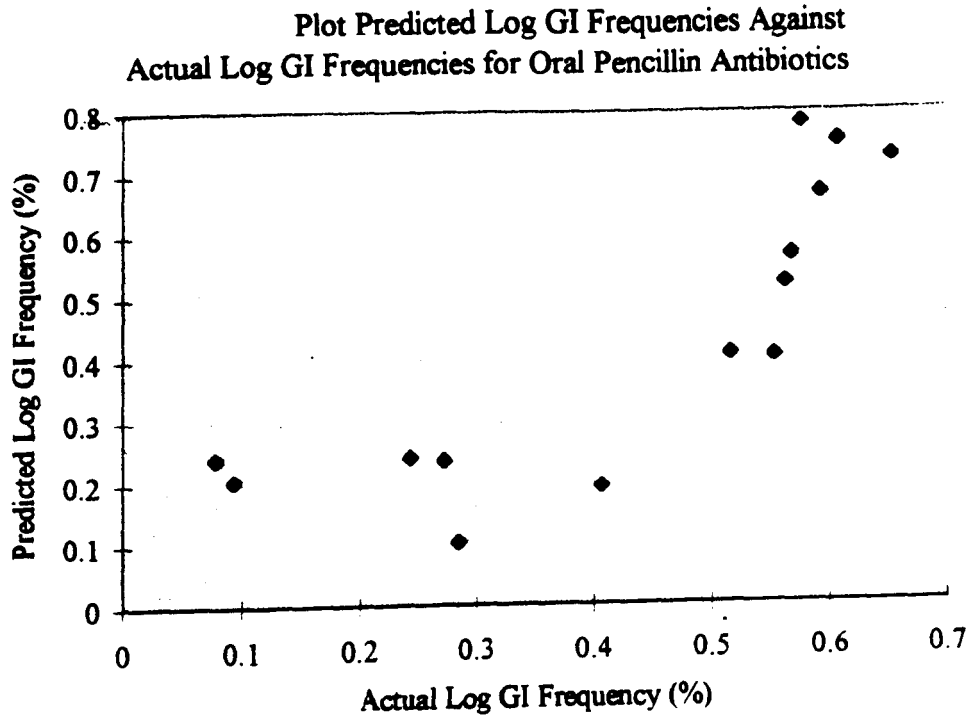
	SA	# CH_3 R1	LogGI
SA	1.0	0.184	-0.265
# CH_3 R1		1.0	0.825
Log_{10} GI			1.0

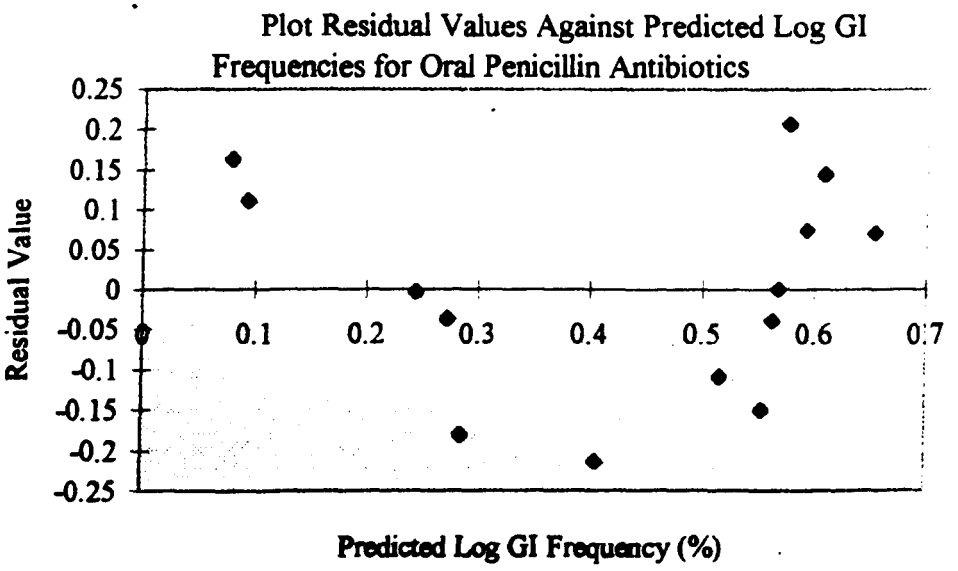
Model 9.22 Validity Plots





Model 9.23 Validity Plots





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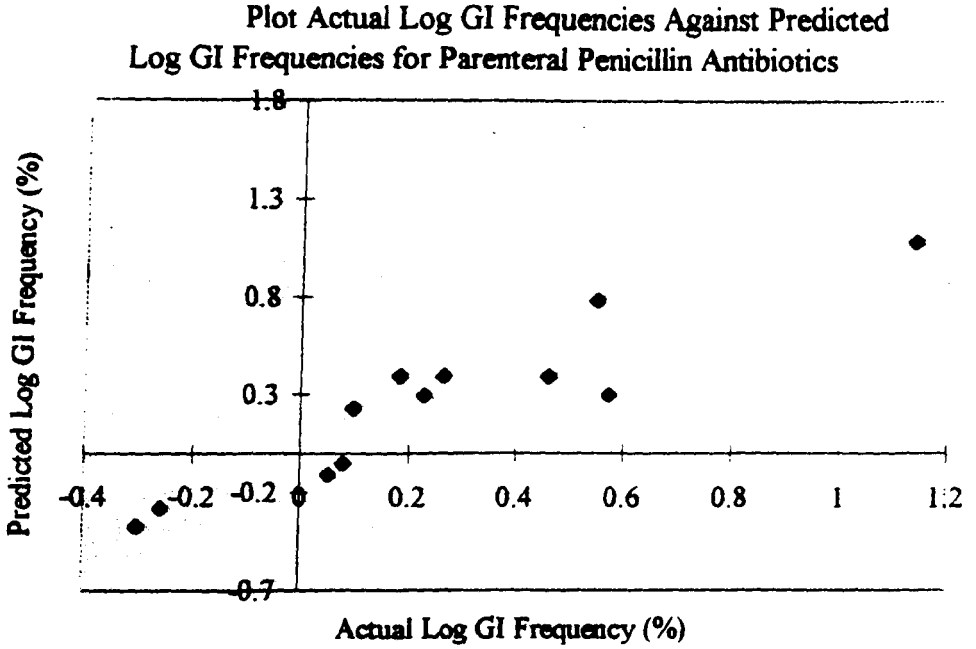

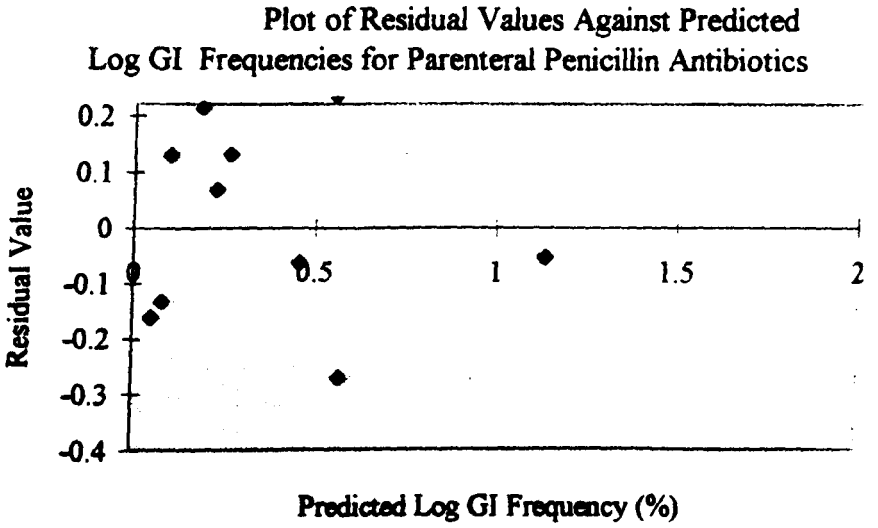

Models 9.25 All Cephalosporins, 9.26 Oral Cephalosporins and 9.27 Parenteral Cephalosporins

	DVz	Σ C=O* Charges	TD 10	SS Cephalex	SS Cephalor	Actual LogGI	All Cephs P. LogGI	All Cephs Resid.	Oral Cephs P.LogGI	Orl Cephs Resid.	Par. Cephs P.LogGI	P.Cephs Resid.
29	0.58	-1.565	0.159	0.780	0.643	0.223	0.486	-0.257	-	-	0.398	-0.175
30	-2.59	-1.170	0.721	0.784	0.690	0.435	0.087	0.348	0.407	0.028	-	-
31	0.31	-1.163	0.207	0.772	0.681	-0.319	-0.005	-0.314	-	-	-	-
33	-1.40	-1.157	0.494	0.704	0.614	-0.097	0.110	-0.207	-	-	0.036	-0.133
34	-0.56	-1.161	0.100	0.683	0.657	0.695	-0.139	0.834	0.793	-0.098	-	-
35	-5.88	-1.162	0.942	0.716	0.792	-0.444	-0.408	-0.036	-	-	-0.477	0.033
37	-0.85	-1.492	0.590	0.681	0.773	-0.347	-0.321	-0.026	-	-	-	-
38	7.44	-1.126	0.884	0.683	0.663	-0.119	-0.431	0.312	0.105	-0.224	-	-
39	5.58	-1.156	0.601	0.739	0.663	-0.569	-0.193	-0.376	-	-	-0.299	-0.270
40	3.64	-1.528	0.788	0.753	0.742	-0.319	-0.104	-0.215	-	-	-0.205	-0.114
41	3.83	-1.126	0.750	0.669	0.603	0.173	-0.129	0.302	-	-	-0.223	0.396
42	-4.73	-1.661	0.706	0.697	0.666	0.322	0.393	-0.071	-	-	0.332	-0.010
43	2.48	-2.363	0.469	0.651	0.652	0.507	0.662	-0.155	-	-	0.580	-0.073
44	-6.45	-1.535	0.700	0.658	0.625	0.00	0.393	-0.393	-	-	0.342	-0.342
45	6.82	-1.975	0.962	0.738	0.668	0.428	0.404	0.024	-	-	0.299	0.129
46	3.99	-1.497	0.654	0.745	0.736	0.045	-0.138	0.183	-	-	-0.240	0.285

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Models 9.25, 9.26 and 9.27 continued

	DVz	Σ C=O* Charges	Total Dipole	SS Cephalex	SS Cephalor	Actual LogGI	All Cephs P. LogGI	All Cephs Resid.	Oral Cephs P.LogGI	Orl Cephs Resid.	Par. Cephs P.LogGI	P.Cephs Resid.
47	4.79	-1.162	0.877	0.663	0.641	-0.409	0.294	-0.115	-	-	-0.394	-0.015
48	-1.28	-1.587	0.596	0.820	0.872	-0.032	-0.223	0.191	-	-	-0.314	0.282
49	-4.77	-1.563	0.750	0.658	0.653	0.021	0.255	-0.234	0.183	-0.162	-	-
50	-2.33	-1.489	0.604	0.710	0.669	0.631	0.207	0.424	0.393	0.238	-	-
51	-0.18	-1.154	0.959	0.771	0.701	0.161	0.164	-0.003	0.177	-0.016	-	-
52	-0.83	-1.183	1.014	0.758	0.668	0.342	0.298	0.044	0.109	0.233	-	-
53	-9.71	-1.831	0.893	0.685	0.600	0.919	0.902	0.017	-	-	0.868	0.051
54	-1.21	-1.560	0.157	0.737	0.599	0.270	0.579	-0.309	-	-	0.508	-0.238
55	-1.62	-1.121	0.475	0.682	0.771	0.558	0.228	-0.330	0.462	0.096	-	-
56	-1.12	-1.529	0.489	0.775	0.699	0.740	0.270	0.470	0.595	0.145	-	-
57	-1.00	-1.121	0.393	0.767	0.683	-0.108	-0.021	-0.087	-	-	-0.102	-0.006
58	0.52	-1.560	1.026	0.668	0.689	0.405	-0.018	0.423	-	-	-0.102	0.507
59	-1.32	-1.552	0.312	0.793	0.818	-0.143	-0.117	-0.026	-	-	-0.204	0.061
60	1.69	-1.919	0.263	0.629	0.711	0.554	0.038	0.516	0.565	-0.011	-	-
61	5.01	-1.126	0.814	0.724	0.712	-0.638	-0.432	-0.206	-	-	-1.668	-0.332
62	2.35	-1.159	0.788	1.000	0.692	0.522	0.533	-0.011	0.687	-0.165	-	-

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Models 9.25, 9.26, 9.27 Continued

	DVz	Σ C=O* Charges	Total Dipole	SS Cephalex	SS Cephalo r	Actual LogGI	All Cephs P. LogGI	All Cephs Resid.	Oral Cephs P.LogGI	Orl Cephs Resid.	Par. Cephs P.LogGI	P.Cephs Resid.
63	2.88	-1.162	0.626	0.692	1.000	-2.000	-1.549	-0.451	-	-	0.258	-0.413
64	0.24	-1.535	0.558	0.756	0.658	-0.155	0.340	-0.495	-	-	-	-
65	1.91	-1.910	0.526	0.662	0.659	0.398	0.320	0.078	0.386	0.012	-	-
66	-3.13	-1.535	0.634	0.562	0.632	-0.097	-0.006	-0.091	-	-	-0.070	--0.027
67	-7.25	-1.161	0.793	0.745	0.679	0.389	0.153	0.236	-	-	0.098	0.291
68	2.00	-1.161	0.649	0.677	0.618	-0.119	-0.001	-0.038	-	-	-0.169	0.050
69	-2.08	-1.533	0.621	0.669	0.603	0.373	0.376	-0.003	-	-	0.309	0.064
70	-3.77	-1.487	0.603	0.563	0.531	0.086	0.370	-0.284	0.163	-0.077	-	-

Model 9.25 Correlation Matrix

	DVz	Σ Charge C=O*	SS Cephalor	SS Cephalex	LogGI
DVz	1.0	-0.008	0.134	0.060	-0.311
Σ Charge C=O*		1.0	0.105	0.278	-0.416
SS Cephalor			1.0	0.325	-0.576
SS Cephalex				1.0	0.066
LogGI					1.0

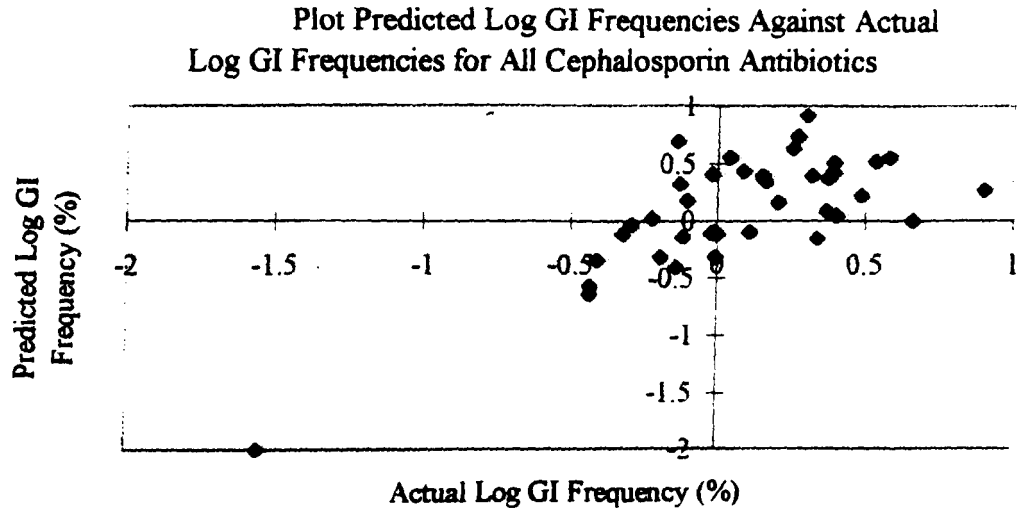
Model 9.26 Correlation Matrix

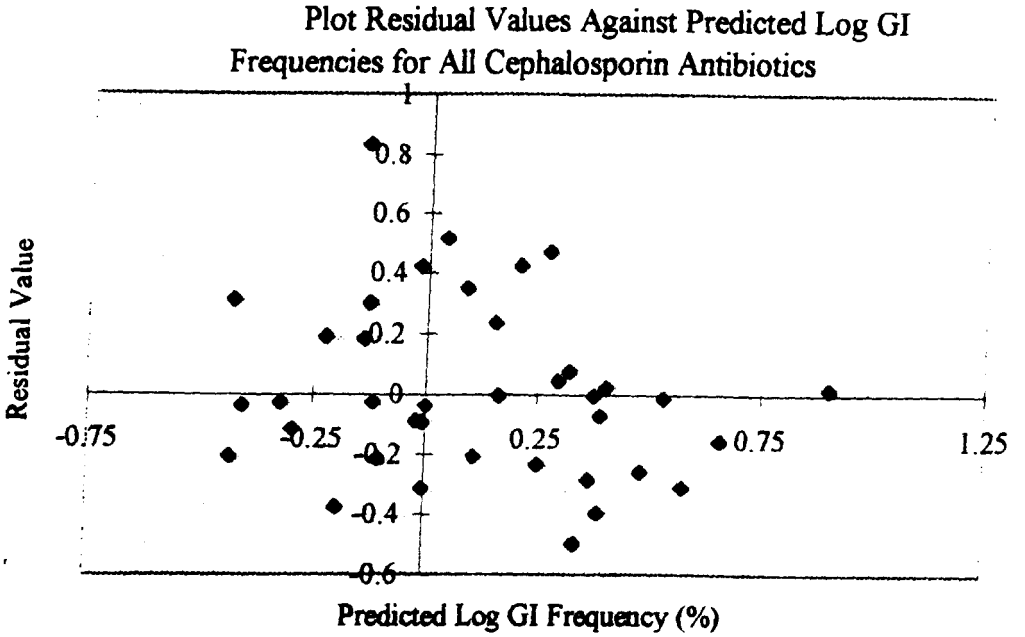
	TD	SS Cephalex	LogGI
TD	1.0	0.376	-0.623
SS Cephalex		1.0	0.288
LogGI			1.0

Model 9.27 Correlation Matrix

	DVz	Σ Charge C=O*	SS Cephalor	SS Cephalex	LogGI
DVz	1.0	0.022	0.121	0.110	-0.334
Σ Charge C=O*		1.0	0.138	0.073	-0.540
SS Cephalor			1.0	0.338	-0.691
SS Cephalex				1.0	-0.047
LogGI					1.0

Model 9.25 Validity Plots





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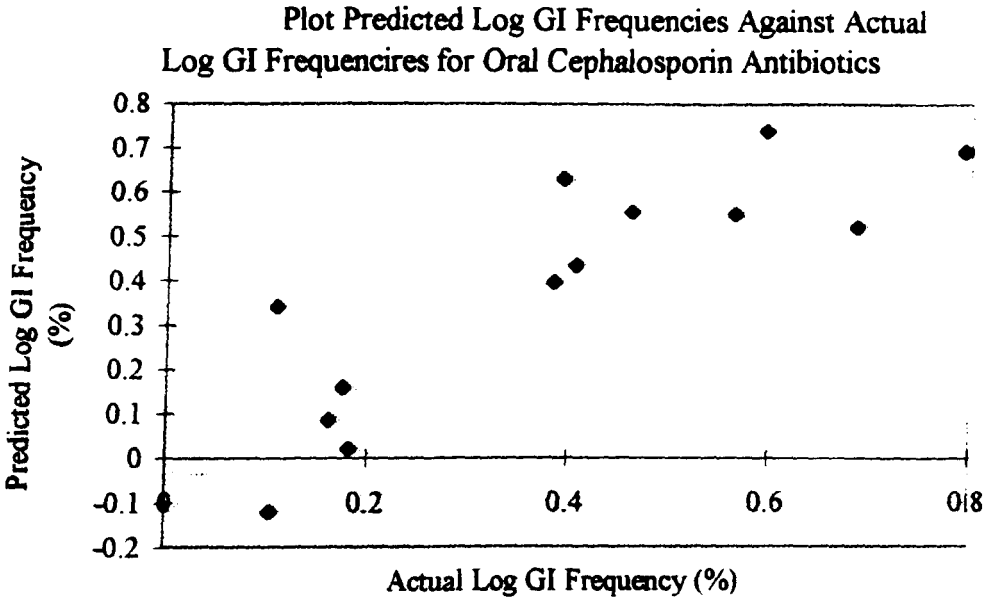

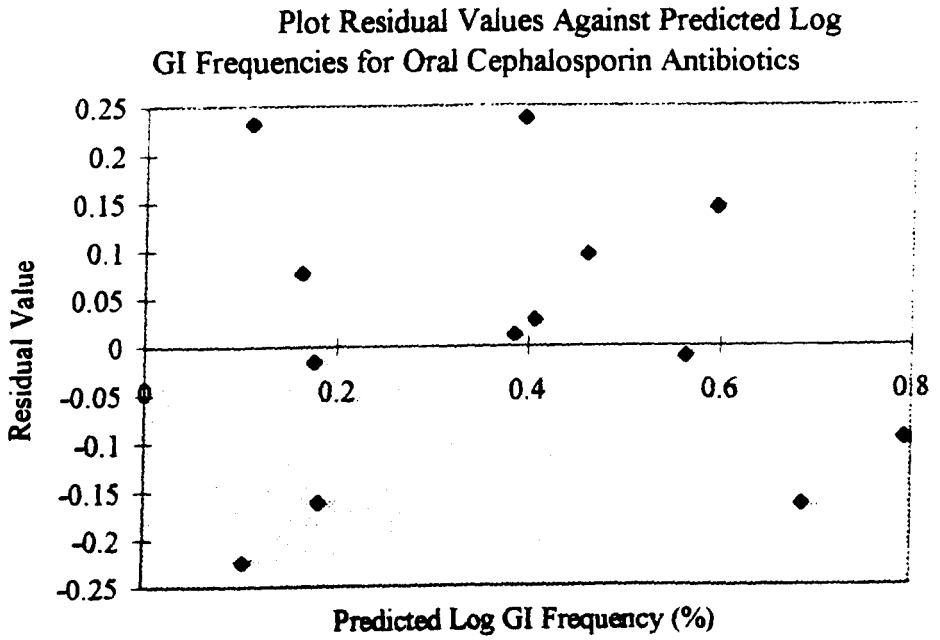

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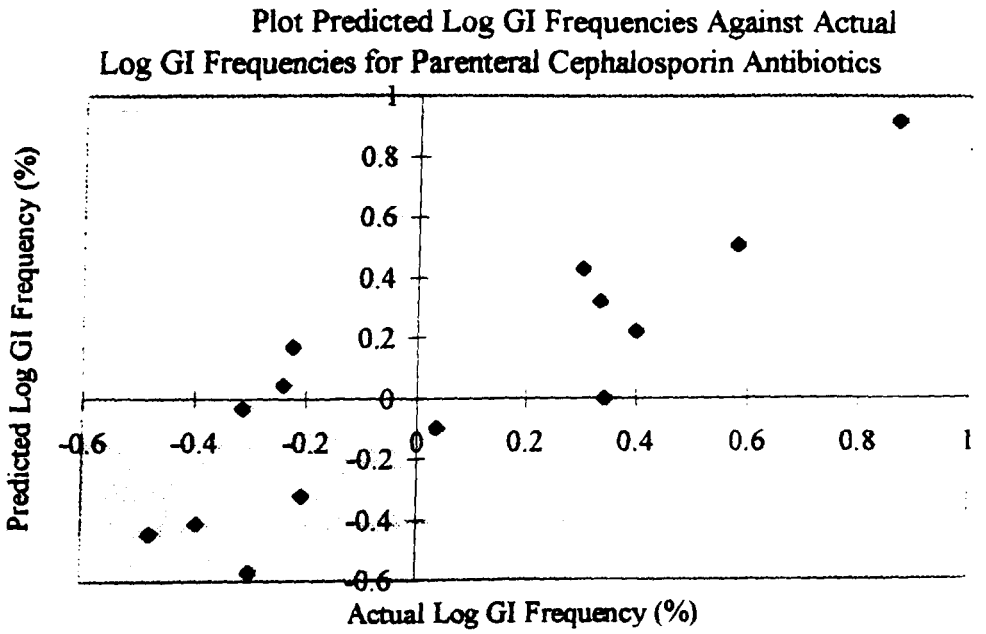

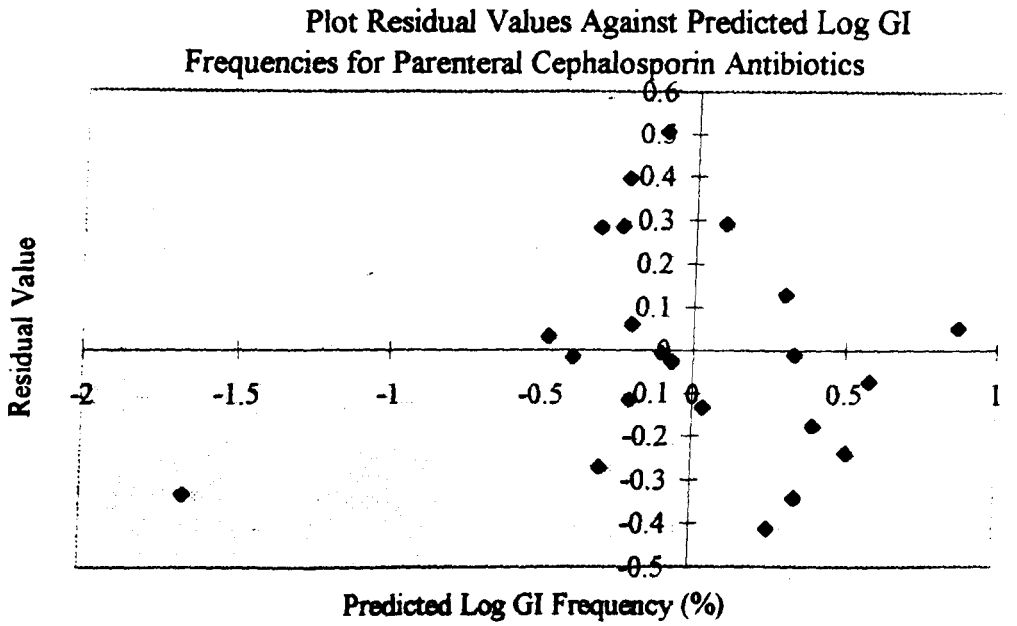

APPENDIX 5 Therapeutic Dose Levels and Plasma Half-Lives of the 70 β-Lactam Antibiotics Investigated in 9.0 [193]

HRTDD = highest recommended therapeutic daily dose in grammes.

Penicillin	Mode of Admin.	CAS Number	HRTDD (g)	Plasma Half-Life (hours)	Peak Plasma Conc for HRTDD @ $t1/_2$ (μg/ml)
Amdinocillin	parenteral	32889-01-7	2.0	1.0	60
Amdinocillin-pivoxil	oral	32887-03-9	1.6	1.0	20
Amoxycillin	oral	26787-78-0	4.0	1.5	80
Ampicillin	oral	69-53-4	5.0	1.5	60
Apacillin	parenteral	63469-19-2	9.0	1.0	180
Azidocillin	oral	17243-38-8	1.5	0.5	15
Azlocillin	oral	37091-66-0	6.0	1.0	-
Bacampicillin	oral	50972-17-3	2.4	0.5	120
Benzylpenicillin	parenteral	61-33-6	1.2	0.5	24
Carbenicillin	parenteral	4697-36-3	4.0	1.5	94
Carindacillin	oral	35531-88-5	3.06	1.5	71
Carfecillin	oral	27025-49-6	3.0	1.5	70
Cyclacillin	oral	3485-14-1	2.0	1.5	-
Cloxacillin	parenteral	61-72-3	2.0	0.5	56
Dicloxacillin	oral	3116-76-5	1.0	0.5	38
Epicillin	oral	26774-90-3	2.0	1.5	-
Flucloxacillin	parenteral	5250-39-5	1.0	1.0	15
Lenampicillin	oral	86273-18-9	2.0	1.5	60
Methicillin	parenteral	61-32-5	6.0	10.5	108
Mezlocillin	parenteral	51481-65-2	16.0	0.5	400
Nafcillin	parenteral	147-52-4	4.0	0.5	32
Oxacllin	parenteral	66-79-5	6.0	0.5	72
Phenoxymethylpenicillin	oral	87-08-1	2.0	0.5	20
Piperacillin	parenteral	61477-96-1	8.0	1.0	160
Pivampicillin	oral	33817-20-8	2.0	1.5	180
Talampicillin	oral	47747-56-8	1.5	1.5	60
Temocillin	parenteral	66148-78-5	4.0	4.5	-
Ticarcillin	parenteral	34787-01-4	20.0	1.2	600

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Appendix 5 continued

Cephalosporin	Mode of Admin.	CAS Number	HRTDD	Plasma Half-Life (hours)	Peak Plasma Conc for HRTDD @ $t1/_2$ (mg/ml)
Cefacetrile	parenteral	10206-21-0	4.0	0.8	-
Cefaclor	oral	53994-73-3	4.0	0.5	104
Cefadroxil	oral	50370-12-2	2.0	1.5	60
Cefaloglycin(e)	oral	-	-	-	-
Cefamandole	parenteral	34444-01-4	12.0	-	300
Cefatrizine	oral	51627-14-6	1.0	1.0	30
Cafazolin	parenteral	25953-19-9	4.0	1.8	240
Cefepirome	parenteral	-	-	-	-
Cefixime	oral	79350-37-1	0.4	4.0	6
Cefmenoxime	oral	65085-01-1	4.0	1.0	100
Cefmetazole	parenteral	56796-20-4	4.0	1.5	276
Cefminox	parenteral	75481-73-1	6.0	1.0	360
Cefodizime	parenteral	69739-16-8	2.0	1.0	50
Cefonicid	parenteral	61270-58-4	1.0	4.5	126
Cefoperazone	parenteral	62893-19-0	4.0	2.0	194
Ceforanide	parenteral	60925-61-3	2.0	3.0	140
Cefotetan	parenteral	69712-56-7	4.0	4.6	280
Cefotaxime	parenteral	63527-52-6	6.0	1.0	150
Cefotiam	parenteral	61622-34-2	4.0	1.2	100
Cefoxitin	parenteral	35607-66-0	6.0	1.0	300
Cefpiramide	oral	70797-11-4	4.0	4.5	194
Cefpodoxime	oral	80210-62-4	0.4	4.0	6
Cefprozil	oral	92665-29-7	1.0	1.4	26
Cefroxadine	oral	51762-05-1	2.0	1.0	72
Cefsulodin	parenteral	62587-73-9	4.0	1.6	-
Ceftazidime	parenteral	72558-82-8	6.0	2.0	534
Cefteram	oral	82547-58-8	2.0	1.5	180
Ceftibuten	oral	97519-39-6	0.4	4.0	6

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Appendix 5 continued

Cephalosporin	Mode of Admin	CAS Number	HRTDD (g)	Plasma Half-Life (hours)	Peak Plasma Conc for HRTDD @ $t1/_2$ (mg/ml)
Ceftizoxime	parenteral	68401-81-0	4.0	1.7	156
Ceftriaxone	parenteral	73384-59-5	2.0	9.0	160
Cefuroxime	parenteral	55268-75-2	2.25	1.2	216
Cefuroxime-axetil	oral	64544-07-6	1.0	6.0	35
Cefuzonam	oral	82219-78-1	-	-	-
Cephalexin	oral	15686-71-2	6.0	1.0	72
Cephaloridine	parenteral	50-59-9	2.0	0.8	40
Cephalothin	parenteral	153-61-7	4.0	0.8	80
Cephamycin	oral	-	-	-	-
Cephapirin	parenteral	21593-23-7	4.0	0.8	80
Cephradine	parenteral	38821-53-3	4.0	1.0	136
Flomoxef	parenteral	99665-00-6	-	-	-
Latamoxef	parenteral	64952-97-2	4.0	1.0	100
T-2588	oral	-	-	-	-

APPENDIX 6 mg/ml Quantities of β-Lactam Antibiotics Used to Give 3mM and HRTDD concentrations for ELISA Analysis

Antibiotic	mg/ml to give HRTDD	Mwt (g)	mg/ml to give 3mM	Ratio HRTDD/3mM
Amoxycillin	4.0	356.4	1.07	3.74
Ampicillin	5.0	371.4	1.11	4.50
Azlocillin	6.0	483.5	1.45	4.05
Bacampicillin	2.4	502.0	1.51	1.58
Benzylpenicillin	1.0	356.4	1.07	1.00
Carbenicllin	4.0	422.4	1.27	3.15
Cloxacillin	2.0	475.9	1.43	1.40
Dicloxacillin	1.0	492.3	1.48	0.66
Epicillin	2.0	351.4	1.05	1.91
Methicillin	6.0	-	-	-
Nafcillin	4.0	436.5	1.31	3.05
Oxacillin	6..0	423.4	1.27	4.72
Piperacillin	8.	539.5	1.62	4.94
Ticarcillin	20.0	428.4	1.29	15.50
Cefaclor	4.0	367.8	1.10	3.64
Cefadroxil	2.0	363.4	1.09	1.84
Cafamandole	12.0	484.5	1.45	8.28
Cefazolin	4.0	476.5	1.43	2.80
Cefoperazone	4.0	667.6	2.00	2.00
Cefotaxime	6.0	-	-	-
Cefoxitin	6.0	450.4	1.35	4.44
Cefsulodin	4.0	554.5	1.66	2.41
Ceftazidime	6.0	-	-	-
Ceftriaxone	2.0	598.5	1.80	1.11
Cefuroxime	2.25	446.4	1.34	1.68
Cephalexin	6.0	-	-	-
Cephalothin	4.0	418.4	1.26	3.18
Cephaloridine	2.0	415.5	1.25	1.60
Cephapirin	4.0	445.4	1.34	3.00
Cephradine	4.0	349.4	1.05	2.67
Latamoxef	4.0	520.5	1.56	2.56

APPENDIX 7 Compounds, Descriptor Variable Values, Actual & Predicted Cross-Reactivity Values, Residual Values, Correlation Matrices and Validity Plots for QSARs Generated in 10.3

Cross-Reactivity at 3mM Concentration

Models 10.1 All β-Lactams, 10.2 Oral β-Lactams, 10.3 Parenteral β-Lacams
10.4 All Penicillins, 10.5 Oral Penicillins, 10.6 Parenteral Penicillins
10.7 All Cephalosporins, 10.8 Oral Cephs. & 10.9 Parenteral Cephs.

	Admin ID	Sterimol $B1_{(C=O)}$	ASA	SA	DVx	DVy
3	3	2.264	0.686	31.64	-2.52	1.24
4	3	2.137	0.668	28.70	-3.19	0.11
7	3	2.462	0.847	37.42	-2.16	3.14
8	3	2.637	0.602	33.94	-2.04	0.69
9	4	2.151	0.660	27.34	-3.78	0.78
10	4	3.313	0.709	30.99	-3.76	0.17
14	4	1.819	0.816	35.60	-4.46	5.28
15	3	1.880	0.777	36.97	-2.99	-0.06
16	3	1.500	0.695	30.36	-3.80	4.07
19	4	1.500	0.735	32.56	-1.31	4.62
21	4	3.342	0.789	33.14	-2.26	6.92
22	4	3.491	0.761	32.46	-0.04	2.49
24	4	3.565	0.947	41.11	-4.15	2.93
28	4	3.620	0.677	29.73	-2.79	-0.68
30	3	3.326	0.913	30.08	2.53	2.42
31	3	1.992	0.904	29.29	-4.04	0.51
33	4	4.186	0.653	35.31	-3.15	2.80
35	4	2.261	0.771	35.85	-1.38	-1.19
43	4	3.318	0.911	53.13	-4.50	0.78
46	4	1.987	0.675	33.78	3.60	3.61
48	4	2.473	0.935	33.16	-1.69	5.58
53	4	4.025	0.839	39.34	1.80	-0.12
54	4	3.910	0.792	46.48	0.66	-0.73
58	4	4.619	0.790	46.18	2.16	9.41
59	4	3.408	0.882	35.32	1.71	0.39
62	3	2.234	0.870	29.45	-3.42	3.86
63	4	2.915	1.103	34.73	-0.23	5.63
64	4	2.590	0.637	32.60	0.91	5.26
66	4	2.757	0.675	31.81	-2.43	2.64
67	4	3.323	0.923	28.88	-2.45	1.94
69	4	3.588	0.864	47.50	7.23	2.48

	Actual $\sqrt{Xr}$	All βLs Predicted $\sqrt{Xr}$	All βLs Residual	Oral βLs Predicted $\sqrt{Xr}$	Oral βLs Residual	Parenteral βLs Pred. $\sqrt{Xr}$	Parenteral βLs Residual.
3	3.02	4.91	-1.089	-	-	5.23	-2.18
4	6.87	5.16	1.71	5.80	0.17	-	-
7	4.38	3.99	0.39	4.24	0.14	-	-
8	4.31	4.26	0.05	4.85	-0.54	-	-
9	10.00	6.82	3.18	-	-	6.80	3.20
10	8.91	6.57	2.34	-	-	6.06	2.85
14	6.80	5.43	1.37	-	-	5.59	1.21
15	3.56	4.22	-0.66	4.35	-0.79	-	-
16	4.23	2.07	2.16	-	-	-	-
19	5.26	5.91	-0.65	-	-	4.61	0.66
21	3.77	5.84	-2.07	-	-	4.97	-1.20
22	4.93	5.49	-0.56	-	-	4.00	0.93
24	4.38	5.44	-1.06	-	-	4.41	-0.03
28	6.73	6.55	0.18	-	-	5.87	0.87
30	0.10	-0.30	0.40	-0.883	0.93	-	-
31	0.10	2.56	-2.46	1.33	-1.23	-	-
33	2.57	3.82	-1.25	-	-	5.00	-2.43
35	0.10	2.83	-2.73	-	-	-	-
43	1.87	2.85	-0.98	-	-	2.33	-0.46
46	1.61	1.83	-0.22	-	-	1.48	0.13
48	2.65	1.58	1.07	-	-	4.68	-2.03
53	0.89	1.74	-0.85	-	-	1.80	-0.91
54	3.36	2.86	0.50	-	-	3.15	0.25
58	1.05	2.32	-1.27	-	-	0.35	0.71
59	0.10	1.61	-1.51	-	-	2.60	-2.50
62	4.46	4.15	0.31	4.04	0.43	-	-
63	3.27	0.80	2.47	-	-	3.67	-0.40
64	3.35	2.98	0.37	-	-	3.50	-0.15
66	3.55	3.94	-0.39	-	-	5.30	-1.75
67	2.37	1.21	1.16	-	-	3.44	-1.07
69	0.10	-1.07	1.17	-	-	-2.40	2.50

Model 10.1 Correlation Matrix

	DVx	Sterimol B1	ASA	Admin ID	√3mM Xr
DVx	1.0	0.581	0.251	0.243	-0.561
Sterimol B1		1.0	0.314	0.274	-0.705
ASA			1.0	0.122	-0.442
Admin ID				1.0	0.033
√3mM Xr					1.0

Model 10.2 Correlation Matrix

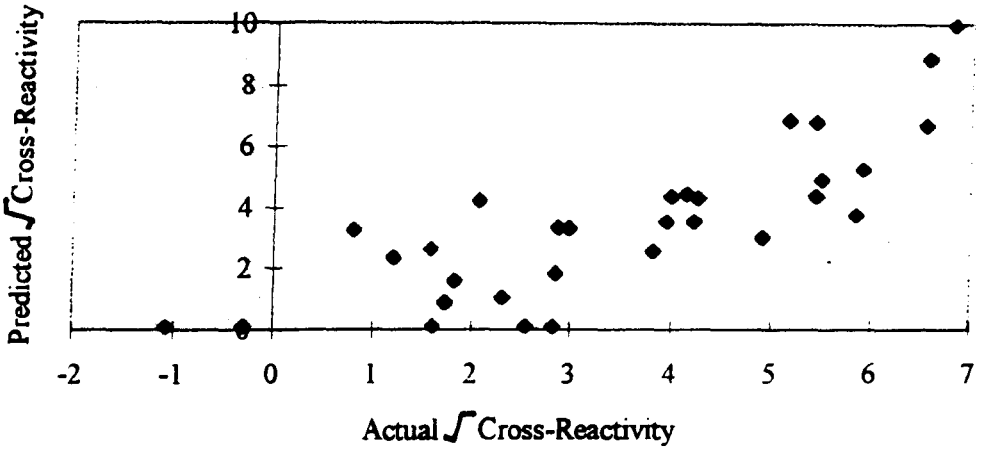
	Sterimol B1	ASA	√3mM Xr
Sterimol B1	1.0	0.382	-0.847
ASA		1.0	-0.689
√3mM Xr			1.0

Model 10.3 Correlation Matrix

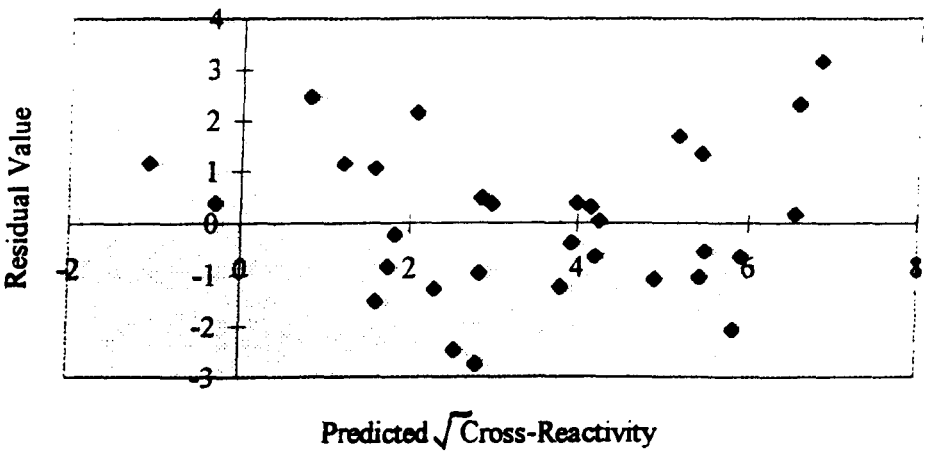
	SA	DVx	√3mM Xr
SA	1.0	0.231	-0.585
DVx		1.0	-0.654
√3mM Xr			1.0

Model 10.1 Validity Plots

Plot of Predicted $\sqrt{}$Cross-Reactivity Against Actual $\sqrt{}$ Cross-Reactivity of All β-Lactams at 3mM Concentration

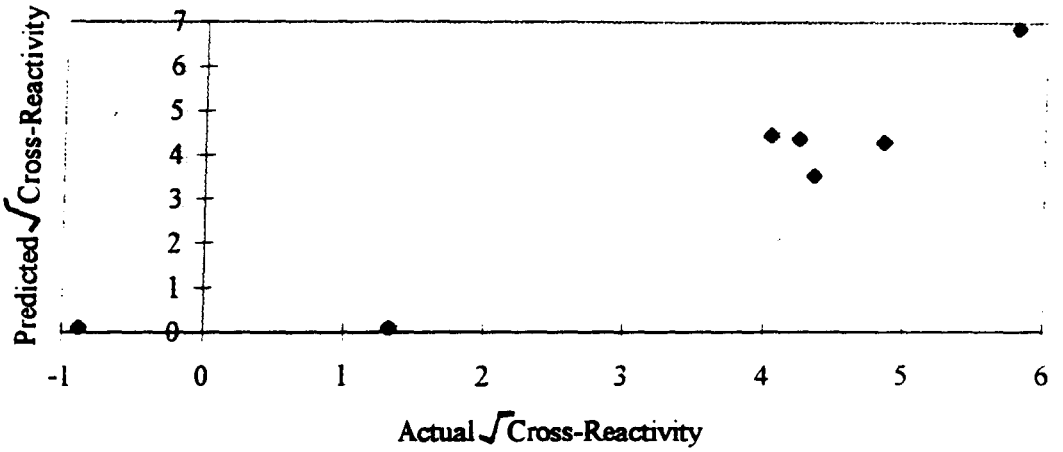


Plot of Residual Values Against Predicted $\sqrt{}$ Cross-Reactivities of All β-Lactams at 3mM Concentration

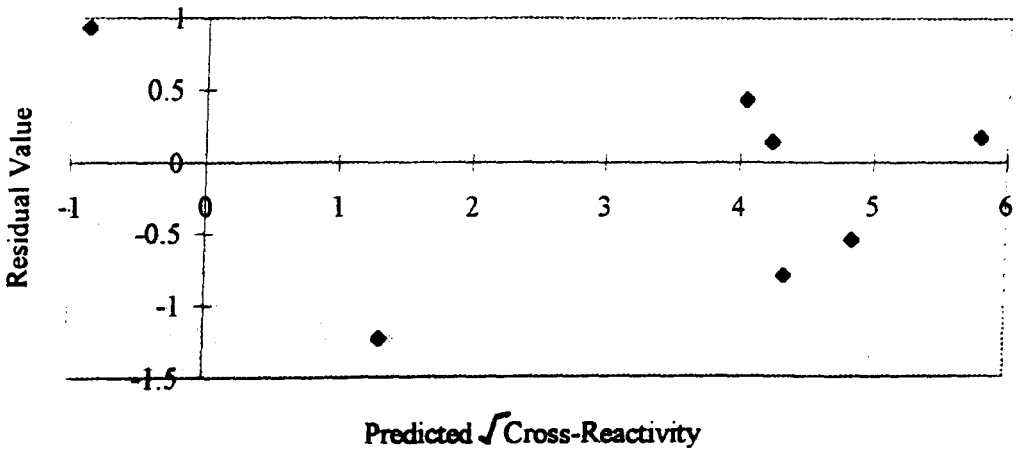


Model 10.2 Validity Plots

Plot of Predicted $\sqrt{}$ Cross-Reactivity Against Actual $\sqrt{}$ Cross-Reactivity of Oral β-Lactams at 3mM Concentration

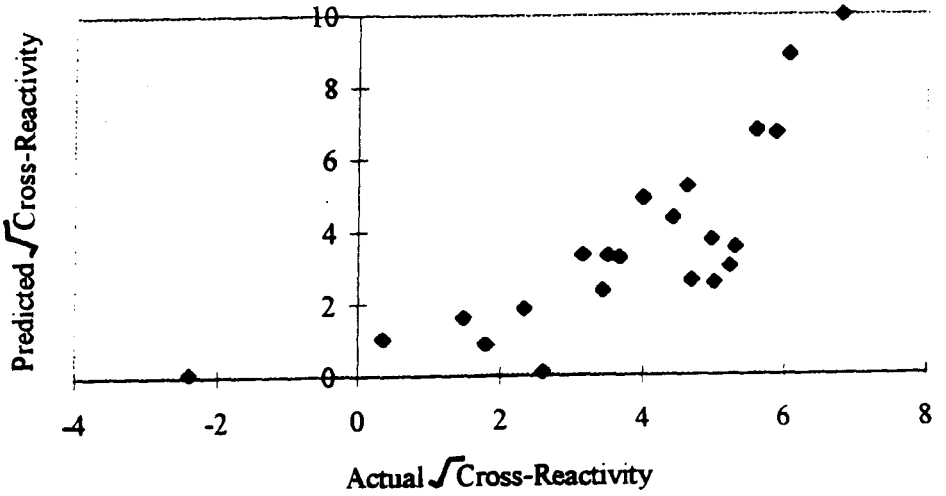


Plot of Residual Values Against Predicted $\sqrt{}$ Cross-Reactivity for Oral β-Lactams at 3mM Concentration

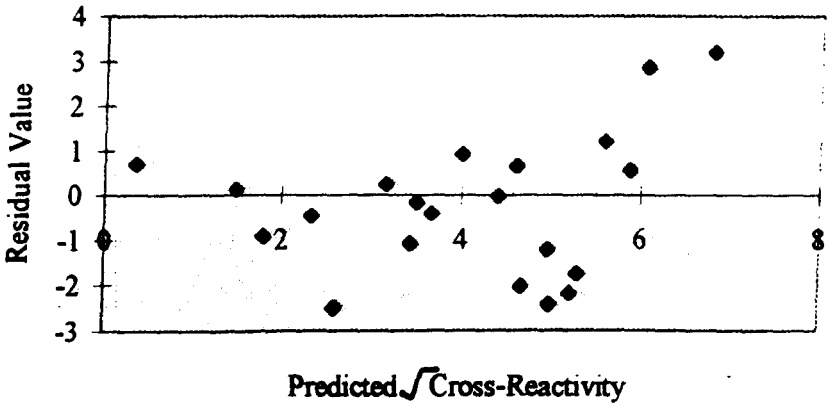


Model 10.3 Validity Plots

Plot of Predicted $\sqrt{}$Cross-Reactivity Against Actual $\sqrt{}$Cross-Reactivity of Parenteral β-Lactams at 3mM Concentration



Plot of Residual Values Against Predicted $\sqrt{}$Cross-Reactivity Vlaues for Parenteral β-Lactams at 3mM Concentration



	Actual $\sqrt{Xr}$	All Pens Predicted $\sqrt{Xr}$	All Pens. Residual	Oral Pens Predicted $\sqrt{Xr}$	Oral Pens Residual	Parenteral Pens Pred. $\sqrt{Xr}$	Parenteral Penicillins Residual.
3	3.02	-	-	-	-	-	-
4	6.87	0.82	-1.30	6.62	0.25	-	-
7	4.38	3.91	0.47	3.73	0.65	-	-
8	4.31	5.18	-0.87	4.89	-0.58	-	-
9	10.00	9.19	0.82	-	-	6.70	0.31
10	8.91	7.66	1.25	-	-	8.12	0.80
14	6.80	6.50	0.30	-	-	6.90	-0.91
15	3.56	4.76	-1.20	3.88	-0.32	-	-
16	4.23	-	-	-	-	-	-
19	5.26	5.12	0.14	-	-	5.51	-0.25
21	3.77	-	-	-	-	-	-
22	4.93	4.13	0.80	-	-	4.50	0.43
24	4.38	4.08	0.30	-	-	4.40	-0.02
28	6.73	7.44	-0.71	-	-	7.90	-1.17

Model 10.4 Correlation Matrix

	SA	DVx	$\sqrt{3}$mM Xr
SA	1.0	-0.105	-0.741
DVx		1.0	-0.438
$\sqrt{3}$mM Xr			1.0

Model 10.5 Correlation Matrix

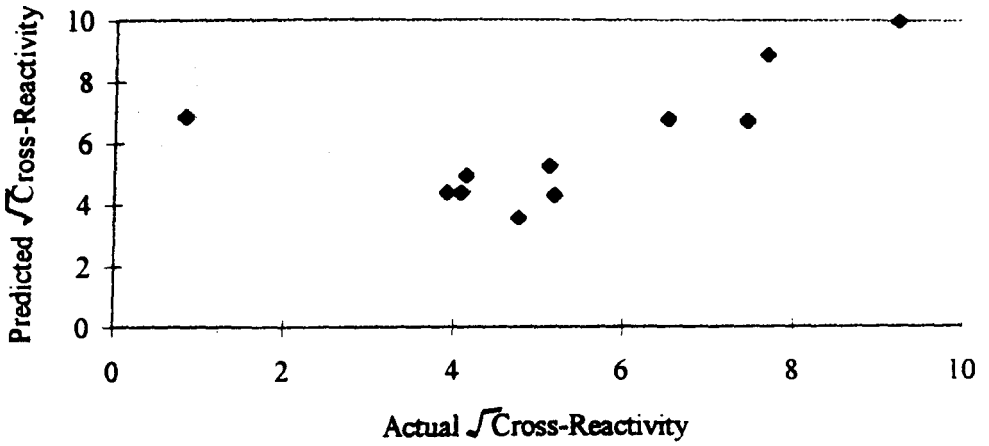
	SA	$\sqrt{3}$mM Xr
SA	1.0	-0.924
$\sqrt{3}$mM Xr		1.0

Model 10.6 Correlation Matrix

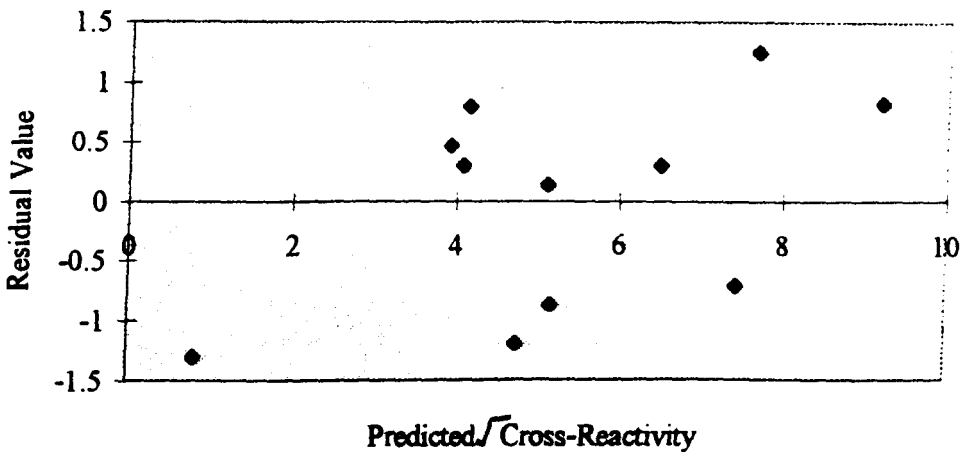
	SA	DVx	$\sqrt{3}$mM Xr
SA	1.0	-0.234	-0.715
DVx		1.0	-0.447
$\sqrt{3}$mM Xr			1.0

Model 10.4 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivity Values for All Penicillins at 3mM Concentration

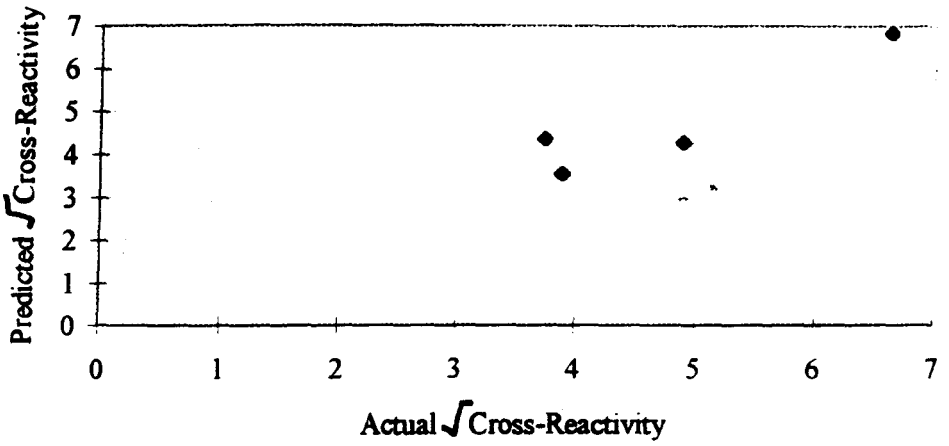


Plot of Residual Values Against Predicted √Cross-Reactivity Values for All Penicillins at 3mM Concentration

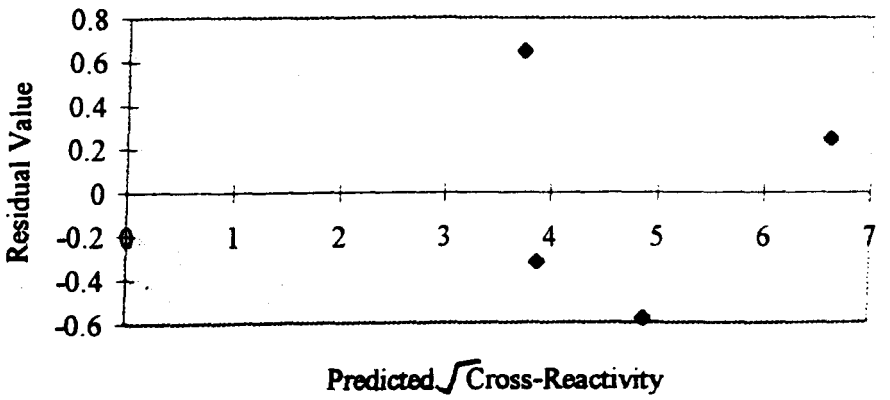


Model 10.5 Validity Plots

Plot of Predicted Against Actual $\sqrt{}$ Cross-Reactivities of Oral Penicillins at 3mM Concentration

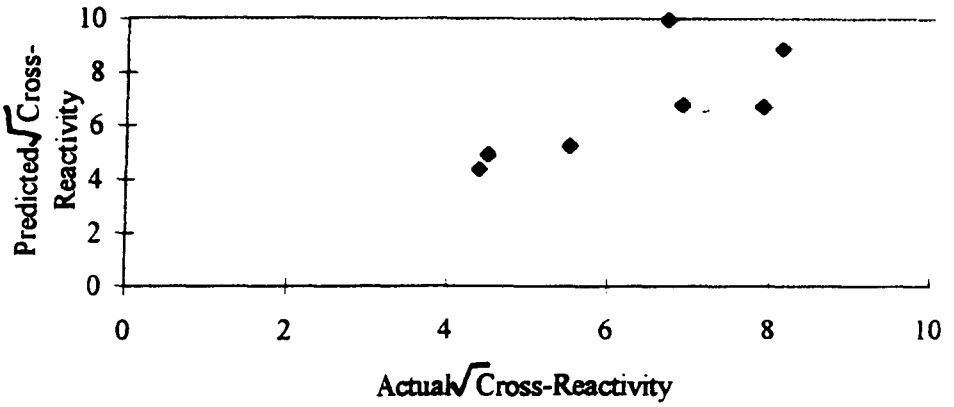


Plot of Residual Values Against Predicted $\sqrt{}$ Cross-Reactivity Values of Oral Penicillins at 3mM Concentration

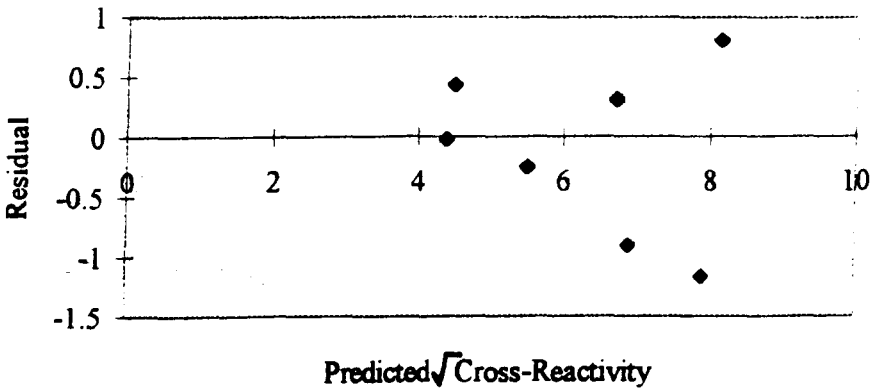


Model 10.6 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivity Values of Parenteral Penicillins at 3mM Concentration



Plot of Residual Values Against √Cross-Reactivities of Parenteral Penicillins at 3mM Concentration



	Actual $\sqrt{Xr}$	All Cephs. Predicted $\sqrt{Xr}$	All Cephs. Residual	Oral Cephs. Predicted $\sqrt{Xr}$	Oral Cephs. Residual	Parenteral Cephs. Pred. $\sqrt{Xr}$	Parenteral Cephs. Residual.
30	0.10	1.30	-1.20	1.75	-	-	-
31	0.10	1.61	-1.51	-0.61	-1.65	-	-
33	2.57	2.61	-0.04	-	-	2.68	-0.11
35	0.10	0.20	-0.10	-	-	0.62	-0.52
43	1.87	1.84	0.03	-	-	2.06	-0.19
46	1.61	1.47	0.14	-	-	1.74	-0.13
48	2.65	3.75	-1.10	-	-	3.59	-0.94
53	0.89	0.14	0.75	-	-	0.50	0.39
54	3.36	2.98	0.38	-	-	4.06	-0.70
58	1.05	-	-	-	-	-	-
59	0.10	0.42	-0.32	-	-	0.73	-0.63
62	4.46	3.20	1.26	3.52	0.94	-	-
63	3.27	3.48	-0.21	-	-	3.34	-0.07
64	3.35	3.07	0.28	-	-	2.97	0.38
66	3.55	2.39	1.17	-	-	2.47	1.08
67	2.37	1.07	1.30	-	-	1.26	1.11
69	0.10	0.41	-0.31	-	-	0.59	-0.49

Model 10.7 Correlation Matrix

	DVx	DVy	$\sqrt{3}$mM Xr
DVx	1.0	0.006	-0.408
DVy		1.0	0.707
$\sqrt{3}$mM Xr			1.0

Model 10.8 Correlation Matrix

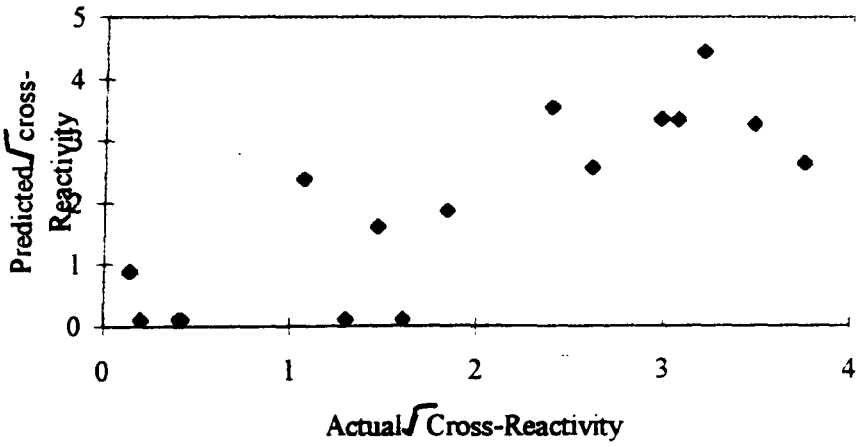
	DVy	$\sqrt{3}$mM Xr
DVy	1.0	0.823
$\sqrt{3}$mM Xr		1.0

Model 10.9 Correlation Matrix

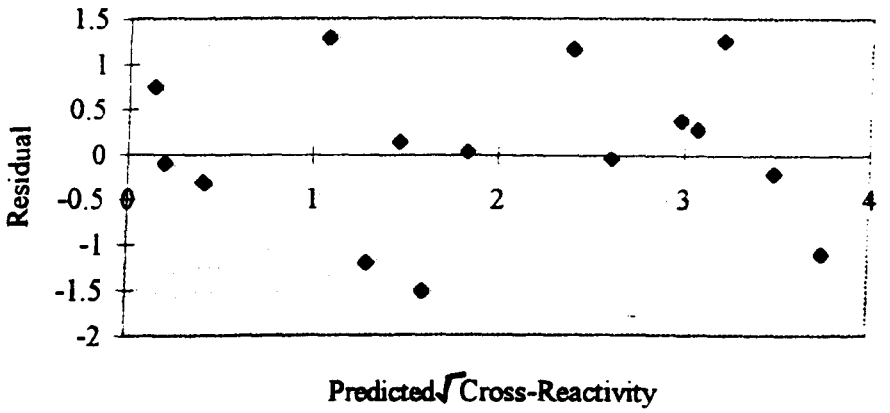
	DVx	DVy	$\sqrt{3}$mM Xr
DVx	1.0	-0.025	-0.465
DVy		1.0	0.746
$\sqrt{3}$mM Xr			1.0

Model 10.7 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivities of All Cephalosporins at 3mM Concentration

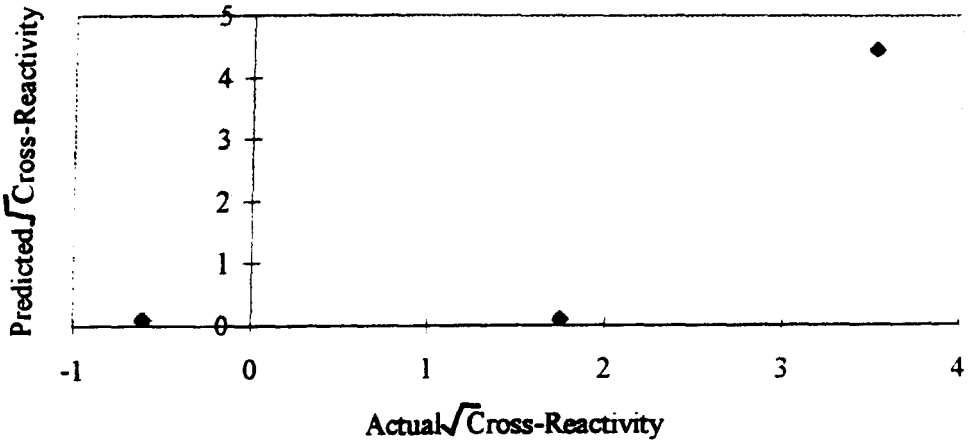


Plot of Residual Values Against Predicted√ Cross-Reactivities of All Cephalosporins at 3mM Concentration

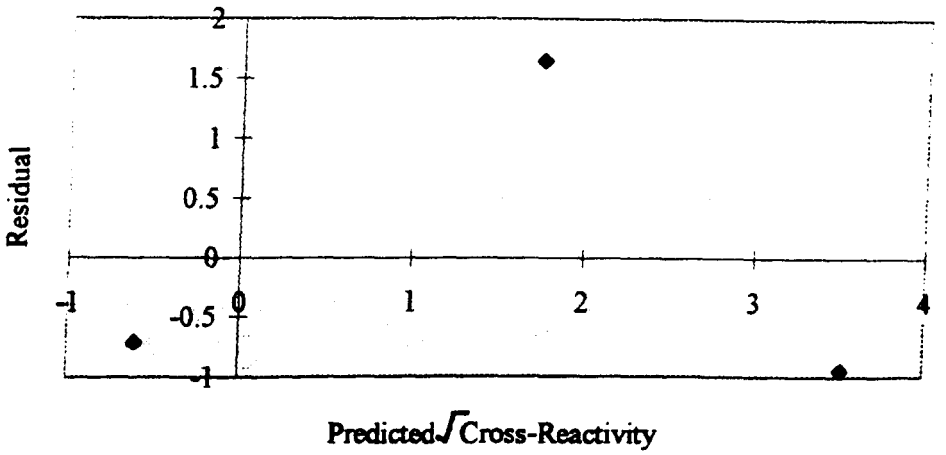


Model 10.8 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivities of Oral Cephalosporins at 3mM Concentration

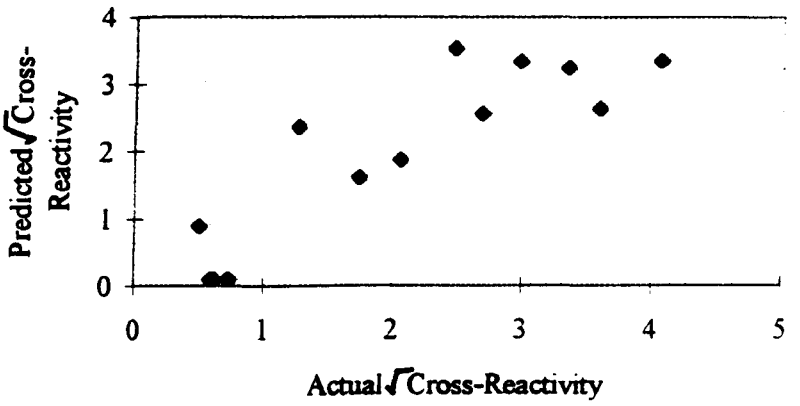


Plot of Residual Values Against predicted √ Cross-Reactivities of Oral cephalosporins at 3mM Concentration

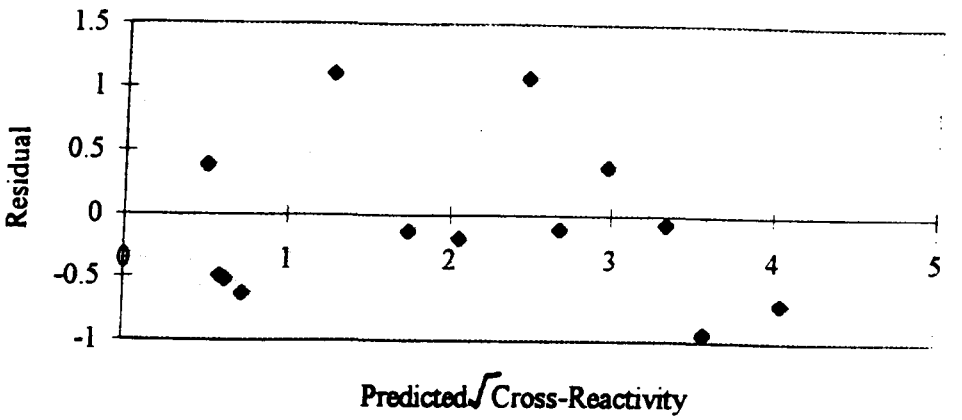


Model 10.9 Validity Plots

Plot of Predicted Against Actual $\sqrt{}$ Cross-Reactivity Values of Parenteral Cephalosporins at 3mM Concentration



Plot of Residual Values Against Predicted $\sqrt{}$ Cross-Reactivity Values of Parenteral Cephalosporins at 3mM Concentration



Cross-Reactivity at Concentration Equivalent to HRTDD

Models 10.10 All β-Lactams, 10.11 Oral β-Lactams, 10.12 Parenteral β-Lacams
10.13 All Penicillins, 10.14 Oral Penicillins, 10.15 Parenteral Penicillins
10.16 All Cephalosporins, 10.17 Oral Cephs. & 10.18 Parenteral Cephs.

	Admin ID	HOMO	Sterimol L (CH_3)	SA	Sterimol B4 (C=O)	# CH_3	SS BP	DVx	# CH_3 R1
3	3	-9.003	1.124	31.64	8.763	2	0.987	-2.52	0
4	3	-9.177	1.125	28.70	8.160	2	0.991	-3.19	0
7	3	-8.942	1.576	37.42	10.860	2	0.876	-2.16	0
8	3	-9.030	0.372	33.94	8.344	2	0.890	-2.04	0
9	4	-9.522	1.007	27.34	8.169	2	1.000	-3.78	0
10	4	-8.989	1.225	30.99	8.106	2	0.848	-3.76	0
14	4	-8.698	1.378	35.60	9.911	3	0.668	-4.46	1
15	3	-9.115	1.301	36.97	10.150	3	0.693	-2.99	1
16	3	-9.050	1.084	30.36	5.758	2	0.889	-3.80	0
19	4	-8.475	1.282	32.56	9.210	4	0.725	-1.31	2
21	4	-9.079	0.657	33.14	11.640	3	0.724	-2.26	1
22	4	-9.085	0.656	32.46	8.150	3	0.827	-0.04	1
24	4	-8.987	0.651	41.11	13.170	3	0.573	-4.15	1
28	4	-8.970	1.245	29.73	8.077	2	0.770	-2.79	0
30	3	-9.032	0.421	30.08	6.420	0	0.870	2.53	0
31	3	-8.720	0.612	29.29	7.140	1	0.864	-4.04	0
33	4	-8.872	0.616	35.31	6.217	1	0.806	-3.15	0
35	4	-8.445	0.784	35.85	11.220	1	0.663	-1.38	0
43	4	-9.176	0.862	53.13	14.300	2	0.574	-4.50	1
46	4	-8.795	0.436	33.78	10.540	2	0.695	3.60	1
48	4	-9.277	0.422	33.16	8.819	1	0.702	-1.69	0
53	4	-8.939	0.697	39.34	7.298	0	0.760	1.80	0
54	4	-8.979	0.849	46.48	9.779	2	0.667	0.66	2
58	4	-9.092	0.863	46.18	9.963	2	0.534	2.16	1
59	4	-9.159	0.425	35.32	9.337	1	0.676	1.71	1
62	3	-9.520	0.431	29.45	8.582	1	0.790	-3.42	0
63	4	-9.229	0.399	34.73	8.596	0	0.638	-0.23	0
64	4	-9.165	0.422	32.60	8.411	1	0.672	0.91	0
66	4	-8.931	0.762	31.81	10.560	1	0.649	-2.43	0
67	4	-9.182	0.438	28.88	5.634	1	0.831	-2.45	0
69	4	-9.401	0.704	47.50	7.642	2	0.716	7.23	0

	Actual $\sqrt{Xr}$	All βLs Predicted $\sqrt{Xr}$	All βLs Residual	Oral βLs Predicted $\sqrt{Xr}$	Oral βLs Residual	Parenteral βLs Pred. $\sqrt{Xr}$	Parenteral βLs Residual
3	6.57	4.26	2.31	4.15	2.43	-	-
4	5.60	5.79	-0.19	5.11	0.50	-	-
7	5.88	5.14	0.74	6.09	-0.21	-	-
8	0.10	0.05	0.05	0.50	-0.40	-	-
9	10.00	10.34	-0.34	-	-	7.82	2.18
10	9.31	8.00	1.31	-	-	7.82	1.49
14	5.71	6.14	-0.43	-	-	6.53	-0.82
15	3.59	4.75	-1.16	5.65	-2.06	-	-
16	4.14	4.56	-0.42	4.20	-0.06	-	-
19	2.76	5.02	-2.26	-	-	5.33	-2.57
21	3.13	4.96	-1.83	-	-	-	-
22	5.44	5.13	0.31	-	-	5.64	-0.20
24	5.23	2.87	2.36	-	-	3.16	2.07
28	8.90	8.09	0.81	-	-	7.32	1.58
30	0.10	0.96	-0.86	0.76	-0.66	-	-
31	0.10	0.51	-0.41	0.01	0.09	-	-
33	4.87	3.19	1.68	-	-	-	-
35	1.67	1.62	0.05	-	-	3.36	-1.69
43	1.79	2.63	-0.84	-	-	2.26	-0.47
46	1.87	2.14	-0.28	-	-	1.09	0.79
48	3.78	4.82	-1.04	-	-	5.94	-2.16
53	3.74	3.19	0.55	-	-	5.34	-1.60
54	3.09	2.80	0.29	-	-	3.59	-0.50
58	4.25	3.55	0.70	-	-	2.51	1.74
59	3.82	3.77	0.05	-	-	3.40	0.43
62	2.90	3.92	-0.06	3.49	0.37	-	-
63	5.53	4.13	1.39	-	-	-	-
64	5.22	4.32	0.34	-	-	4.78	-0.12
66	3.76	4.96	-1.20	-	-	4.68	-0.92
67	2.51	2.86	-0.35	-	-	3.06	-0.55
69	2.51	4.15	-1.64	-	-	1.75	0.76

Model 10.10 Correlation Matrix

	SA	HOMO	Sterimol L	Admin ID	√HRTDD Xr
SA	1.0	-0.039	-0.008	0.042	-0.281
HOMO		1.0	0.270	0.099	-0.287
Sterimol L			1.0	0.153	0.500
Admin ID				1.0	0.234
√HRTDD Xr					1.0

Model 10.11 Correlation Matrix

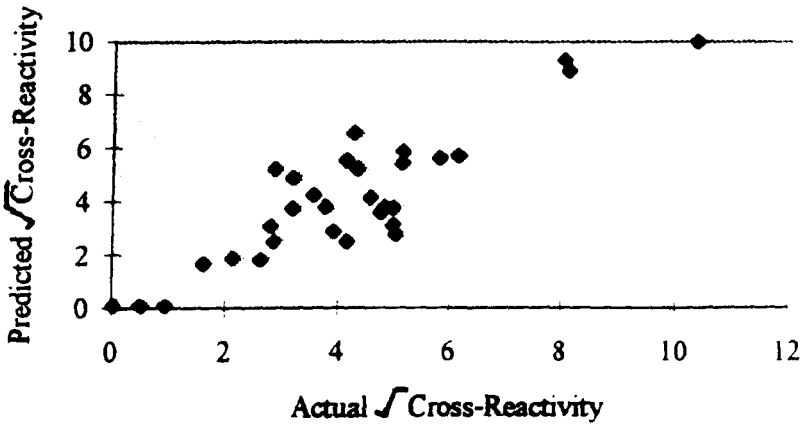
	Sterimol L	HOMO	√HRTDD Xr
Sterimol L	1.0	0.178	0.775
HOMO		1.0	-0.296
√HRTDD Xr			1.0

Model 10.12 Correlation Matrix

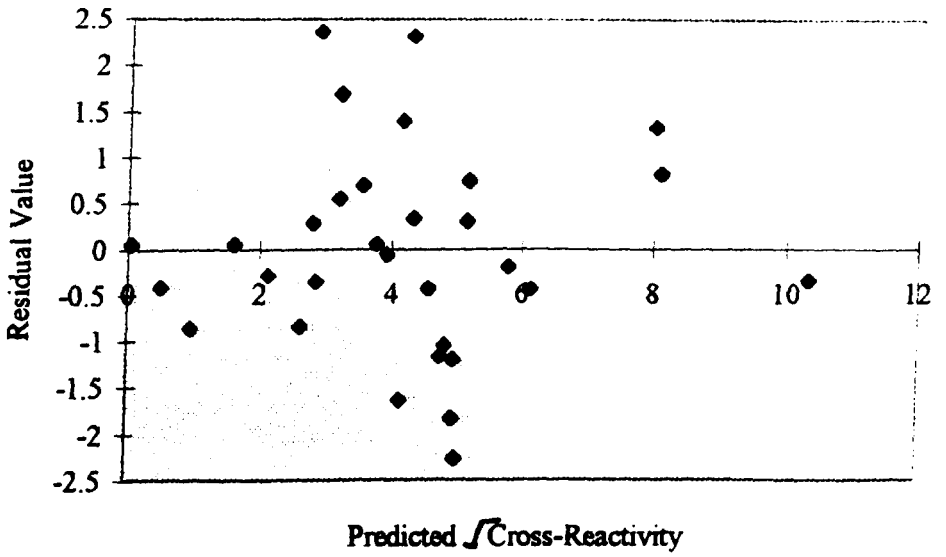
	DVx	Sterimol B4	√HRTDD Xr
DVx	1.0	-0.396	-0.471
Sterimol B4		1.0	-0.423
√HRTDD Xr			1.0

Model 10.10 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivity of All β-Lactams at HRTDD

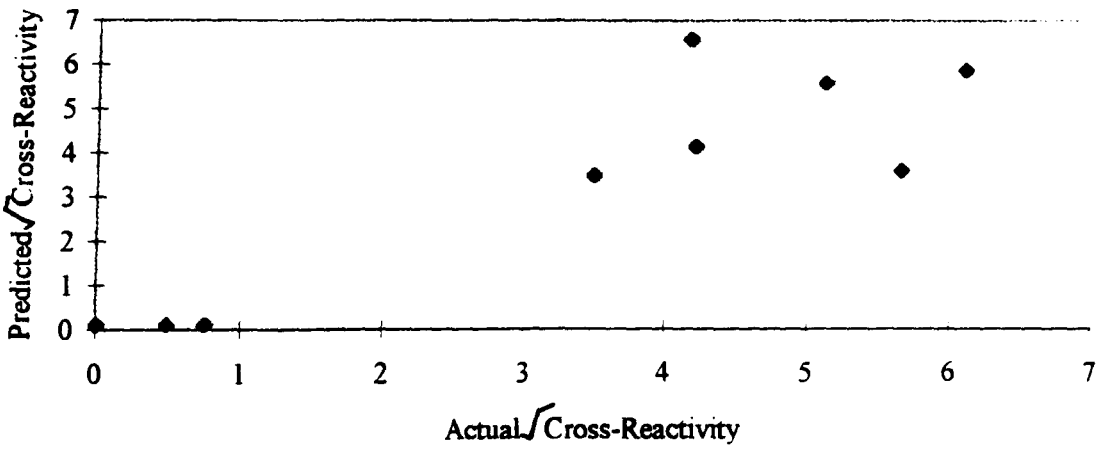


Plot of Residual Values Against Predicted √ Cross-Reactivities of All β-Lactams at HRTDD

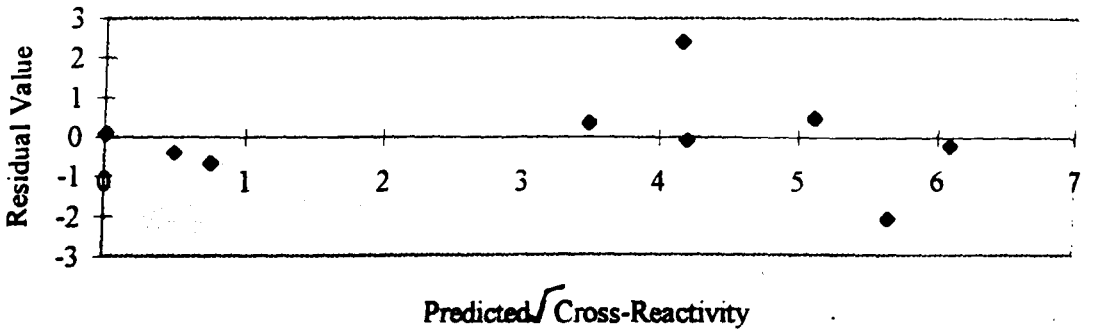


Model 10.11 Validity Plots

Plot of Predicted Against Actual $\sqrt{}$ Cross-Reactivities of Oral β-Lactams at HRTDD

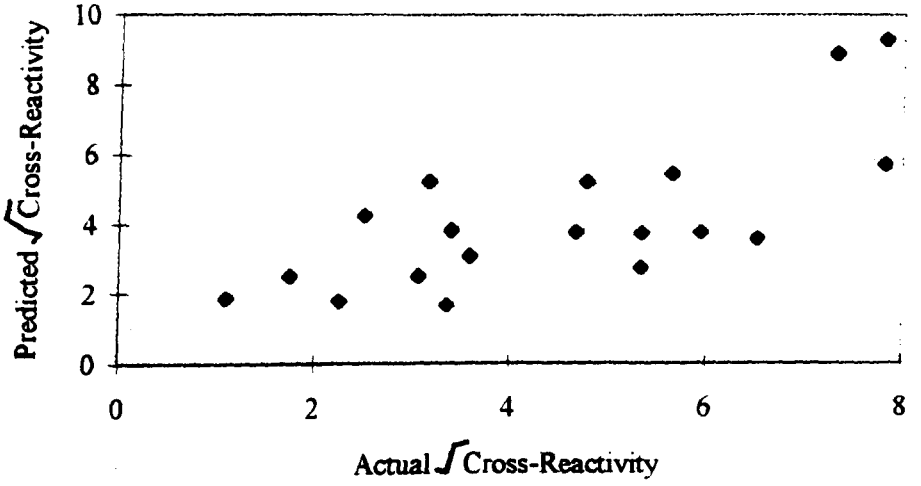


Plot of Residual Values Against Predicted $\sqrt{}$ Cross-Reactivities of Oral β-Lactams at HRTDD

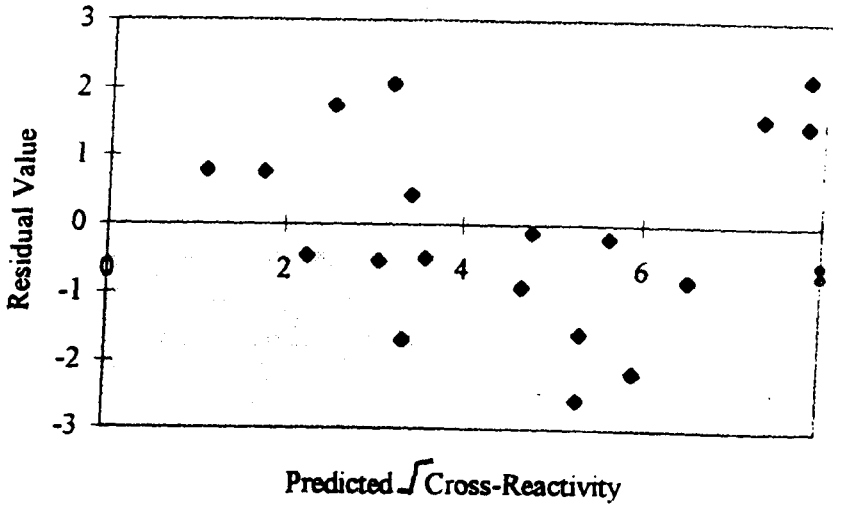


Model 10.12 Validity Plots

Plot of Predicted Against Actual $\sqrt{}$Cross-Reactivity of Parenteral β-Lactams at HRTDD



Plot of Residual Values Against Predicted $\sqrt{}$Cross-Reactivity Values for Parenteral β-Lactams at HRTDD



	Actual √Xr	All Pens Predicted √Xr	All Pens. Residual	Oral Pens Predicted √Xr	Oral Pens Residual	Parenteral Pens Pred. √Xr	Parenteral Penicillins Residual
3	6.57	5.88	-0.69	5.62	-0.95	-	-
4	5.60	5.88	-0.28	5.62	-0.02	-	-
7	5.88	5.88	0.00	5.62	0.26	-	-
8	0.10	-0.40	0.50	0.39	-0.29	-	-
9	10.00	9.67	0.33	-	-	9.06	0.94
10	9.31	0.67	0.65	-	-	9.06	0.25
14	5.71	5.53	0.19	-	-	5.39	0.32
15	3.59	2.74	0.85	3.01	0.58	-	-
16	4.14	5.88	--1.74	5.62	1.48	-	-
19	2.76	2.39	0.38	-	-	1.73	1.03
21	3.13	5.53	--2.40	-	-	5.39	-2.26
22	5.44	5.53	-0.09	-	-	5.39	0.05
24	5.23	5.53	-0.30	-	-	5.39	-0.16
28	8.90	8.67	0.24	-	-	9.06	-0.16

Model 10.13 Correlation Matrix

	CH_3	Admin	√HRTDD Xr
CH_3	1.0	0.172	-0.772
Admin		1.0	0.378
√HRTDD Xr			1.0

Model 10.14 Correlation Matrix

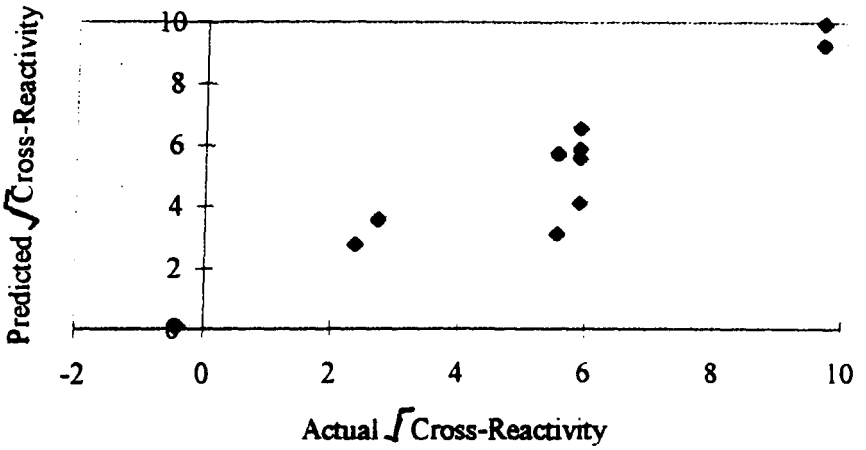
	CH_3	√HRTDD Xr
CH_3	1.0	-0.933
√HRTDD Xr		1.0

Model 10.15 Correlation Matrix

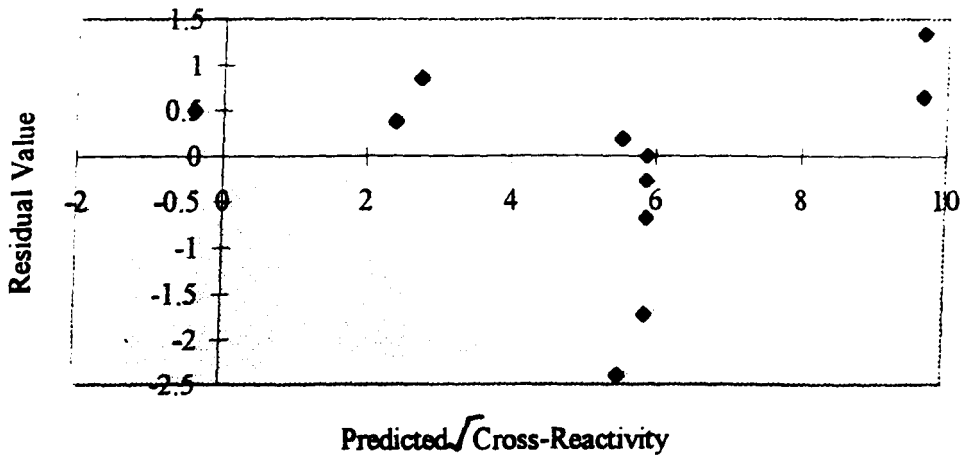
	CH_3	√HRTDD Xr
CH_3	1.0	-0.930
√HRTDD Xr		1.0

Model 10.13 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivity Values for All Penicillins at HRTDD

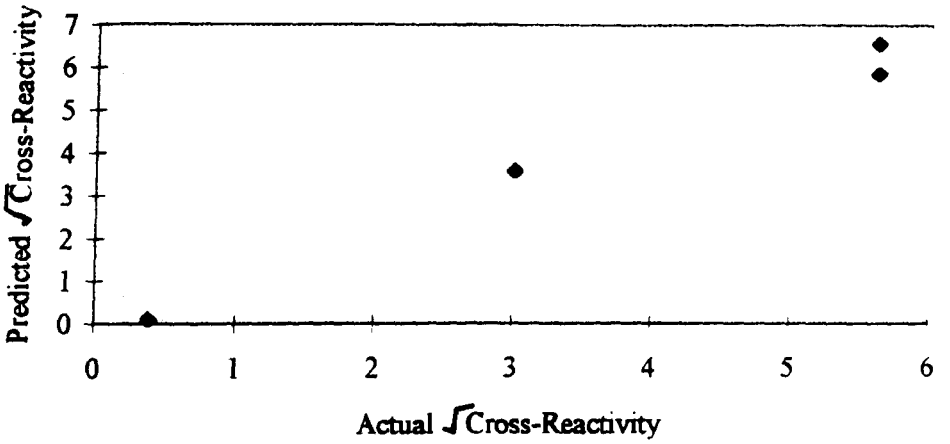


Plot of Residual Values Against Predicted √Cross-Reactivity Values for All Penicillins at HRTDD

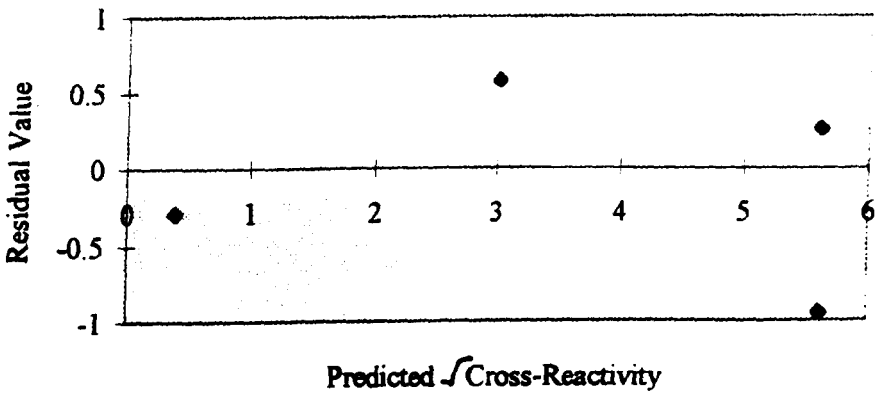


Model 10.14 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivities of Oral Penicillins at HRTDD

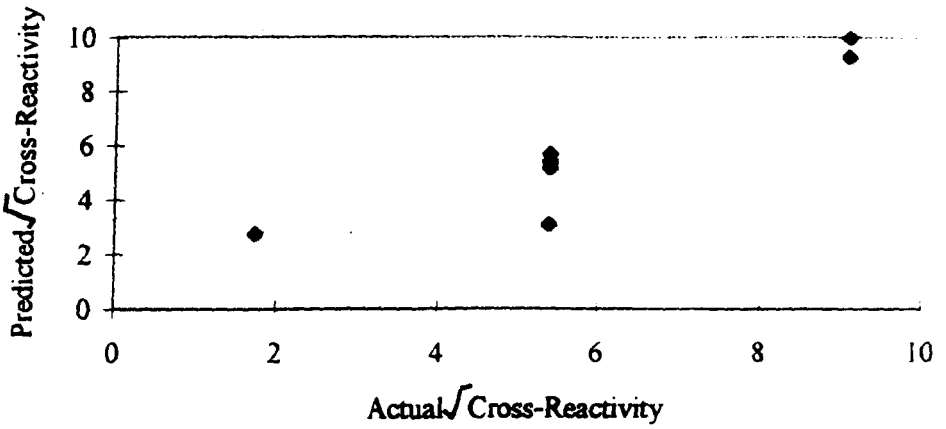


Plot of Residual Values Against Predicted √Cross-Reactivity Values of Oral Penicillins at HRTDD

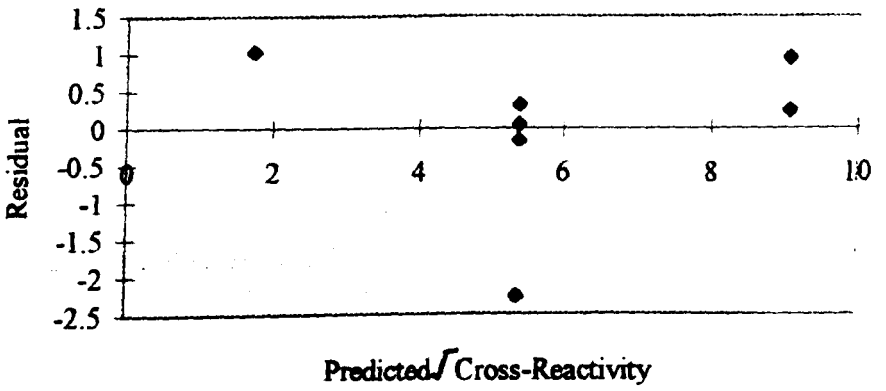


Model 10.15 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivity Values of Parenteral Penicillins at HRTDD



Plot of Residual Values Against √Cross-Reactivities of Parenteral Penicillins at HRTDD



	Actual √Xr	All Cephs. Predicted √Xr	All Cephs. Residual	Oral Cephs. Predicted √Xr	Oral Cephs. Residual	Parenteral Cephs. Pred. √Xr	Parenteral Cephs. Residual.
30	0.10	-0.45	0.35	-0.44	0.54	-	-
31	0.10	0.08	-0.18	0.91	-0.81	-	-
33	4.87	-	-	-	-	-5.64	0.77
35	1.67	-2.15	0.48	-	-	-1.45	0.22
43	1.79	-1.88	0.09	-	-	-1.36	0.43
46	1.87	-1.83	-0.04	-	-	-2.56	0.78
48	3.78	-3.32	-0.46	-	-	-4.01	0.23
53	3.74	-3.02	-0.72	-	-	-3.09	0.65
54	3.09	-3.47	0.38	-	-	-3.42	0.33
58	4.25	-	-	-	-	-3.69	0.56
59	3.82	-3.62	-0.20	-	-	-3.67	0.15
62	2.90	-	-	3.59	0.27	-	-
63	5.53	-5.55	0.03	-	-	-5.09	0.43
64	5.22	-3.89	-0.77	-	-	-4.61	0.05
66	3.76	-3.25	-0.51	-	-	-3.02	0.74
67	2.51	-2.86	0.35	-	-	-3.65	1.14
69	2.51	-4.06	1.55	-	-	-	-

Model 10.16 Correlation Matrix

	SS BP	Sterimol B4	√HRTDD Xr
SS BP	1.0	0.814	0.561
Sterimol B4		1.0	-0.028
√HRTDD Xr			1.0

Model 10.17 Correlation Matrix

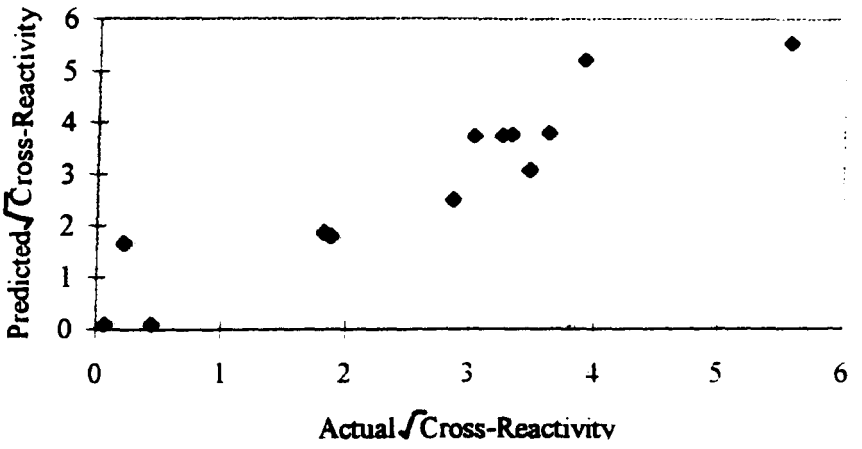
	Sterimol B4	√HRTDD Xr
Sterimol B4	1.0	0.945
√HRTDD Xr		1.0

Model 10.18 Correlation Matrix

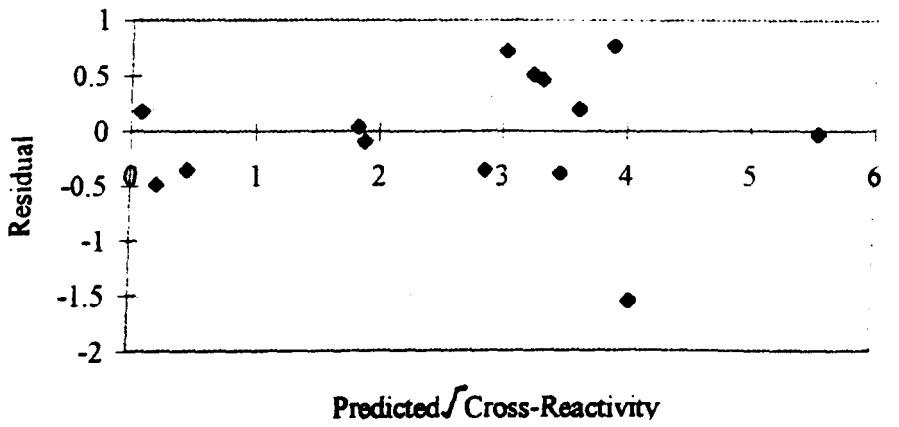
	DVx	Sterimol B4	√HRTDD Xr
DVx	1.0	0.092	0.311
Sterimol B4		1.0	-0.721
√HRTDD Xr			1.0

Model 10.16 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivities of All Cephalosporins at HRTDD

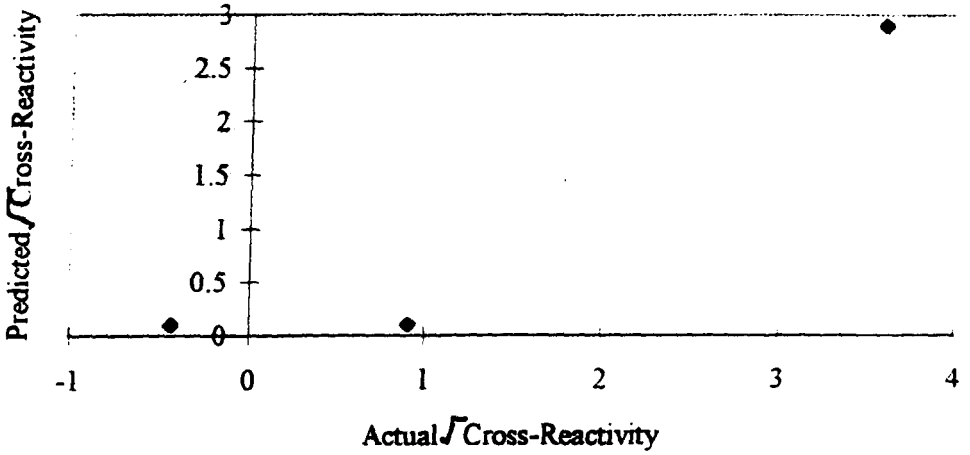


Plot of Residual Values Against Predicted √Cross-Reactivities of All Cephalosporins HRTDD

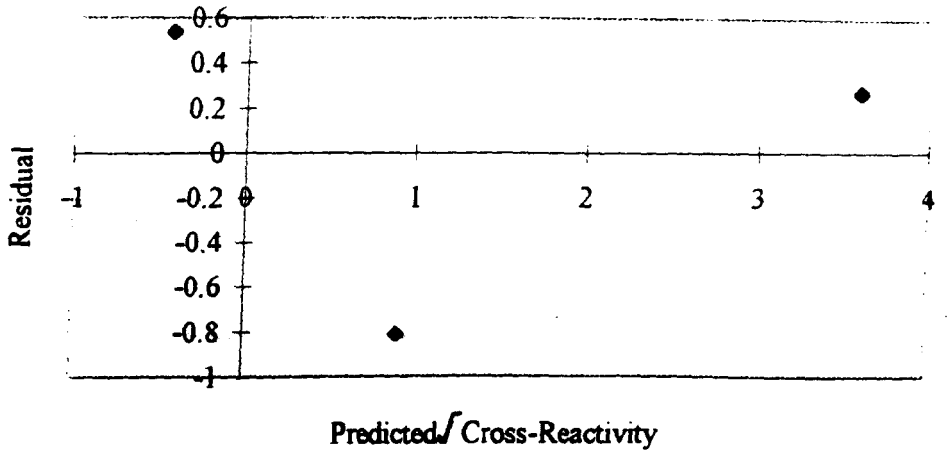


Model 10.17 Validity Plots

Plot of Predicted Against Actual $\sqrt{}$ Cross-Reactivities of Oral Cephalosporins at HRTDD

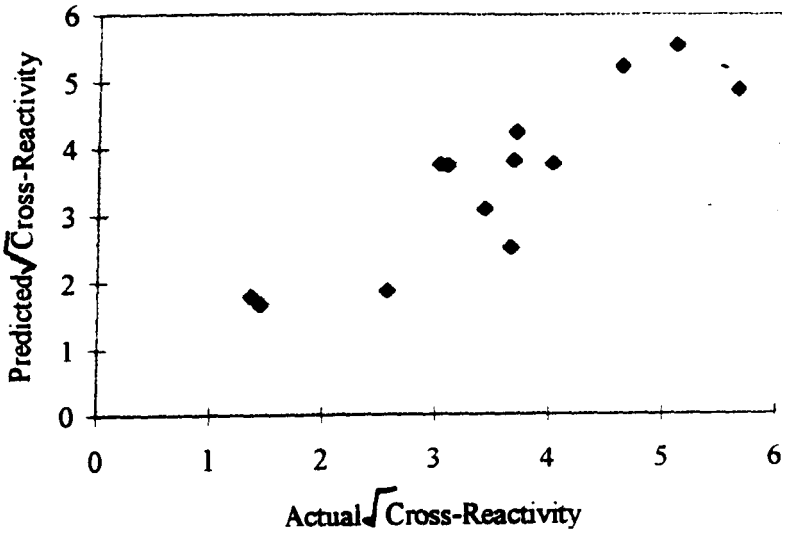


Plot of Residual Values Against Predicted $\sqrt{}$ Cross-Reactivities of Oral Cephalosporins at HRTDD



Model 10.18 Validity Plots

Plot of Predicted Against Actual √Cross-Reactivity Values of Parenteral Cephalosporins at HRTDD



Plot of Residual Values Against Predicted√ Cross-Reactivity Values of Parenteral Cephalosporins at HRTDD