Cross-species scaling of cardiovascular safety pharmacology using PKPD modelling and simulation

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Abstract

One of the fundamental factors determining the success of a new drug candidate is the identification of a dose range that is both efficacious and safe. This is achieved via the use of pharmacokinetic (PK) and pharmacodynamic (PD) data to establish dose-(concentration)-response relationships for the various drug effects. Modelling and simulation (M&S) techniques are now commonly used to analyse clinical PKPD data as part of a model-based drug development framework, however, their use in the pre-clinical stages is limited and within the safety sciences is virtually non-existent. Application of such techniques to pre-clinical safety pharmacology data may help to improve the predictability of adverse effects compared to conventional methods. The aim of this project is to develop PKPD models using safety pharmacology data, which can be used to predict cardiovascular (CV) effects in man.

Non-linear mixed effects modelling was used to analyse the PKPD relationships for the haemodynamic effects of N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), milrinone and doxazosin in three species (rat, dog and guinea-pig). PK data were described by compartmental models and PD data by either direct, effect compartment or indirect response models with stimulatory or inhibitory effects characterised by non-linear ($E_{\text{max}}$) or linear (slope) functions. Baseline mean blood pressure (MBP) and heart rate (HR) either displayed circadian rhythms, which were described by cosine functions for dog or a biorhythm model specifically developed for rats, or an anaesthetic effect in guinea-pigs, which was described using a linear function. A sequential modelling approach was taken where PK and baseline values were fixed when fitting the PD data. Human responses to the 3 compounds were predicted using the PD values determined from each animal model. Human PK and baseline were fixed, pharmacological values were set as the animal values and physiological values were scaled according to body weight using an exponent of -0.25.

Adequate fits to PK, baseline and PD data were generally observed, although issues with sampling times and dose ranges restricted the model options in a number of cases. For L-NAME and milrinone, where a delay in effect was observed, indirect models gave better fits than effect-compartment models. Doxazosin had direct effects in all species. Some issues with model fit were due to the fixed baseline, therefore a simultaneous fit of baseline and PD data would be recommended. However, the complexity of the rat baseline model could lead to unusual profiles with this method and thus it is not recommended for standard use. Generally values agreed with literature observations although no consistency in the PD values was observed across species or between MBP and HR. In most cases human response was under-predicted but there was no consistent pattern regarding the level of under-prediction across compounds. The extent of human effect on HR by L-NAME and milrinone was successfully predicted using dog values. Unfortunately dog was not predictive for doxazosin or MBP for any compound. Time course of effect was not predicted successfully in any of the cases. It was theorised that more complex models containing the CV feedback mechanisms may be required for some of the unsuccessful predictions.

Overall PKPD modelling can be used to describe CV safety pharmacology data in different species and if study design and modelling issues were addressed, greater accuracy could be obtained. Dog showed the best potential for prediction of CV measures and predictions might be improved with the use of CV models that incorporate accurate feedback mechanisms. If greater model complexity is restricted to physiological aspects the use of such models in safety pharmacology may also help to give more insight into mechanism of action.
Declaration

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Chapter 1

Introduction
1 Introduction

This project is concerned with the potential application of pharmacokinetic-pharmacodynamic (PKPD) modelling, currently a well established methodology within the therapeutic development of new drugs, to usage within the safety sciences. More specifically the project aims to establish the potential of utilising safety data from pre-clinical species to predict the safety profile of new compounds in man via a cross-species scaling approach.

In the following sections PKPD modelling and cross-species scaling and their current use in industry and academia will be discussed. An introduction to safety pharmacology, specifically relating to the cardiovascular system will also be given. Information regarding the background of the compounds used in this project are presented as an introduction to each results chapter.

1.1 PKPD modelling

1.1.1 Pharmacokinetics and pharmacodynamics

Pharmacokinetics (PK) and pharmacodynamics (PD) are the two disciplines that determine the relationship between dose and effect, and the mechanisms that characterise it. In their most simple terms pharmacokinetics is described as “what the body does to the drug” whereas pharmacodynamics is “what the drug does to the body” (Holford et al., 1982).

In more specific terms the field of pharmacokinetics is concerned with quantifying the relationship between an administered dose and the resulting concentrations in the body (Machado et al., 1999). The literal meaning of the word pharmacokinetics is the “movement” of drug throughout the body (Aarons, 1999a). This movement is governed by the processes of absorption, distribution, metabolism and excretion (ADME) and it is through their quantification that an understanding of the relationship between dose and concentration can be obtained.

Pharmacokinetic data are comprised of drug concentrations measured over a set time-course following dosing. Concentrations from the site of action of the drug can rarely be measured and therefore plasma/blood (as an accessible body fluid) is typically used as a substitute, although on occasion data from tissues may also be available (Aarons, 2005). The resulting concentration-time profile(s) are analysed to derive parameters that explain the ADME processes in mathematical terms, such as clearance (CL), volume of
distribution (V) etc. This can be performed using a so-called non-compartmental analysis or through fitting of a model to the data.

Pharmacodynamics is concerned with describing the relationship between drug concentration and effect (Machado et al., 1999). This effect can be any pharmacological action including both beneficial and adverse responses and can be expressed as a direct or indirect measure of efficacy/safety (Derendorf et al., 2000). Effect measures can be classified as biomarkers, surrogate end points or clinical outcome. Clinical outcome is a direct measure of desired/undesired effect of the drug treatment (e.g. survival, cure, time to an event etc). Biomarkers are biochemical (e.g. cytokines, glucose, ACE activity etc.) or physiological (e.g. blood pressure, heart rate, pupil dilation etc.) functions that are measures of some pharmacological effect of the drug but not necessarily directly linked to the clinical outcome. However, if a biomarker is strongly predictive of the clinical outcome then it is classed as a surrogate end point. Although clinical outcome is the ideal PD measure, it is often difficult to quantify and may require a large number of samples due to its typically categorical nature (Derendorf et al., 2000; Derendorf et al., 1999). Therefore, biomarkers or surrogate end points are often used as they can be measured in a shorter time frame, using more robust measures (Colburn et al., 2003).

PD data may also present in a number of different forms. Continuous data are the most informative, although depending on the nature of the pharmacological response it may present as categorical, count or time to event data, amongst others (Derendorf et al., 2000).

Like PK data, quantification of the relationship between concentration and effect is obtained by applying a mathematical model. However, PD data usually consists of responses measured over a time-course and so is typically modelled in conjunction with a PK model (via a PKPD model) to fully characterise the dose-concentration-effect relationship.

1.1.2 Modelling of PK/PD data

Models are often described as a simplified description of reality (Holford et al., 1982). How simplified a model is will depend on the amount and quality of the data available and for what it will be used for. Models can be descriptive i.e. be valid for only a specific set of circumstances (patients, designs etc.), or predictive, where variables are included that allow the model to predict different outcomes dependent on the values of
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those variable used (Rajman, 2008). Models can also be described as mechanistic, where parameters related to physical entities (in this case physiological/biological aspects) are incorporated, or empirical where such attributes are absent. In general those models that are more mechanistic will prove to be more predictive.

1.1.2.1 PK models

In terms of PK models there is a clear hierarchy of models (Aarons, 2005). The simplest empirical models are the sum of exponentials, which are purely a description of the concentration-time profile, with parameters describing concentration coefficients and rate constants. These models are adequate to describe the data and derive primary PK parameters such as CL and V but are not useful for prediction purposes.

Compartmental models describe the body as a series of theoretical compartments. Although the compartments do not represent real spaces within the body, when a physiological parameterisation is used, the data can be defined in terms of volumes and clearances. These terms can be more easily related to (patho-)physiological processes that affect the variability in PK and hence be used for prediction of other scenarios. Compartmental models are the most commonly used models for PK data analysis (Balant et al., 2000).

Physiologically-based pharmacokinetic (PBPK) models are mechanistic and are formed by a series of compartments representative of actual tissues and organs in the body. These compartments are arranged anatomically and linked via the vascular system. The parameters that are incorporated into the model are of two types: physiological (e.g. blood flows, tissue volumes etc.) and drug-specific (e.g. tissue/blood partition coefficients, intrinsic clearance, protein binding etc.). A series of mass-balance differential equations utilise these parameters to define the concentration-time course in each tissue and the blood/plasma. A criticism of PBPK models has been that they are complex with requirement for many parameters. However, the model can exhibit different levels of complexity, with certain organs “lumped” together if so required (Rowland et al., 2004). In addition, advances have been made with methods for the prediction of input parameters (Jones et al., 2006). The separation of physiological parameters from the drug effect allows both the mechanistic insight into the properties of a compound and prediction of PK in numerous different circumstances.
1.1.2.2 PD models

In terms of PD models, the majority are still empirically-based. The most commonly used PD model, which describes non-linear concentration-effect relationships, is the sigmoidal $E_{\text{max}}$ model (Equation 1.1):

$$E = \frac{E_{\text{max}} \cdot C_p^n}{EC_{50}^n + C_p^n}$$

Equation 1.1

Where $E$ is effect as a function of $E_{\text{max}}$, which is the maximum effect, $C_p$ is the plasma drug concentration, $EC_{50}$ is the concentration of drug producing half maximum effect and $n$ which is referred to as the shape factor. The $E_{\text{max}}$ model is based on receptor theory therefore $E_{\text{max}}$ reflects the efficacy of the drug and $EC_{50}$ the potency. The parameter $n$ can be thought of as the number of molecules binding but in practice is simply used to give a better fit to the data (Derendorf et al., 1999). Since drug responses are usually measured as a change from a baseline state, the baseline value ($E_0$) is often incorporated into the model to allow for potential variability to be included.

There are other simpler PD models that are related to the sigmoidal $E_{\text{max}}$ model, namely the simple $E_{\text{max}}$ model, where $n=1$, the linear model, log-linear model and the fixed effect model (Holford et al., 1982). These models are however less commonly used as it is recognised that they are typically only valid within a certain concentration range (Csajka et al., 2006). When data is not of a continuous nature other models may also be used such as the logistic regression model often used for binary or ordered categorical data (Machado et al., 1999). This model is similar to the $E_{\text{max}}$ model but is linear in terms of concentration rather than log concentration.

The $E_{\text{max}}$ model is derived from the classical receptor occupancy theory under equilibrium conditions (Mager et al., 2003). This assumes that receptor binding is rapid and reversible. In some instances binding occurs more slowly and becomes a rate-limiting step for drug effect. In these cases the binding kinetics are required (Equation 1.2).

$$\frac{dRC}{dt} = k_{\text{on}} \cdot (R - RC) \cdot C - k_{\text{off}} \cdot R$$

Equation 1.2
Where $RC$ is the drug-receptor complex, $RT$ is the total receptor concentration, $C$ is the concentration of drug at the site of action, $k_{on}$ is the second order rate constant for receptor association and $k_{off}$ is the first order rate constant for receptor dissociation.

The drug effect is then assumed to be directly proportional to the fraction of occupied receptors, which is the same assumption as inherent in the $E_{max}$ model. It may be more realistic to assume that effect is related to the drug-receptor complex in a non-linear fashion, in which case the operational model of pharmacological agonism (Black et al., 1983) may be used (Equation 1.3).

$$E = \frac{E_m \cdot \tau^n \cdot C^n}{(K_D + C)^n + \tau^n \cdot C^n}$$

Equation 1.3

Where $E$ is the pharmacological effect, $E_m$ is the maximum system response (i.e. the maximum effect that can be observed in the biological system), $K_D$ is the dissociation equilibrium constant ($k_{off}/k_{on}$) and $C$ and $n$ are as described in equations 1.1 and 1.2.

$\tau$ is the so-called “transducer ratio”, the ratio of $RT/K_E$, where $K_E$ is concentration of the RC that elicits half the maximum effect and $RT$ and RC are as in equation 1.2. This transducer ratio is a measure of the efficiency of receptor occupancy/activation to pharmacological effect.

The operational model of agonism had been used successfully in a number of PKPD modelling exercises for different agonists (Garrido et al., 2000; Van Der Graaf et al., 1997; Zuideveld et al., 2004).

Use of models such as equations 1.2 and 1.3 enables a more mechanistic approach to PD modelling as they separate out the properties of the drug (receptor affinity and intrinsic efficacy) and the biological system (receptor density and the function relating receptor activation to response). However the proportional or non-linear function used to describe the translation of receptor activation to pharmacological response (known as transduction) may not be sufficient to describe the complex processes involved. Transduction can involve a cascade of events involving a variety of chemical mediators, signals, enzymes etc. The time frame for the turnover of such processes can vary and so may become the rate-limiting step for drug response (Mager et al., 2008).

A family of four “indirect response” (or turnover) models, where drug response can be related to the stimulation or inhibition of factors controlling the input/production or
determinants of loss were developed by Dayneka et al., (1993). The general model describing the rate of change of the response over time (turnover) when no drug is present is described in equation 1.4:

\[
\frac{dR}{dt} = k_{in} - k_{out} \cdot R
\]

Equation 1.4

Where \( k_{in} \) is the zero order rate constant for production of the response (R) and \( k_{out} \) is the first order rate constant for its loss. Stimulation or inhibition of \( k_{in} \) or \( k_{out} \) is then applied via non-linear functions similar to the E\(_{max}\) model or a simpler linear effect function.

These models can be used to account for delays in response due to transduction processes and the rate constants are considered system specific. Where the transduction process is complex, a series of linked or cascading turnover models can be used to describe the intermediary processes (Ramakrishnan et al., 2002). Alternatively signal transduction models based on the transit compartment model can be used (Sun et al., 1998). It must be noted that to develop these more complex models, biomarkers for the various stages are required.

Models for tolerance or homeostatic feedback mechanisms can also be incorporated into turnover models to characterise complex response time profiles (Danhof et al., 2005).

1.1.2.3 PKPD models

As mentioned in section 1.1.1 modelling of PD data generally happens in conjunction with PK data via an integrated PKPD model. This approach dispels the need for simultaneous concentration and effect measurements as the PK part of the model provides a continuous concentration-time profile (Holford et al., 1982). A step-wise approach modelling PK data first (as opposed to simultaneous modelling of PK and PD data), is most often undertaken since the PK model is often better understood and more reliable estimates of its parameters can be obtained (Derendorf et al., 1999; Holford et al., 1982). The concentrations provided by the PK model then act as input to the PD model along with the effect data.

The concentration used in the PD models should in theory be the concentration at the effect site, however in practice the concentration measured in blood/plasma is used due to its accessibility. Under steady-state conditions the concentration between the
blood/plasma and effect site will be in equilibrium, however under non-steady-state conditions distribution to the site of action may represent a rate-limiting step for producing the effect. This is typically reflected in a delay in the time course of the pharmacological effect relative to the plasma concentration and can be observed as an anti-clockwise “hysteresis” loop in a plasma concentration vs. effect plot as shown in Figure 1.1.

![Figure 1.1: A typical anti-clockwise hysteresis observed between plasma concentration and effect data.](image)

Depending on the rate-limiting step, there are other potential reasons for observing a hysteresis loop (e.g. slow receptor binding or transduction processes) but if it is due to delayed distribution to the effect site, then a link between the PK and PD models will be required.

The “biophase” or “effect compartment” model was introduced by (Sheiner et al., 1979). The model is based on the recognition that the time course of the effect could be used to determine the changes in concentration at the effect site. To achieve this, a theoretical effect compartment attached to the central compartment of PK model was used. The concentration in the effect compartment is linked to the central (plasma) compartment by a first order process as described in Equation 1.5.

\[
\frac{dC_e}{dt} = k_{1e} \cdot C_p - k_{e0} \cdot C_e
\]

Equation 1.5

Where \( C_e \) and \( C \) are the drug concentrations in the effect and plasma compartments respectively, and \( k_{1e} \) and \( k_{e0} \) are the first–order rate constants into and out of the hypothetical effect compartment. \( k_{1e} \) and \( k_{e0} \) are set equal to each other so as to avoid affecting the PK (Danhof et al., 2007a). The resulting \( C_e \) values are utilised in the PD model.
As discussed by Danhof et al., (2007b), a limitation of this model is that it is not truly mechanistic. While it may work well for drugs that distribute to their site of action via passive diffusion, factors such as physiochemical properties, binding and functionality of transporter proteins may restrict a drug’s distribution to the biophase. At present there are a number of proposed semi-parametric and non-parametric approaches to be used as alternatives for modelling effect compartment kinetics, however the only mechanistic alternative is to use a PBPK model in conjunction with a mechanism-based PKPD model (Danhof et al., 2008).

1.2 PKPD M&S in industry

The role of modelling and simulation (M&S) within the pharmaceutical industry has strengthened over the years and there are many papers that review the use of PKPD M&S within the different development phases (Aarons et al., 2001; Chien et al., 2005; Gomeni et al., 2001; Miller et al., 2005; Rajman, 2008; Sheiner et al., 2000). The “learn-confirm” paradigm of Sheiner (1997) can be seen as the initial driving force for the increase in use of M&S in industry. It was proposed as an efficient and rational approach to clinical drug development and as Miller et al., (2005) describe it, learn-confirm has now become the “catch-phrase” of clinical pharmacology. With the FDA’s Critical path whitepaper (FDA-US, 2004) highlighting that “model-based drug development” offers an important approach to improving decision-making, M&S has now expanded throughout the various stages of development.

As noted by Rajman (2008), to make most effective use of PKPD M&S in drug development models should be developed in the early, preferably pre-clinical stages of development. The models can then be continuously updated and refined with new data throughout the clinical phases and assist in the decision making for the next stage.

1.2.1 Pre-clinical M&S: cross-species scaling

Examples of how PKPD M&S techniques have been implemented in pre-clinical and early clinical development are rarely published in the literature due to confidentiality issues. Modelling at the pre-clinical stage is often used to help with the selection of the candidate to take into the clinic and the decision of what doses to use. Prediction of the human dose-response relationship is key for such decisions and requires techniques for accurate “scaling” of the animal data. Data from compounds of the same class can be used as comparators, with the relative difference between the comparator’s efficacy and potency in animals compared to man applied to the new compound. PK parameters are...
usually scaled allometrically (see 1.2.1.1). Chien et al., (2005) describe an example where this type of approach was taken and proved successful. However, they do highlight that if there is a lack of comparator data then this type of empirical efficacy scaling would not be feasible and that a mechanistic approach would be required.

1.2.1.1 Cross-species scaling of PK parameters

The prediction of human PK parameters, particularly clearance is central to the selection of a dose for first in man (FIM) studies. There are a number of techniques that can be used but the two main approaches utilising animal data are allometric scaling and use of PBPK models. Allometric scaling is an empirical approach, examining the relationships between PK parameters and body size, without necessarily understanding the underlying mechanisms. PBPK as explained in section 1.1.2.1 is a mechanistic approach.

The concept of allometric scaling is based on the observation that many anatomical and physiological parameters can be related to the size of the animal (Lin, 1995). More specifically they can be related to body weight via the exponential function described in Equation 1.6.

\[ Y = a \cdot WT^b \]  

Equation 1.6

Where \( Y \) is an anatomical or physiological parameter, \( WT \) is the body weight, \( a \) is the allometric coefficient and \( b \) is the allometric exponent.

It has been shown that physiological parameters, such as glomerular filtration and hepatic blood flow, and anatomical parameters, such as liver and kidney size, can be allometrically scaled. Since these parameters can be directly related to drug disposition, the belief is that PK parameters can be scaled in the same way (Ings, 1990).

The allometric relationship for a PK parameter is derived via linear regression of a log-log plot of the kinetic parameter vs. body weight of various pre-clinical species (Lin, 1995). The exponents of \( CL \), \( V \) and \( t_{1/2} \) are often found to be close to 0.75, 1 and 0.25 respectively (Ings, 1990).

Where data from multiple species are not available PK parameters can also be scaled according to the single species scaling approach (equation 1.7).
Where $P$ is the pharmacokinetic parameter of interest, $WT$ is body weight and $b$ is a physiologically relevant exponent, typically assumed to be 0.75 for clearance and 1 for volume (Hosea et al., 2009).

There are many examples in the literature where a PK allometric relationship has been successfully derived and predicts the value in human accurately but there are also many that show failures. In general those drugs that show success are where PK parameters are directly related to physiological and anatomical parameters i.e. those drugs primarily renally cleared or those with high hepatic extraction where clearance approximates liver blood flow. Conversely, those that fail are often directly affected by biochemical parameters, such as protein binding and enzyme activity, which do not express a direct allometric relationship i.e. low extraction compounds, where clearance is predominantly mediated by cytochrome P450 enzymes (Lin, 1995).

An alternative approach to the empiricism of allometry is to utilise PBPK models. Since these models are mechanistic, the parameters are divided into physiological and drug-specific terms. In addition, the structure is essentially common to all mammals, and therefore these models are ideal for cross-species scaling (Rowland et al., 2004). Jones et al., (2006) describe an approach for predicting human PK using PBPK models with animal and in vitro data. In this approach, the model is validated in pre-clinical species using data relevant to that species. If the model can predict the outcome in these animals, then there is confidence that predictions of human PK (using the relevant human data) will be sufficiently accurate. The approach was compared with an allometric approach and found to be superior.

1.2.1.2 Cross-species scaling of PD parameters

In contrast to PK parameters, very little work has been published on scaling of PD parameters. As described in section 1.2.1, the typical industry approach is to use relative data from a same in class compound but this is only valid if a comparator drug is available (Chien et al., 2005). The general expectation for scaling PD parameters is that the rates of biological turnover processes should be predictable between species based on allometry, whereas intrinsic capacity ($E_{\text{max}}$) and sensitivity ($EC_{50}$) tend to be similar across species (Mager et al., 2008). However, if a simple $E_{\text{max}}$ model is used, such
parameters could actually incorporate properties of the biological system (such as receptor expression and transduction) as well as the drug properties (see section 1.1.2.2). Thus, this expectation for drug parameters may be incorrect.

There are two published examples where an allometric analysis for PD parameters has been performed. One for metocurine, an acetylcholine receptor (AchR) antagonist (Gronert et al., 1995) causing muscle relaxation and the other S-ketoprofen, a cyclooxygenase (COX) 1 and 2 inhibitor (Lepist et al., 2004) for decreasing inflammation. The results of the 2 analyses differed with Gronert et al., (1995) showing an allometric relationship for IC$_{50}$ with an exponent close to 0.25, whereas Lepist et al., (2004) showed no relationship between IC$_{50}$ and body weight. The reasons for this difference in observation may be a result of the model used and its link to the effect measure. Gronert et al., (1995) used an effect compartment model to describe the delay in metocurine concentration and muscle twitch response, whereas Lepist et al., (2004) used indirect response models to describe S-ketoprofen effect on thromboxane B2 (TXB2) and prostaglandin E2 (PGE2) levels. Theoretically if the observed delay in muscle twitch response was actually at the transduction rather than distribution stage, the IC$_{50}$ values may be influenced by such physiological processes and thus incorrectly appear to have a relationship with weight.

In addition to the allometric analysis, there are examples where extrapolation of human response from rat models was investigated.

Yassen et al., (2007) used a receptor binding model with a linear transduction function and effect compartment to describe the antinociceptive and respiratory depressant effects of the opioid µ receptor agonist buprenorphine. Receptor binding kinetics were shown to be equivalent in rats and human and k$_{e0}$ scaled allometrically with an exponent close to -0.25, the value expected for biological rate constants.

Mager et al., (2009) describe a model for the effects of rHuEpo on the concentrations of reticulocytes (RET), red blood cells (RBC) and haemoglobin (Hb), three biomarkers for anaemia. The model contains steps for the processes involved in erythropoesis and is based on a series of indirect response models integrated with cell life span concepts. Pharmacological effect was incorporated via a stimulatory E$_{max}$ function. With the use of rat values for the pharmacological terms, physiological life span terms allometrically scaled with an exponent of 0.124 (previously determined in 20 species) and human
baseline values for RET, RBC and Hb, it was shown that the human effect on all three biomarkers could be accurately predicted.

Zuideveld et al., (2007) investigated the hypothermic effects of two 5HT\textsubscript{1A} agonists, flesinoxan and buspirone using a set-point model. This model is based on an indirect response model with a feedback mechanism incorporated. In addition the effect of flesinoxan on cortisol levels was described using a simple indirect model. In both cases pharmacological effect was incorporated via a sigmoid $E_{\text{max}}$ function. The physiological rate constants were scaled from rats using an exponent of -0.25 and rat values were used for the pharmacological terms, except for flesinoxan $E_{C50}$. A previous study had shown the affinity to be underestimated, thus a factor of 30 was applied to the rat value. Prediction of the human hypothermic response was successful for both flesinoxan and buspirone. Prediction of flesinoxan’s effect on human cortisol levels was also successful with respect to the extent although not the time-course of the response.

These examples show that if the pharmacological effect can be sufficiently separated from the physiological processes, successful translation of animal to man is possible. Complex transduction and feedback processes may be required, which demands a thorough understanding of the mechanism of action and relevant biomarkers. Even then, there may be issues with the pharmacological values as shown by Zuideveld et al., (2007). Some studies investigating animal to human PD translation have only looked at receptor occupancy aspects (Bourdet et al., 2012) and others require adjustment of pharmacological values by differences in \textit{in vitro} binding (Chang et al., 2011). However, it appears with enough knowledge about the mechanism of action, cross-species translation is possible.

\subsection*{1.2.2 M&S in clinical development: population PK/PD}

Once a drug has entered human clinical trials the data obtained can be used to confirm/update/refine the pre-clinical models. M&S has many wide and varied uses within the clinical development program relating to dosing and sampling regimens, assessing variability and covariates for PK and PD, impact of disease progression, different populations etc. One of the key M&S methods is that of population PK/PD, which is an approach used to quantify and explain the inter-individual variability of the PK and PD parameters within a (target) population (Aarons, 1999b). During phase II/III trials there are a considerable number of individuals studied and so it is not feasible to collect a great number of data points from each person. Therefore, sparse sampling
techniques are used, where data is collected from a few time points in each individual and these time points vary between individuals. The population approach analyses all the data together to define the distribution of PKPD parameters in the population. This is achieved using non-linear mixed effects modelling (Aarons, 1999b).

1.2.2.1 Non-linear mixed effects modelling

The term “mixed effects modelling” refers to the use of models which combine both structural (fixed effects) and variance (random effects) elements. The structural model describes the overall trend of the observations in the population with respect to a set of parameters. The fixed effects parameter estimates are the average values within the defined population. The variance model typically contains two random effect components describing inter-individual variability and residual error. Inter-individual variability describes the difference between the parameter estimates for an individual and the average population values. Residual error describes the difference between the observations and the predictions made using an individual’s parameter estimates. Residual error can represent a number of factors including assay error, experimental error, intra-individual variability and model misspecification.

A general mixed effect model used for PKPD problems is described in Equation 1.8

\[ y_{ij} = f(\Theta_i, x_{ij}) + \varepsilon_{ij} \quad \text{Equation 1.8} \]

\[ y_{ij} \] is the \( j \)th observation for the \( i \)th individual, \( f(\cdot) \) denotes the structural model, which is typically non-linear for PK or PD models (hence non-linear mixed effects) and is a function of the parameters, \( \Theta_i \) and the independent variables \( x_{ij} \) (e.g. time, dose). \( \varepsilon_{ij} \) is the residual error, which is assumed to be normally distributed with a mean of 0 and a variance of \( \sigma^2 \). \( \Theta_i \) defines a vector of parameters (\( \theta \)) for an individual \( i \). Individual parameter values are determined from the population mean estimate using an inter-individual variability term as described in Equation 1.9

\[ \theta_i = \theta + \eta_i \quad \text{Equation 1.9} \]

\( \theta_i \) is the individual value for the parameter, \( \theta \) is the mean population parameter estimate and \( \eta_i \) describes the difference between them i.e. the inter-individual variability. For each parameter, \( \eta_i \) is assumed to be normally distributed with a mean of 0 and a variance of \( \omega^2 \).
The variance terms ($\varepsilon$ and $\eta$) can be applied in a number of ways. In Equations 1.7 and 1.8 both terms are additive and thus remain constant. They may also be applied in a proportional manner, where they are dependent on the predictions or a combination of additive and proportional. Inter-individual variability terms are typically applied in an exponential manner as in equation 1.10

$$\theta_i = \theta \cdot e^{\eta}$$

Equation 1.10

1.2.2.2 Model fitting and parameter estimation

There are a number of software packages that can be used to fit the model to the data. They can differ in the statistical method used to estimate the parameters, including parametric and non-parametric maximum-likelihood (ML) and Bayesian methods, but most utilise the parametric ML approach. The maximum likelihood approach, as its name suggests, estimates the parameter values that make the observations most likely given the selected model. The maximum likelihood objective function is minus twice the log of the likelihood (-2LL). Thus maximum likelihood estimates are the parameter values that minimise the -2LL objective function.

Monolix is a software package that has been designed for non-linear mixed effects modelling and adopts the parametric maximum likelihood approach. Until recently it was unique in respect to the optimisation algorithm used for minimisation. For non-linear mixed effect models, the structural and variance parameters are estimated simultaneously. However, computation of the likelihood function is difficult due to the non-linearity of the inter-individual random effects. Many software packages use methods to linearise the model with respect to its random effects but this is not the case in Monolix. The optimisation algorithm used is known as the stochastic approximation estimation maximisation (SAEM) algorithm and for use in fitting non-linear mixed effects models is coupled with a Markov Chain Monte Carlo (MCMC) procedure. Each iteration of the algorithm consists of three different steps. Firstly the MCMC method (the Hastings-Metropolis algorithm) computes a sequence of random samples from the individual parameter probability distributions. The second stage is a stochastic approximation of the likelihood function and in the third (maximisation) stage the population parameters are updated. During the MCMC stage more than one sequence of samples can be obtained by increasing the number of Markov Chains. The likelihood function is computed using another Monte-Carlo approach known as importance
sampling (nb. An estimate of the likelihood can also be obtained using the linearisation approach and both can be reported in the output).

Monolix reports population values for fixed and random effects with their standard errors, individual parameter estimates and the minimised objective function.

1.3 Safety pharmacology

Pharmacology studies are traditionally associated with the discovery of properties related to therapeutic use i.e. efficacy (Claude et al., 2004). The concept of safety pharmacology however, is concerned with the adverse effects that drugs exert on organ functions and that standard toxicological testing methods do not easily detect (Bass et al., 2004).

1.3.1 Evolution of the discipline

The first mention of organ function testing in appeared in Japanese regulatory documentation as early as 1975 (MHW-Japan, 1975). This was subsequently extended to give more specific guidelines forming part of the non-clinical study requirements released in 1995 (MHW-Japan, 1995). Around the same time, an increasing number of drugs were being withdrawn from the market due to safety reasons (Lasser et al., 2002). The withdrawal of the antihistamine drug terfenadine is the classic example cited for this time period. It became a concern for public health after it was discovered that in rare cases it could induce torsades de pointes (TdP), a potentially life threatening arrhythmia. Up until this time, it had been believed such a pre-disposition would only be limited to cardiovascular drugs (Pugsley et al., 2008). In addition it would not have been picked up by the toxicity study methodology employed at that time. Therefore industry and regulatory authorities began to recognise that further specific safety studies were required to help protect individuals receiving new chemical entities (NCEs) from adverse effects related to organs/systems.

During the 1990s, the Japanese guidelines, as the most thorough of the time, were globally adopted as the basis of organ function testing (Kinter et al., 2002). In 1998, the EU, US and Japanese regulators (EMEA, FDA, MHW) each produced draft concept papers on “safety pharmacology”, which were debated at a discussion group that later became the safety pharmacology society (Bass et al., 2004). Later that year the Japanese authorities proposed the implementation of global safety pharmacology guidelines to the steering committee of the International Conference on Harmonisation of technical
requirements for registration of pharmaceuticals for human use (ICH). This was accepted and in 2000 the harmonised ICH S7A guidelines (ICH-S7A, 2000) were finalised and adopted by the global regulators over 2000-1 (Bass et al., 2004). The aim of the guidance was to establish the procedures (e.g. experimental models, study designs and data collection methods) to be undertaken in the assessment of safety pharmacology endpoints (Kinter et al., 2002).

The ICH S7A guidelines define safety pharmacology as “those studies that investigate the potential undesirable effects of a substance on physiological functions in relation to exposure in the therapeutic range and above”. The functions of the cardiovascular, respiratory and central nervous systems are considered vital to life and thus constitute the focus of the “core-battery” of tests, which must be performed prior to any human dosing. In addition, supplemental studies on the renal/urinary system, autonomic nervous system, gastro-intestinal system or other systems such as skeletal muscle, immune or endocrine function or the potential for dependency, may also be studied if there is a reason for concern.

1.3.2 Cardiovascular safety pharmacology

The cardiovascular system is one of the main causes of concern in safety testing with 45% of the 47 drugs withdrawn from the market between 1975 and 2007 due to cardiovascular toxicities (Stevens et al., 2009). According to the ICH S7A guidance, the cardiovascular system function should be assessed by measurements of blood pressure (BP), heart rate (HR) and the electrocardiogram (ECG). In addition if an adverse effect is found and follow-up studies are required then cardiac output (CO), ventricular contractility, vascular resistance and the effect of endogenous/exogenous substance on the cardiovascular system may also be evaluated. However, these studies are not specifically designed to assess TdP risk, which is the main cause of drug withdrawal due to cardiovascular reasons (Stevens et al., 2009).

Delayed ventricular repolarisation is the major drug-related risk factor in the occurrence of ventricular arrhythmias such as TdP (De Ponti et al., 2000). Ventricular repolarisation is determined by cardiac action potential duration (APD) and is a complex process involving influx and efflux of various ions through a number of channels. A substance’s ability to prolong the APD can be observed in the ECG recording as a prolongation of the QT interval (the beginning of the QRS complex to the end of the T wave on an ECG trace), which covers the duration of ventricular
depolarisation and subsequent repolarisation. As an extension to the ICH S7A, the ICH S7B guidelines were specifically developed to direct the nonclinical assessment of delayed ventricular repolarisation potential (ICH-S7B, 2005). These were finalised in 2005 and adopted by the EMEA and FDA that year, although not by the Japanese authorities until 2009. In parallel, the ICH E14 guidance for clinical evaluation of pro-arrhythmic risk was developed, describing the “thorough QT study” requirement (ICH-E14, 2005).

1.3.2.1 Current Practices

The S7B guidelines recommend an integrated approach to assessing the delayed ventricular repolarisation (QT prolongation) risk, with the minimum requirement of an in vitro $I_{Kr}$ assay and an in vivo QT assay. $I_{Kr}$ stands for the rapid delayed rectifier potassium current, crucial for repolarisation of cardiac action potentials and conducted through potassium channels encoded by the human ether-a-go-go-related gene (hERG). Inhibition of the hERG channel is the most common mechanism of drug induced QT prolongation (ICH-S7B, 2005). Further studies on ion channels other than the hERG channel, may also be undertaken, however this is much less common (Lindgren et al., 2008).

The in vivo QT assay, as the name suggests, is generally designed to measure the QT interval from an ECG, which the most common endpoint for ventricular repolarisation. The recommendation is to design the assay to also meet the objectives of the S7A guidance, to help reduce the number of animals used. A recent survey of safety pharmacology practices within the pharmaceutical industry showed that the most common species used for the in vivo assay was the dog (Lindgren et al., 2008). This is likely due to the fact that the electrophysiology of the dog heart and the functionality of the canine $I_{Kr}$ channel (cERG) are similar to human (Wang et al., 2003). Since the S7A guidance indicates a preference for animals to be in a conscious, unrestrained state, radiotelemetry is typically used to simultaneously collect ECG and haemodynamic parameters. Animals are generally chronically instrumented with telemetry transmitters, consisting of an inter-arterial catheter to measure blood pressure and a Lead II ECG, although more recently the use of non-invasive telemetry jackets has been proposed (Prior et al., 2009). Benefits of the model include the ability to dose via the oral route, (typically the clinical route of administration), administer increasing single doses on separate occasions (typically via a Latin-square crossover design) and to record the parameters over an extended period of time (typically 24 hours), which all fit with S7A
design recommendations (De Clerck et al., 2002; Lindgren et al., 2008; Ollerstam et al., 2007). The main limitation appears to be the potential for higher doses to induce side-effects such as emesis or sedation/excitation (De Clerck et al., 2002). This may cause difficulty in reaching the expected exposure range, which ideally includes and exceeds the therapeutic range.

Other species may also be used for the \textit{in vivo} QT assay, although this does not include mice or rats since the ion channels involved in repolarisation differ in these rodent species (ICH-S7B, 2005). The most common species after dogs are non-human primates (NHP)/monkeys (Lindgren et al., 2008). Increasing use of monkeys in toxicological studies led to the need for a cardiovascular safety model in this species (Guth, 2007) and models using invasive (Authier et al., 2007) and non-invasive (Mitchell et al., 2010) telemetry techniques have both been validated. Due to problems with primate availability, the mini-pig is another species that has been suggested as an alternative. A mini-pig model which simultaneously measures cardiovascular and respiratory safety parameters has been proposed (Authier et al., 2011). This approach to combine different aspects of the core-battery requirements has also been proposed in monkeys (Ingram-Ross et al., 2012) and in dogs where cardiovascular and neurological parameters were obtained (Moscardo et al., 2009).

In addition to the S7B minimum requirements, specific assays measuring action potential duration (APD) are often employed both \textit{in vitro} and \textit{in vivo} (Lindgren et al., 2008). Purkinje fibre, Langendorff heart and papillary muscle from dog, rabbit and guinea-pig are the most commonly used \textit{in vitro} models. \textit{In vivo} APD assays are less commonly studied but when they are dog or guinea-pig are generally the species selected and parameters obtained are monophasic action potential duration along with the ECG measurements. Animals are often anaesthetised in such studies to allow pacing of the heart rate (Wisialowski et al., 2006). The use of anaesthetised animals is generally limited as many anaesthetics can affect the normal function of the cardiovascular system or produce drug interactions (Mitchell et al., 2010), and the study duration is restricted. However there are benefits in that higher dose levels via the intravenous route can be administered.

1.3.2.1.1 Heart rate and blood pressure

Heart rate is another key parameter in cardiovascular safety pharmacology since it has an inverse relationship with QT interval (ICH-S7B, 2005). Each heart beat is recorded
on the ECG trace as the R-R interval. Thus the faster the heart rate, the shorter the R-R interval and the shorter the QT interval (Piotrovsky, 2005). Correction of QT interval for changes in heart rate (denoted QTc) is important for accurately detecting drug-induced QT changes, since HR is one of the major sources of QT variability (Ollerstam et al., 2007; Piotrovsky, 2005). The HR-QT relationship varies both between and within species. Various correction formulas (e.g. Bazett, Fridericia, Van der Water) have been proposed and often used to correct human data; however these assume the same correction for all individuals, which is inconsistent with actual observations (Piotrovsky, 2005). It has been shown that individual corrections are superior, particularly in dogs, which exhibit large variations in heart rate (Ollerstam et al., 2007). Large, rapid intra-individual variations are also common in dogs due to emotional and physical responses and variability is further complicated by the presence of respiratory sinus arrhythmia (differences in heart rate on inhalation and exhalation). In addition to the correction method used, it must also be recognised that there is a delay in QT changes following abrupt changes in HR. In humans, it takes approximately 2-3 minutes for QT interval to adapt to HR changes, whereas the work of Ollerstam et al., (2007) showed that in dogs, 90% adaptation was reached after 1.5 minutes. Without accounting for this delay, there is a risk of under/overestimating any effect of a compound on QT interval.

In addition to being an essential correction factor for QT interval, HR itself is an important parameter to monitor for drug induced changes, since higher HR has been associated with mortality (Kannel et al., 1987). Tachycardia has been associated with various non-cardiovascular drugs, such as adrenergic agonists used in non-cardiovascular conditions (e.g. decongestants, asthma medications) and a number of antidepressants such as Duloxetine and Venlafaxine (Raj et al., 2009).

Blood pressure (BP) is also a key parameter, measured as part of the cardiovascular core battery tests. Maintenance of a stable arterial blood pressure within a certain physiological range is vital to ensure adequate perfusion of all organs/tissues without causing damage to the vascular system. In addition, even relatively small increases in blood pressure can increase mortality due to stroke and ischaemic heart disease (Lewington et al., 2002). Hypertension has been reported as an adverse effect for compounds for various indications including antidepressants, NSAIDs and decongestants (Raj et al., 2009). In addition compounds used to treat erectile
dysfunction and benign prostatic hypertrophy have been reported to cause or worsen hypotension.

Although most focus has been on ventricular repolarisation and QT, recognising drug induced effects on haemodynamic parameters are also crucial to determining cardiovascular safety. However, it has been recognised that the recommendations in some areas of the S7A documentation are vague (Pugsley et al., 2008) and there has also been criticism of the lack of focus on haemodynamic and other non QT-related risk (Picard et al., 2011). It is therefore vital that the studies that are performed can be adequately translated to human risk.

1.3.3 Non-clinical to clinical translation of safety pharmacology

The S7A guidance indicates that the choice of non-clinical experimental models, endpoints and study designs “should be relevant to the prediction of the potential human response”. Although a great deal of effort has been put into the design of such studies, translational aspects have been limited to determining firstly whether the models have the statistical power to detect a response (Sivarajah et al., 2010) and secondly if they have appropriate sensitivity, specificity and predictive capacity determined qualitatively by comparing rates of true and false negative and positive signals (Valentin et al., 2009). Quantitatively, the current best practice for the magnitudes of effect that would be a cause for concern in animals are the same as human values for HR and BP (Leishman et al., 2012). Values for QTc in dogs differ slightly to human values due to more comprehensive study of the QT translation area. The reliability of the predictions from non-clinical studies are vital as are often used for decision making e.g. if the studies indicate a compound is likely to have a positive signal in the thorough QT study then the clinical development of that compound would not proceed (Leishman et al., 2012). The S7A guidelines make reference to defining the dose(concentration)-response relationship of any observed adverse effect and the majority of companies will test doses that give multiples (most commonly 10, 30 and 100 times) the expected clinical concentration (Lindgren et al., 2008). Interpretation of the dose-response relationship is not always straightforward through and techniques such as PKPD modelling may be required to truly uncover the potential risk.

It has been noted that PKPD modelling could aid safety pharmacology studies in many ways (e.g. with respect to interpretation of exposure and time-response data, minimising animal use, investigating different study designs etc.) to aid the selection of drug
candidates (Cavero, 2007). A recent analysis of the trends in presentation topics at the safety pharmacology society has also shown that both PKPD relationships and pre-clinical to clinical translation are increasingly popular themes (Redfern et al., 2011). There is still a lack of literature publications showing examples of these topics though. One recent example showed how PKPD modelling techniques could be used to determine the dose-response relationship of a compound that caused PR and QRS interval prolongation in dogs and monkeys, when a delay between concentration and effect was observed (Fleury et al., 2011). Another looked retrospectively at whether the increased HR response observed in dogs would have been able to predict the effect seen in man (Langdon et al., 2010). The study showed that a reasonable prediction of the effect in humans could be achieved but that a difference of approximately 2-fold between human and dog effect parameters observed. This agrees well with the work of Ollerstam et al., (2006) that showed an approximate 2-fold difference between the potency of dofetilide’s QT interval prolongation in dog and human. There has also been success with the use of in vitro assays (hERG) along with the operational model of pharmacological agonism for the prediction of QT prolongation (Jonker et al., 2005).

Successful prediction of early clinical studies is vital to ensure that the risk of adverse effects in humans is sufficiently low, to both protect the participants and to prevent further development of unsafe compounds. Prediction of adverse effects in patient populations, where underlying conditions may affect their response, is the ultimate aim. It has been recognised that the use of PKPD modelling may help in this area (Bass et al., 2011), as disease models could be incorporated. Problems may still exist, particularly with the use of QT interval as only a biomarker of TdP risk (Piotrovsky, 2005) but with more studies now performed in the discovery (before or during lead optimisation) stages (Lindgren et al., 2008) with a variety of animal models (Fryer et al., 2012; Marks et al., 2012), it is hoped that adverse effects can be predicted early on and any risk to patients determined prior to late-phase clinical trials.

1.4 Aims and Objectives

The aim of this project was to determine the predictability of human cardiovascular response from pre-clinical safety pharmacology data using a modelling approach.

The objectives were:
Chapter 1: Introduction

Develop PKPD models for the effect of cardiovascular reference compounds with different mechanisms of action on blood pressure and heart rate in various pre-clinical animal models (rat, dog and guinea-pig)

Predict the human cardiovascular response using a cross-species scaling approach.
Chapter 2
General Methods
2 General Methods

2.1 Data

The pre-clinical data used in this project were kindly provided by Pfizer Ltd (Sandwich, UK). Cardiovascular measures and plasma concentrations were obtained during a series of safety pharmacology studies carried out at several Pfizer Global R&D sites between 2004 and 2009. The studies were performed in 6 different animal models, namely conscious rat, conscious ambulatory dog, conscious sling restrained dog, isoflurane anaesthetised dog, urethane/α-chloralose anaesthetised dog and anaesthetised guinea-pig, using 4 cardiovascular reference compounds with different mechanisms of action: \(\text{N}^\text{G}\)-nitro-L-arginine methyl ester (L-NAME), milrinone, doxazosin and verapamil. Not all animal models were studied for each compound and there were issues with missing data for a few studies. The project therefore focussed on a comparison of 3 of the animal models (conscious rat, conscious ambulatory dog and anaesthetised guinea-pig), which were available and complete for 3 of the 4 compounds (L-NAME, milrinone and doxazosin). Details of the studies which were not used and reasons why are presented in appendix 1.1.

2.1.1 Study designs

2.1.1.1 \(\text{N}^\text{G}\)-nitro-L-arginine methyl ester (L-NAME)

2.1.1.1.1 Rat study

The L-NAME rat study was performed with a parallel design in conscious animals. Cardiovascular parameters were measured in four separate groups of male Sprague Dawley rats (weight: 416-505g) receiving either a single dose of 10, 30 or 100 mg/kg L-NAME (n=6 for each group) or vehicle (n=5). Plasma concentrations were measured in a satellite group of age and lot-matched animals (weight: 324-373g), pre-cannulated for blood sampling via the tail vein. These satellite animals also received a single dose of 10, 30 or 100 mg/kg L-NAME (n=3 for each group). All doses were administered in a 0.5% methylcellulose suspension via oral gavage.

Cardiovascular parameters were recorded via pre-implanted telemetry devices in unrestrained animals. Food and water were allowed ad libitum and animals were exposed to an artificial 12 hour light-dark cycle running from 6am to 6pm. Doses were administered at 7.30am with parameters recorded from 1 hour pre-dose to 23 hours post-dose. The cardiovascular parameters measured/derived were heart rate (HR),
systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP). Parameters were reported in Excel® as 10 minute means.

Blood samples were collected from the cannulated rats pre-dose and at 0.5, 1, 2, 4, 8 and 24 hours following dosing. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of NG-nitro-L-arginine (L-NOARG), the active metabolite. The data set was not complete for all timings: particularly there was a complete absence of 4 hour measurements for the 30mg/kg dose and 8 hour measurements for the 100mg/kg dose. Additional data points were also absent, leaving a total of 34 concentrations from 9 animals.

2.1.1.1.2 Dog study

The L-NAME dog study was performed with a cross-over design in conscious animals. Cardiovascular parameters were measured in four male Beagle dogs (weight: 7-15kg) each receiving single doses of 10, 20 & 40 mg/kg L-NAME or vehicle on 4 occasions at least 7 days apart. The same animals also received additional single 10 and 40 mg/kg L-NAME doses on a further 2 occasions, at which time blood samples were collected for determination of L-NOARG plasma concentrations. All doses were administered orally in gelatine capsules. Individual animals were dosed according to the schedule described in table 2.1

Table 2.1: Dosing schedule for L-NAME dog study

<table>
<thead>
<tr>
<th>Study leg</th>
<th>Study day</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dog 1</td>
</tr>
<tr>
<td>PD</td>
<td>1 [0-24h]</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8 [168-192h]</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>15 [336-360h]</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>22 [504-528h]</td>
<td>20</td>
</tr>
<tr>
<td>PK</td>
<td>29 [672-696h]</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>36 [840-864h]</td>
<td>10</td>
</tr>
</tbody>
</table>

Cardiovascular parameters were recorded via pre-implanted telemetry devices (PhysioTel®, DSI, Inc.) in unrestrained animals. A standard diet of dry food was provided once daily, approximately 6 hours post-dose and water was allowed *ad libitum*. Animals were exposed to an artificial 12 hour light-dark cycle running from...
6am to 6pm. Doses were administered at 9am (±15mins) with parameters recorded from approximately 1 hour pre-dose to 22 hours post-dose. The cardiovascular parameters measured/derived were SBP, DBP & MBP; left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), and the index of contractility, maximum rate of rise in left ventricular pressure (LV +dP/dt); PR, QRS, QT & QTc (QT corrected for HR) intervals (from the ECG). In addition HR was derived from both pressure signals (left ventricular and arterial) and the ECG. Parameters were logged by the data acquisition system (Ponemah, DSI Inc.) as one minute mean values from complete cardiac cycles. Raw data were then transferred to Excel where they were converted to 15 minute means.

On PK study days blood samples were collected from the jugular or cephalic vein pre-dose and at 1, 2, 4, 6 and 24 hours post-dose for all animals. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of L-NOARG. Although there was a wash-out period of 7 days between each dose, some residual L-NOARG was detected in the pre-dose samples in 3 cases. In addition to the blood samples obtained on the PK study days, additional samples were obtained pre-dose and at 6 hours on the CV study days. A total of 80 concentration points for 4 animals were available.

2.1.1.1.3 Guinea-pig study

The L-NAME guinea-pig study was performed with a semi-parallel design in anaesthetised animals. Male Hartley guinea-pigs (weight: 400-650g), were anaesthetised with pentobarbital 40mg/kg (i.p.). Each animal was then placed on a heating pad, a tracheotomy was performed and the animals ventilated mechanically. The right jugular vein and left carotid artery were cannulated for drug delivery and blood pressure monitoring/blood sampling respectively. In addition, a thoracotomy was performed to allow set-up of electrodes for ECG monitoring and probes for recording monophasic action potential (MAP) signals. Cardiovascular parameters were measured in animals receiving either doses of L-NAME (n=7) or vehicle (n=7). On the same occasion, blood samples were obtained for the determination of plasma concentrations. Multiple ascending doses of L-NAME were administered in a solution of 5% dextrose water via intravenous infusion. Doses were escalated in 4 phases, in which each phase consisted of a loading dose administered as a 5 min infusion followed by a maintenance dose infused over 10 minutes. The dose escalation protocol is described in table 2.2
Table 2.2: Dosing schedule for L-NAME guinea-pig study

<table>
<thead>
<tr>
<th>Study phase</th>
<th>Dose (µg/kg)</th>
<th>Loading (5min)</th>
<th>Maintenance (10min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Cardiovascular parameters were measured continuously for approximately 80 minutes. Drug administration was initiated approximately 20 minutes into recording to allow a stable baseline to be established. Part of the experimental protocol involved a pacing procedure, during which the heart was stimulated to calculate alternans (beat-to-beat variations in monophasic action potential duration (MAPD)). These pacing procedures were repeated twice during baseline and for the last 5 minutes of each maintenance infusion. The cardiovascular parameters measured/derived were HR, SBP, DBP & MBP; MAP duration from the maximum rate of depolarization to 50% and 90% repolarization (MAPD50, MAPD90). ECG data were collected but not reported due to poor signal quality. Parameters were logged by the data acquisition system (Notocord Systems Inc.). Data were then transferred to Excel as one minute mean values from complete cardiac cycles. Data collected during the pacing procedure were removed.

Blood samples were collected 5 minutes into each maintenance infusion. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of both L-NAME and L-NOARG. Concentrations of L-NAME were very low or negligible, and therefore only the L-NOARG concentrations were considered for modelling. A total of 28 concentration points for 7 animals were available.

2.1.1.2 Milrinone

2.1.1.2.1 Rat study

The milrinone rat study was performed with a predominately cross-over design (but with PK in parallel) in conscious animals. Cardiovascular parameters were measured in eight male Sprague Dawley rats receiving single doses of 0.35, 3.5 & 10.5 mg/kg milrinone or vehicle on separate occasions at least 48 hours apart. Individual animals
were dosed according to the schedule described in table 2.3. Plasma concentrations were measured in a satellite group of age and lot-matched animals, pre-cannulated for blood sampling. These satellite animals received a single dose of either 0.35, 3.5 or 10.5 mg/kg milrinone (n=3 for each group). All doses were administered in a 0.5% methylcellulose suspension via oral gavage.

Table 2.3: Dosing schedule for milrinone rat study

<table>
<thead>
<tr>
<th>Study day</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat 1</td>
</tr>
<tr>
<td>1 [0-24h]</td>
<td>0</td>
</tr>
<tr>
<td>3 [48-72h]</td>
<td>10.5</td>
</tr>
<tr>
<td>22 [504-528h]</td>
<td>3.5</td>
</tr>
<tr>
<td>24 [552-576h]</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*Data from all doses for Rat 4 and for the 3.5mg/kg doses for Rats 5 & 7 were excluded due to poor signal quality, therefore n=7 for vehicle, 0.35 & 10.5mg/kg; n=5 for 3.5mg/kg.

Cardiovascular parameters were recorded via pre-implanted telemetry devices (PhysioTel®, DSI, Inc.) in unrestrained animals. Food and water were allowed *ad libitum* and animals were exposed to an artificial 12 hour light-dark cycle running from 6am to 6pm. Doses were administered at 10am with parameters recorded from approximately 1 hour pre-dose to 24 hours post-dose. The cardiovascular parameters measured/derived were HR, SBP, DBP & MBP; QA (a measure of contractility), PR, QRS, QT & RR intervals from the ECG. Parameters were logged by the data acquisition system (Ponemah, DSI Inc.) as mean values from complete cardiac cycles, every 30 seconds. Raw data were then transferred to Excel where they were converted to 15 minute means.

Blood samples were collected from the cannulated rats pre-dose and at 0.25, 0.5, 1, 2, 4, 10.5 and 24 hours following dosing. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of milrinone. The data set was complete for all timings but measurements were below the limit of quantification for 2 animals at 24 hours for the 0.35mg/kg dose. This left a total of 61 concentrations from 9 animals.

2.1.1.2.2 Dog study
The milrinone dog study was performed with a cross-over design in conscious animals. Cardiovascular parameters were measured in four male Beagle dogs (weight: 14.4-18kg) receiving single doses of 0.03, 0.1 & 0.3mg/kg milrinone or vehicle on separate occasions at least 72 hours apart. The same animals also received additional single 0.1mg/kg milrinone on a further occasion, at which time blood samples were collected for determination of plasma concentrations. All doses were administered as a solution in purified water via oral gavage. Individual animals were dosed according to the schedule described in table 2.4

Table 2.4: Dosing schedule for milrinone dog study

<table>
<thead>
<tr>
<th>Study leg</th>
<th>Study day</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dog 1</td>
</tr>
<tr>
<td>PD</td>
<td>1 [0-24h]</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4 [72-96h]</td>
<td>0.3*</td>
</tr>
<tr>
<td></td>
<td>8 [168-192h]</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>11 [240-264h]</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>15 [336-360h]</td>
<td>0.3</td>
</tr>
<tr>
<td>PK</td>
<td>18 [408-432h]</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*On study day 4, dogs 1 and 4 received the wrong treatments; therefore the 2nd treatment for all dogs was repeated on study day 8. Results from study day 8 were then used for Dogs 1, 2 & 4. #Dog 3 was severely ectopic on study day 8; therefore the results from study day 4 were used for this animal.

Cardiovascular parameters were recorded via pre-implanted telemetry devices (Konigsberg Instruments, Inc.) in unrestrained animals. A standard diet of dry food was provided once daily, approximately 6 hours post-dose and water was allowed ad libitum. Animals were exposed to an artificial 12 hour light-dark cycle running from 7am to 7pm. Doses were administered at 9am (±15mins) with parameters recorded from approximately 1 hour pre-dose to 23 hours post-dose. The cardiovascular parameters measured/derived were SBP, DBP & MBP; LVSP, LVEDP, & LV +dP/dt; PR, QRS, QT & QTc intervals. HR was derived from both pressure signals and the ECG. Parameters were logged by the data acquisition system (Ponemah, DSI Inc.) as one minute mean values from complete cardiac cycles. Raw data were then transferred to Excel where they were converted to 15 minute means.

On PK study days blood samples were collected from the cephalic vein pre-dose and at 0.25, 0.5, 1, 1.5, 2, 4, 6 and 24 hours post-dose for all animals. Following separation of
plasma from whole blood, the samples were analysed to obtain plasma concentrations. The data set was complete for all timings but measurements were below the limit of quantification for dog 4 at 4 hours and for all animals at 6 and 24 hours post-dose. This left a total of 23 concentrations from 4 animals.

2.1.1.2.3 Guinea-pig study

The milrinone guinea-pig study was performed with a semi-parallel design in anaesthetised animals. Male Hartley guinea-pigs (weight: 450-750g), were anaesthetised with pentobarbital 40mg/kg (i.p.). The surgical procedures where the same as for the L-NAME study, with the exception of an extra probe inserted into the left ventricle to measure left ventricular pressure. Cardiovascular parameters were measured in animals receiving either doses of milrinone (n=8) or vehicle (n=8). On the same occasion, blood samples were obtained for the determination of plasma concentrations. The dosing protocol was the same as the L-NAME study with the exception of the actual doses administered, which are described in table 2.5. All doses of milrinone were administered as a solution in 0.9% saline via intravenous infusion. Cardiovascular parameters were measured continuously for approximately 120 minutes. Drug administration was initiated approximately 30 minutes into recording to allow a stable baseline to be established. Pacing procedures were followed as in the L-NAME study. The cardiovascular parameters measured/derived were HR, SBP, DBP & MBP; LVSP, LV +dP/dt; MAPD30, MAPD50, MAPD90. ECG data were also collected but not reported. Parameters were logged by the data acquisition system (Ponemah, DSI Inc.). Data were then transferred to excel as one minute mean values from complete cardiac cycles. Data collected during the pacing procedure were removed.

Table 2.5: Dosing schedule for milrinone guinea-pig study

<table>
<thead>
<tr>
<th>Study phase</th>
<th>Dose (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loading (5min)</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>288</td>
</tr>
</tbody>
</table>
Blood samples were collected 5 minutes into each maintenance infusion. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of milrinone. A total of 32 concentration points for 8 animals were available.

2.1.1.3 Doxazosin

2.1.1.3.1 Rat study

The doxazosin rat study was performed with a predominately cross-over design (but with PK in parallel) in conscious animals. Cardiovascular parameters were measured in eight male Wistar Han rats receiving single doses of 1, 10 & 30mg/kg doxazosin or vehicle on separate occasions at least 72 hours apart. Individual animals were dosed according to the schedule described in table 2.6. Plasma concentrations were measured in a satellite group of age and lot-matched animals, pre-cannulated for blood sampling. These satellite animals received a single dose of either 1, 10 or 30mg/kg doxazosin (n=3 for each group). All doses were administered in a 0.5% methylcellulose suspension via oral gavage.

Table 2.6: Dosing schedule for doxazosin rat study

<table>
<thead>
<tr>
<th>Study day</th>
<th>Dose (mg/kg)</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
<th>Rat 5</th>
<th>Rat 6</th>
<th>Rat 7</th>
<th>Rat 8*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 [0-24h]</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>4 [72-96h]</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8 [168-192h]</td>
<td>10</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11 [240-264h]</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1#</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*Rat 8 was found dead during the study and so data from this animal were not analysed. In addition data for Rat 6 at the 1mg/kg dose were excluded due to poor signal quality. Therefore, n=6 for 1mg/kg and n=7 for vehicle and 10 & 30 mg/kg

Cardiovascular parameters were recorded via pre-implanted telemetry devices (PhysioTel®, DSI, Inc.) in unrestrained animals. Food and water were allowed ad libitum and animals were exposed to an artificial 12 hour light-dark cycle running from 6am to 6pm. Doses were administered at 9am with parameters recorded from approximately 1 hour pre-dose to 24 hours post-dose. The cardiovascular parameters measured/derived were HR, SBP, DBP & MBP. Parameters were logged by the data acquisition system (Ponemah, DSI Inc.) as mean values from complete cardiac cycles.
every 30 seconds. Raw data were then transferred to Excel where they were converted to 15 minute means.

Blood samples were collected from the cannulated rats pre-dose and at 0.5, 1, 2, 4, 6 and 24 hours following dosing. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of doxazosin. The data set was complete for all timings but measurements were below the limit of quantification for all 3 animals at 24 hours for the 1mg/kg dose. This left a total of 51 concentrations from 9 animals.

2.1.1.3.2 Dog study

The doxazosin dog study was performed with a cross-over design in conscious animals. Cardiovascular parameters were measured in four male Beagle dogs (weight: 13.8-17.8kg) receiving single doses of 0.1, 0.3 & 1mg/kg doxazosin or vehicle on separate occasions at least 72 hours apart. The same animals also received additional single 0.3mg/kg doxazosin on a further occasion, at which time blood samples were collected for determination of plasma concentrations. All doses were administered as a suspension in 20% PEG 200 via oral gavage. Individual animals were dosed according to the schedule described in table 2.7

<table>
<thead>
<tr>
<th>Study leg</th>
<th>Study day</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dog 1</td>
</tr>
<tr>
<td>PD</td>
<td>1 [0-24h]</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4 [72-96h]</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>8 [168-192h]</td>
<td>0.1*</td>
</tr>
<tr>
<td></td>
<td>11 [240-264h]</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>15 [336-360h]</td>
<td>0</td>
</tr>
<tr>
<td>PK</td>
<td>18 [408-432h]</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*On study day 8, dog 1 experienced episodes of ectopic beats and runs of ventricular tachycardia, which persisted throughout the monitoring period. This arrhythmia affected the CV parameters therefore these data were excluded and the treatment for dog 1 repeated on study day 11.

Cardiovascular parameters were recorded via pre-implanted telemetry devices (Konigsberg Instruments, Inc.) in unrestrained animals. A standard diet of dry food was provided once daily, approximately 6 hours post-dose and water was allowed ad
Animals were exposed to an artificial 12 hour light-dark cycle running from 7am to 7pm. Doses were administered at 9am (±15mins) with parameters recorded from approximately 1 hour pre-dose to 23 hours post-dose. The cardiovascular parameters measured/derived were SBP, DBP, MBP, LVSP, LVEDP, LV +dP/dt; PR, QRS, QT & QTc intervals. HR was derived from both pressure signals and the ECG. Parameters were logged by the data acquisition system (Ponemah, DSI Inc.) as one minute mean values from complete cardiac cycles. Raw data were then transferred to Excel where they were converted to 15 minute means.

On PK study days blood samples were collected from the cephalic vein pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6 and 24 hours post-dose for all animals. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of doxazosin. The data set was complete for all timings but measurements were below the limit of quantification for dog 1 at 0.5 hours and for dogs 2 & 3 at 24 hours post-dose. This left a total of 29 concentrations from 4 animals.

2.1.1.3.3 Guinea-pig study

The doxazosin guinea-pig study was performed with a semi-parallel design in anaesthetised animals. Male Hartley guinea-pigs (weight: 450-700g), were anaesthetised with pentobarbital 40mg/kg (i.p.). The surgical procedures were the same as for the L-NAME study. Cardiovascular parameters were measured in animals receiving either doses of doxazosin (n=8) or vehicle (n=8). On the same occasion, blood samples were obtained for the determination of plasma concentrations. The dosing protocol was the same as the L-NAME study with the exception of the actual doses administered, which are described in table 2.8. All doses of doxazosin were administered as a solution in 30% SBE-β-Cyclodextrin via intravenous infusion.

Table 2.8: Dosing schedule for doxazosin guinea-pig study

<table>
<thead>
<tr>
<th>Study phase</th>
<th>Dose (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loading (5min)</td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
</tr>
</tbody>
</table>
Cardiovascular parameters were measured continuously for approximately 120 minutes. Drug administration was initiated approximately 30 minutes into recording to allow a stable baseline to be established. Pacing procedures were followed as in the L-NAME study. The cardiovascular parameters measured/derived were HR, SBP, DBP & MBP; MAPD30, MAPD50, MAPD90. ECG data were also collected but not reported. Parameters were logged by the data acquisition system (Ponemah, DSI Inc.). Data were then transferred to Excel as one minute mean values from complete cardiac cycles. Data collected during the pacing procedure were removed.

Blood samples were collected 5 minutes into each maintenance infusion. Following separation of plasma from whole blood, the samples were analysed to obtain plasma concentrations of doxazosin. A total of 32 concentration points for 8 animals were available.

2.1.1.4 Study design summaries

Summaries of the study designs for each compound are given in tables 2.9 – 2.11. Plots of the data used are provided in appendix 1.2.

Table 2.9: Summary of L-NAME study designs

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosing</th>
<th>PK data</th>
<th>PD data</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat: Sprague Dawley, Conscious</td>
<td>Single p.o. doses: 10, 30 &amp; 100mg/kg</td>
<td>&lt; 7 samples: pre-dose &amp; 0.5, 1, 2, 4, 8, 24h</td>
<td>10min means: 1h pre-dose to 23h post-dose</td>
<td>Parallel (n=19 PD, n=5 vehicle, n=9 PK)</td>
</tr>
<tr>
<td>Dog: Beagle, Conscious</td>
<td>Single p.o. doses: 10, 20 &amp; 40mg/kg (PK: 10 &amp; 40mg/kg)</td>
<td>6 samples: pre-dose &amp; 1, 2, 4, 6, 24h</td>
<td>15min means: 1h pre-dose to 22h post-dose</td>
<td>Crossover with vehicle &amp; PK legs (n=4)</td>
</tr>
<tr>
<td>Guinea pig: Hartley, Anaesthetised</td>
<td>Multiple 5 &amp; 10min i.v. infusions: 2.5, 0.5: 5, 1; 15, 3; 45, 9mg/kg</td>
<td>4 samples: 10, 25, 40, 55min</td>
<td>1min means: 20min pre-dose to 60min post-dose</td>
<td>Semi-parallel (n=7 PD&amp;PK, n=7 vehicle)</td>
</tr>
</tbody>
</table>
Table 2.10: Summary of milrinone study designs

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosing</th>
<th>PK data</th>
<th>PD data</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat: Sprague Dawley, Conscious</td>
<td>Single p.o. doses: 0.35, 3.5 &amp; 10.5mg/kg</td>
<td>8 samples: pre-dose &amp; 0.25, 0.5, 1, 2, 4, 10.5, 24h</td>
<td>15min means: 1h pre-dose to 24h post-dose</td>
<td>Crossover with vehicle leg (n=7); (PK parallel n=9)</td>
</tr>
<tr>
<td>Dog: Beagle, Conscious</td>
<td>Single p.o. doses: 0.03, 0.1 &amp; 0.3mg/kg (PK: 0.1mg/kg)</td>
<td>9 samples: pre-dose &amp; 0.25, 0.5, 1, 1.5, 2, 4, 6, 24h</td>
<td>15min means: 1h pre-dose to 23h post-dose</td>
<td>Crossover with vehicle &amp; PK legs (n=4)</td>
</tr>
<tr>
<td>Guinea pig: Hartley, Anaesthetised</td>
<td>Multiple 5 &amp; 10min i.v. infusions: 6, 1.5; 18, 4.5; 72, 18; 288, 72µg/kg</td>
<td>4 samples: 10, 25, 40, 55min</td>
<td>1min means: 30min pre-dose to 60min post-dose</td>
<td>Semi-parallel (n=8 PD&amp;PK, n=8 vehicle)</td>
</tr>
</tbody>
</table>

Table 2.11: Summary of doxazosin study designs

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosing</th>
<th>PK data</th>
<th>PD data</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat: Wistar Han, Conscious</td>
<td>Single p.o. doses: 1, 10 &amp; 30mg/kg</td>
<td>7 samples: pre-dose &amp; 0.5, 1, 2, 4, 6, 24h</td>
<td>10min means: 1h pre-dose to 24h post-dose</td>
<td>Crossover with vehicle leg (n=7); (PK parallel n=9)</td>
</tr>
<tr>
<td>Dog: Beagle, Conscious</td>
<td>Single p.o. doses: 0.1, 0.3 &amp; 1mg/kg (PK: 0.3mg/kg)</td>
<td>9 samples: pre-dose &amp; 0.5, 1, 1.5, 2, 3, 4, 6, 24h</td>
<td>15min means: 1h pre-dose to 23h post-dose</td>
<td>Crossover with vehicle &amp; PK legs (n=4)</td>
</tr>
<tr>
<td>Guinea pig: Hartley, Anaesthetised</td>
<td>Multiple 5 &amp; 10min i.v. infusions: 1.5, 0.25; 4.5, 0.75; 18, 3; 72, 12µg/kg</td>
<td>4 samples: 10, 25, 40, 55min</td>
<td>1min means: 30min pre-dose to 60min post-dose</td>
<td>Semi-parallel (n=7 PD&amp;PK, n=7 vehicle)</td>
</tr>
</tbody>
</table>
2.1.2 Data formatting

2.1.2.1 Cardiovascular data

A number of different cardiovascular measures were recorded for each study (see section 2.1.1). The measures of interest for this project were blood pressure and heart rate, thus data on all other measures were ignored.

In some cases heart rate was measured via a number of different means. Where left ventricular pressure had been measured the preference was for values derived from this signal. If not then the values derived from the arterial pressure signal were used. Only in very specific cases where there were issues with the other values were data derived from the ECG recording used. This is because the ECG values are generally deemed less accurate. The only cases where HR derived from the ECG were used were for the high doses of milrinone administered to rats 3 & 6 in that study. The HR values derived from the blood pressure in these cases appeared to fall to extremely low levels during the hour after dosing, which did not fit with the profiles of the other rats or effect seen with other doses. The profile of the HR derived from the ECG however was consistent with the remaining data and so was used instead.

In all studies both systolic and diastolic blood pressure were measured. However, since the compounds appeared to have a similar effect on both measures, it was decided that modelling both sets of data would be unnecessary. Instead mean blood pressure was selected as an appropriate measure. Mean blood pressure was reported for some studies but where it hadn’t been it was calculated according to equation 2.1.

\[ MBP = \frac{2 \cdot DBP + SBP}{3} \]

Equation 2.1

2.1.2.1.1 Time formats

Raw data for the cardiovascular measures were converted to summarised formats within Excel (see section 2.1.1.). For the dog and rat studies each time point was 15 minutes and the corresponding response value was the mean of values for the previous 15 minutes (except L-NAME rat where it was 10 minutes). For guinea-pig studies each time point was 1 minute and the corresponding response value was the mean of values for the previous minute. The heart rate and mean blood pressure data points used for modelling were left in these formats. For rat and dog studies, time was reported in hour units and for guinea-pig studies was reported in minute units. Due to the baseline model
used for the rat (where some parameters had rate units of min\(^{-1}\)) the rat time units were converted from hours to minutes.

2.1.2.2 Plasma concentration data

All dosing information and concentration data were converted from mass to molar units using the relevant molecular weight (table 2.12). The main reason for this was to ensure that as part of the PKPD models the compounds’ potency values would be estimated in molar units. In addition for the L-NAME studies, plasma concentration data were for L-NOARG, whilst the actual compound dosed was L-NAME.

Table 2.12: Molecular weights of study compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>L-NAME</th>
<th>L-NOARG</th>
<th>Milrinone</th>
<th>Doxazosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (g/mol)</td>
<td>269.69</td>
<td>219.2</td>
<td>211.23</td>
<td>451.49</td>
</tr>
</tbody>
</table>

2.1.3 Excluded data

2.1.3.1 Exclusions by the study investigator

For the dog studies, the reports for milrinone and doxazosin stated “that any artifacts (e.g. momentary signal loss from radio interference or occasional spontaneous arrhythmias) were identified, and the data excluded before processing into 15min means”. Despite this, very few points appeared to be removed. For the L-NAME dog study a more strict set of criteria were applied to the data. Any blood pressure measurements below 10mmHg or with a standard deviation above 45mmHg or heart rate measurements below 30bpm or above 200bpm were excluded from further analysis. A number of points were excluded from the 15 minute mean calculations and where this left less than 4 points in 15 minutes, the mean value was also excluded.

For the rat studies, no clear indications of any exclusion criteria were provided, however for L-NAME a small number of the 10 minute time points were missing.

For the guinea-pig studies, data collected during the pacing protocol were excluded in all files provided except for L-NAME, which were subsequently excluded prior to modelling.

Other exclusions of data for specific animals or doses and the reasons are described in section 2.1.
2.1.3.2 Exclusions for modelling purposes

For all dog studies, the animals were fed between 3 and 4pm, which caused an increase in blood pressure and heart rate unrelated to drug effect. Since this was approximately 6-7 hours after dosing, it was decided that removing the data points within this time frame shouldn’t affect the profiles and hence the model estimates in any significant way. Another transient increase in MBP and HR was noted for dogs and rats during the first hour after dosing with either vehicle or active compound. Since this was during a key time in the profile, these data were left in the set and the effect of administration modelled (see sections 2.2.4.3 and 2.2.5.3).

For the rat studies, a number of transient increases in blood pressure and heart rate were noted for many of the animals. These occurred sporadically and did not appear to be related to any reported events. Since most were greater than the levels set by the Pfizer safety pharmacology group as the “magnitude of effect seen as a concern” (±>50bpm for HR ±>10mmHg for MBP), it was decided to remove these points for modelling purposes to ensure they did not influence the results. Examples of profiles with these large transient increases are shown in appendix 1.3.1 and 1.3.2. Data during the “lights off” phase of the rats’ profiles were more variable than the “lights on” phase, due to darkness initiating the rats’ active phase. Any period of lower activity during this phase would cause MBP and HR to fall. Since this was both a normal occurrence and consistent across doses, no data were excluded from this time period.

The heart rate and mean blood pressure data for dog displayed more intra-individual variability than rats and could easily vary up to the “magnitude of effect seen as a concern” (±>20bpm for HR ±>10mmHg for MBP) between time points. Therefore the majority of data were left in the set. The only exceptions were for a couple of dogs in the milrinone study where large increases (>50bpm for HR >15mmHg for MBP) were obviously inconsistent with the rest of the profile. These examples are shown in appendix 1.3.3 and 1.3.4.

Where issues with data were noted by the study investigator, the data were usually removed from the files. However in the case of the L-NAME study in dogs there were issues with an unusually high heart rate for one of the animals, which were noted in the study report but not removed from the files. Since this was an unusual occurrence and may affect the results, data from this animal were excluded from modelling. The figure for heart rate in this animal is shown in appendix 1.3.5.
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There were also a few cases for the rat and guinea-pig where data for some of the individual animals displayed either a lack of response (or potentially a response masked by large intra-individual variability) or an inconsistent response when compared to the other animals. This data caused issues when fitting the models and in some cases led to poor precision of estimates. Data from these animals were therefore also partially or totally excluded. The animals involved were rat 2 from the milrinone study, rat 3 from the doxazosin study, guinea-pig 3 from the L-NAME study and guinea-pig 1 from the doxazosin study.

2.1.4 Plasma protein binding data

According to the free drug hypothesis, only unbound drug can bind to the active site of the protein (e.g. receptor, enzyme etc.) on which it exerts its effects. Therefore unbound plasma drug concentrations were required and were calculated using the fraction of drug unbound in plasma.

The extent to which compounds can bind to plasma proteins varies both between compounds and between species. Values for the fraction of unbound drug in plasma (fu) were therefore required for each animal study. In most cases these values were measured as part of the study but in one case the value had to be obtained from the literature (table 2.13).

Table 2.13: Fraction unbound in plasma values for study compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat</th>
<th>Dog</th>
<th>Guinea-pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NOARG</td>
<td>0.696</td>
<td>0.802</td>
<td>0.844</td>
</tr>
<tr>
<td>Milrinone</td>
<td>0.45</td>
<td>0.185*</td>
<td>0.11</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>0.047#</td>
<td>0.06</td>
<td>0.039</td>
</tr>
</tbody>
</table>

*values for individual animals available *data from Kaye et al. 1986

2.1.5 Human data

Since one of the main aims of the project was to determine if values derived from PKPD modelling of drug effect in pre-clinical species could be used to simulate the effect in humans, human PK and PD data were required for each of the compounds studied. These data were collated from the literature after an extensive search. The ideal studies were placebo controlled, measured mean blood pressure and heart rate responses over 24 hours and plasma drug concentrations. Details of the studies used for each
compound are described in sections 3.5.1.1, 4.5.1.1 and 5.5.1.1. In addition, values for the fraction of unbound drug in human plasma were required. Details are given in sections 3.5.2, 4.5.2 and 5.5.2.

2.2 Modelling approach

2.2.1 Non-linear mixed effects modelling (NLMEM)

Modelling of pharmacokinetic and pharmacodynamic data was undertaken using a non-linear mixed effects approach. Thus, both fixed effects (mean population parameter values) and random effects (inter-individual variability and residual error) were estimated (see section 1.2.2.1).

2.2.1.1 Software

The software package utilised for non-linear mixed effects modelling (NLMEM) was Monolix® (Version 4.0.1). Details of the algorithms used within Monolix are described in section 1.2.2.2. Compared to other software packages used for NLMEM, Monolix is relatively new. The first version was available in February 2005; Version 4.0.1 was released in October 2011. Monolix is also available as a full MATLAB version or a stand-alone version. The former was used with MATLAB® version 7.12.0 (R2011a). Graphics are automatically produced as MATLAB figure files but can be altered, which they were for this thesis.

2.2.2 General model development

Modelling followed the sequential PKPD approach, where as the name suggests PK data were fitted first. The PK parameter estimates obtained from the best fit were then implemented as fixed values in integrated PKPD models. Since baseline MBP and HR responses varied over time, models to describe these temporal changes were also required (see section 2.2.4) and were then incorporated into the integrated PKPD models. Parameter estimates from the best baseline model fits were thus also fixed during the fitting of the PKPD model. Depending on the design of each study, the fixed values for PK and baseline parameters were either individual estimates (where the same animals were used), or population estimates (where different animals were used). In some cases, a few baseline parameters were re-estimated in the PKPD model fitting. Details of which estimates were used and when parameters were re-estimated are specified in sections 3.4, 4.4 and 5.4.
Monolix contains a library of commonly used PK and PD models with various parameterisations. The library was used for simple PK models, however all baseline and integrated PKPD models required new models to be constructed. New structural models can be written using MATLAB code however the use of MLXTRAN, the Monolix scripting language, is recommended and thus was used.

Appropriate models were selected based on assessment of the concentration-time or response-time profiles. In the interests of run time, initial model selection was undertaken without the incorporation of inter-individual variability (i.e. effectively adopting a naive pooled approach). Once the structural model had been defined, inter-individual variabilities for structural parameters were incorporated if they improved the model fit.

For the majority of parameters, inter-individual variability was modelled with an exponential error term (see equation 1.10) i.e. with a log-normal distribution. The exceptions to this were where a slope function was used to describe the change in baseline response (see section 2.2.4.1) or an inhibitory $E_{\text{max}}$ ($I_{\text{max}}$) function was used as part of the PD model (see section 2.2.5.1). Since it was possible for a baseline response to increase or decrease, this meant the slope parameter could be either positive or negative. Since a log-normal distribution does not incorporate negative values, inter-individual variability in this slope parameter was modelled with a normal distribution. Since all PD functions were modelled relative to baseline (see section 2.2.5.2), the $I_{\text{max}}$ parameter could only take values between zero and one (since a value of 1 indicates total inhibition). This required a special distribution with limits of 0 and 1 for which the logit-normal distribution was selected.

With respect to residual error, an additive model was used for all cardiovascular response data (after vehicle or drug administration). For PK data, in the first instance a proportional error model was used. If there were any problems with errors or precision, a combined (proportional plus additive) model or in the last instance an additive model were also assessed.

The default settings for the Monolix algorithms were used in all cases, namely: seed number set to 123456, number of iterations set to automatic, number of Markov chains set to automatic (with a minimum size of 50), simulating annealing selected, and the Monte-Carlo size for log-likelihood set to 20000. Standard errors were calculated via
linearization, individual parameters were conditional mode estimates and log-likelihood was calculated via importance sampling.

2.2.2.1 Model assessment

The most important criteria for selection of the best model fit were as follows:

i. Convergence of the estimation algorithm without errors. Matrix errors are rare with Monolix but do occur occasionally. Other errors are often seen with the use of more advanced features such as inter-occasion variability.

ii. Best goodness of fit statistics. For nested models the likelihood ratio test was applied using the reported -2 log-likelihood (-2LL) values. The difference between -2LL values for nested models is approximately $\chi^2$-squared distributed, thus a significant ($p \leq 0.05$) drop in -2LL is 3.84 for 1 degree of freedom (df; number of additional parameters). For non-nested models, the fit was assessed by the Akaike Information Criterion (AIC), where a lower value indicates a better model fit.

iii. Successful calculation of the standard errors. Issues with calculation of the Fisher information matrix (FIM) can result in standard error values that appear as “NaN” (not a number).

iv. Relative standard errors no greater than 50% for the structural parameter estimates. This ensured an acceptable level of precision and confidence intervals that did not include negative values.

v. Good fitting of the diagnostic plots (observed vs. predicted concentration, weighted residual vs. predicted concentration or time) and the individual fits.

2.2.3 PK modelling

2.2.3.1 Structural PK models

Compartmental PK models parameterised by physiological terms i.e. clearances and volumes, were considered adequate models to describe the plasma concentration-time data. Each model had either one or two-compartment disposition and linear elimination. Where the dose had been administered orally, drug absorption was modelled via either a zero or first order process. As an example, the 2-compartment model with first order absorption and its parameters is shown in figure 2.1
Drug is administered into the depot compartment (representative of the gut), absorbed into the central compartment (representative of the plasma), according to a first order rate constant ($k_a$), distributed between central and peripheral (representative of the tissues) compartments, described by an inter-compartmental clearance term ($Q$), and eliminated from the central compartment, described by a clearance term ($CL$). The terms $V_1$ and $V_2$ are the volumes of distribution of the drug in the central and peripheral compartments respectively.

One-compartment models do not have the peripheral compartment or parameters $Q$ and $V_2$. Models for administration via i.v. infusion do not have the gut compartment (or parameter $k_a$) and drug enters the central compartment directly according to the infusion time. For zero-order absorption, the drug is absorbed into the central compartment at a constant rate and the duration of absorption ($T_{k0}$) is estimated instead of $k_a$. Also to note is that for oral administration, parameters will be confounded by bioavailability ($F$) and therefore are “apparent” parameters i.e. $CL/F$, $V_1/F$, $Q/F$ and $V_2/F$.

In Monolix this series of compartmental models with physiological parameterisation are available in the library of PK models.

For L-NAME administration, an additional PK model was also developed and implemented in Monolix using the MLXTRAN complier. This model was based on the 2-compartment model with 1st order absorption described in figure 2.1 but modified to include an additional central compartment. This was to allow the conversion of L-NAME to L-NOARG to be described via an additional parameter, $k_{met}$. The modified 2-compartment PK model is shown in Figure 2.2.
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Figure 2.2: Schematic of the modified 2-compartment 1st-order absorption model with additional central compartment, defined by a physiological parameterisation.

L-NAME is administered into the depot compartment (representative of the gut) and absorbed into the central compartment (representative of the plasma), according to a first order rate constant (\(k_\text{a}\)). It is then converted to L-NOARG via a first order rate constant (\(k_\text{met}\)). L-NOARG is distributed between central and peripheral (representative of the tissues) compartments, described by an inter-compartmental clearance term (Q), and eliminated from the central compartment, described by a clearance term (CL). The terms \(V_1\) and \(V_2\) are the volumes of distribution of L-NOARG in the central and peripheral compartments respectively.

The model can be described by the differential equations defined in equations 2.2 – 2.5:

\[
\frac{dA_1}{dt} = -k_a \cdot A_1 \quad \text{Equation 2.2}
\]

\[
\frac{dA_2}{dt} = k_a \cdot A_1 - k_\text{met} \cdot A_2 \quad \text{Equation 2.3}
\]

\[
\frac{dA_3}{dt} = k_\text{met} \cdot A_2 - k_{12} \cdot A_3 + k_{21} \cdot A_4 - k_\text{el} \cdot A_3 \quad \text{Equation 2.4}
\]

\[
\frac{dA_4}{dt} = k_{12} \cdot A_3 - k_{21} \cdot A_4 \quad \text{Equation 2.5}
\]

where \(k_{12} = Q/V_1\), \(k_{21} = Q/V_2\) and \(k_\text{el} = CL/V_1\)

\(dA_x/dt\) is the rate of change in drug amount in compartment \(x\), \(A_x\) is the amount of drug in compartment \(x\) (1 = depot, 2 = L-NAME central, 3 = L-NOARG central, 4 = L-NOARG peripheral), \(k_{12}\) and \(k_{21}\) are the first-order rate constants for movement of L-NOARG between its central and peripheral compartments and \(k_\text{el}\) is its elimination rate constant.
2.2.4 Modelling of vehicle data

Baseline response data will often be constant with time, but for a number of biochemical and physiological functions circadian rhythms lead to distinctive diurnal patterns. Physiological functions of the cardiovascular system are affected by circadian rhythms; hence parameters such as blood pressure and heart rate vary periodically over 24 hours. Accurate description of the baseline response is required during fitting of PD models to ensure parameter estimates are accurate and unbiased. Fortunately during the safety pharmacology studies used in the project, MBP and HR response data were obtained during administration of vehicle. For the rat and dog studies circadian rhythms were present since measurements of cardiovascular response were taken over a period of 24 hours. For the guinea-pig studies the rhythm could not be defined during the short (90 minute) time frame but changes in baseline were still present. Models to describe the patterns seen in baseline response were therefore required.

2.2.4.1 Structural baseline models

A literature search for models describing circadian rhythms of blood pressure and heart rate data was performed. The search revealed a study where modelling of circadian rhythms of physiological parameters including MBP and HR had been performed in rats (Sallstrom et al., 2005).

The Sallstrom model is based on a classical model known as the Van der Pol oscillator and consists primarily of 2 coupled differential equations as described in Equations 2.6 and 2.7 (modified from original terms in paper):

\[
\frac{dB1}{dt} = \gamma \cdot (B1 - B2) - B1^3 + g(t) \quad \text{Equation 2.6}
\]

\[
\frac{dB2}{dt} = \delta \cdot (B1 - B2) \quad \text{Equation 2.7}
\]

dBx/dt is the rate of change of the variables B1 or B2 with time, B1 is a variable which is related to the physiological parameter, B2 is a complementary variable with no special physiological meaning, \(\gamma\) and \(\delta\) are first-order rate constants and \(g(t)\) is a timekeeper function that introduces a term to force an increase in the physiological parameter during periods of darkness. Thus, \(g(t) = 0\) during periods of light and \(g(t) = d\) during periods of darkness, where \(d\) is a positive constant that describes the difference between light and dark values.
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$B1$ is a generic function and so is scaled to the actual physiological parameter values using Equation 2.8 (modified from original terms in paper):

$$R_0 = R_{av} \cdot (1 + R_{amp} \cdot B1)$$  \hspace{1cm} \text{Equation 2.8}

$R_0$ is the baseline physiological parameter, $R_{av}$ is an average or reference value for the physiological parameter and $R_{amp}$ is the amplitude i.e. the peak to trough difference in the physiological parameter.

The search did not reveal any specific cardiovascular baseline models for dogs. However, there were studies from the physiology literature where the circadian rhythm of blood pressure and heart rate had been captured using cosine functions. Cosine functions are commonly used for modelling physiological and biochemical parameters displaying circadian rhythms, the classic example of which is cortisol secretion. A single cosine function with a 24 hour period is described in Equation 2.9:

$$R_0 = R_{av} + R_{amp} \cdot \cos \left( \frac{2\pi}{24} (T - T_z) \right)$$  \hspace{1cm} \text{Equation 2.9}

$R_0$, $R_{av}$ and $R_{amp}$ are the same as for the Sallstrom model. $T$ is time and $T_z$ is the acrophase parameter, signifying the time at which maximum MBP/HR occurs.

In many cases a single cosine function is sufficient to describe the circadian rhythm. However, in humans studies a second 12 hour phase has been noted and a dual cosine function with 24 and 12 hours periods required for a good fit (Hempel et al., 1998). This dual cosine model is described in equation 2.10.

$$R_0 = R_{av} + R_{amp24} \cdot \cos \left( \frac{2\pi}{24} (T - T_{z24}) \right) + R_{amp12} \cdot \cos \left( \frac{2\pi}{12} (T - T_{z12}) \right)$$  \hspace{1cm} \text{Equation 2.10}

$R_0$, $R_{av}$ and $T$ are the same as for the single cosine model. $R_{amp24}$ and $R_{amp12}$ are the amplitude parameters, and $T_{z24}$ and $T_{z12}$ are the acrophase parameters, for the 24 hour and 12 hour rhythms respectively.

As previously mentioned the timeframe used for the guinea-pig studies was insufficient to see the circadian rhythm. However there were changes in baseline over the study period. Both increases and decreases with time were observed and the changes were approximately linear. Therefore a linear model as described in equation 2.11 was required.
\[ R_0 = R_{\text{Intercept}} + R_{\text{Slope}} \cdot T \]  

Equation 2.11

\( R_0 \) and \( T \) are the same as for previous baseline models. \( R_{\text{Intercept}} \) is the response at time 0 and \( R_{\text{Slope}} \) is the parameter describing the linear change in response over time.

### 2.2.4.2 Administration effect

As noted in section 2.1.3.2, for the dog and rat studies a transient increase in MBP and HR was observed during the first hour after vehicle administration. Since it was a key time in the profile, this effect of administration was modelled using an empirical function describing the onset and offset of the effect, as explained in Sallstrom et al., (2005) and used by Visser et al., (2006). The administration effect model is described in equation 2.12:

\[ R_{\text{Adm}} = k_{\text{Adm}} \cdot P \cdot [T - T_{T_{\text{Adm}}} - k_{\text{Adm}} \cdot [T - T_{T_{\text{Adm}}}] \]  

Equation 2.12

\( R_{\text{Adm}} \) is the administration effect, \( k_{\text{Adm}} \) is the first-order rate constant determining the appearance and disappearance of the MBP/HR elevation, \( P \) is the magnitude of administration effect, \( T \) is time, and \( T_{T_{\text{Adm}}} \) is the time of vehicle administration.

The administration effect was then applied to the baseline model in a relative manner (equation 2.13).

\[ R_{\text{All}} = R \cdot (1 + R_{\text{Adm}}) \]  

Equation 2.13

\( R_{\text{All}} \) is the total response, incorporating the baseline response \( (R_0) \) and the administration effect \( (R_{\text{Adm}}) \).

All baseline models and the administration effect function were written and implemented in Monolix using the MLXTRAN compiler.

### 2.2.5 PKPD modelling

As mentioned in section 2.2.2, PKPD models incorporating both PK and baseline models were used for the fitting of the MBP and HR response to the 3 study drugs. Different PD models were applied, depending on the nature and time course of the response.
2.2.5.1 Structural PD models

Direct effects were described by the simple $E_{\max}$ model (equation 2.14) or the linear effect model (equation 2.15), for non-linear and linear concentration-effect relationships respectively.

\[ E = \frac{E_{\max} \cdot C_p}{EC_{50} + C_p} \quad \text{Equation 2.14} \]

Where $E$ is effect, $E_{\max}$ is the maximum effect (efficacy), $EC_{50}$ is the concentration of drug that produces half the maximum effect (potency) and $C_p$ is the concentration of drug in plasma.

\[ E = E_{\text{slope}} \cdot C_p \quad \text{Equation 2.15} \]

$E$ and $C_p$ are the same as equation 2.14 and $E_{\text{slope}}$ is the linear change in effect.

When effects were negative, $E_{\max}$, $EC_{50}$ and $E_{\text{slope}}$ parameters were instead termed $I_{\max}$, $IC_{50}$ and $I_{\text{slope}}$ to indicate their inhibitory nature.

Where a delay in the time course of the drug effect relative to the plasma concentration was observed a more complex model was required. Often these delays are assumed to be due to delays in distribution to the site of effect. In this case an effect compartment model is used to determine a hypothetical concentration at the effect site (see section 1.1.2.3). The “effect compartment” concentration is determined by equation 2.16 (a variant of equation 1.6):

\[ \frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad \text{Equation 2.16} \]

$dC_e/dt$ is the rate of change in effect compartment concentration with time, $k_{e0}$ is the first–order rate constant for drug movement into and out of the hypothetical effect compartment, $C_p$ is the concentration of drug in plasma and $C_e$ is the concentration of drug in the hypothetical effect compartment.

Drug effect is then related to the effect compartment concentration via non-linear or linear functions, such as equations 2.17 and 2.18 respectively, which were used in this project.
$$E = \frac{E_{\text{max}} \cdot C_e}{EC_{50} + C_e} \quad \text{Equation 2.17}$$

$$E = E_{\text{slope}} \cdot C_e \quad \text{Equation 2.18}$$

$E$, $E_{\text{max}}$, $EC_{50}$, and $E_{\text{slope}}$ are the same as equations 2.14 and 2.15 and $C_e$ is the concentration of drug in the hypothetical effect compartment. Parameters were again termed $I_{\text{max}}$, $IC_{50}$ and $I_{\text{slope}}$ if the effect was negative.

Another possibility for observing a delay is that the effect is generated via an indirect mechanism. Indirect response models (Daynkea et al., 1993) enable the normal turnover of response to be described and influenced by the drug effect (see section 1.2.2.2 and equation 1.2). Since all 3 study compounds cause their effects via changes to factors affecting the production of the BP/HR response, the model considered for this project contained an effect function affecting $k_{\text{in}}$ (the rate constant for production of response).

The model is shown in Figure 2.3

![Figure 2.3: Schematic of the indirect response model with stimulation/inhibition of the rate of production of the response ($k_{\text{in}}$).](image)

The turnover of the response ($R$) is maintained by $k_{\text{in}}$, the zero order rate constant for its production, and $k_{\text{out}}$ is the first order rate constant for its loss. The stimulatory or inhibitory effect function is applied to $k_{\text{in}}$.

The model can be described by the differential equation defined in Equation 2.19:

$$\frac{dR}{dt} = (k_{\text{in}} \cdot (I \pm E)) - k_{\text{out}} \cdot R \quad \text{Equation 2.19}$$

Where $dR/dt$ is the rate of change of the response with time, $E$ is a function describing the stimulatory (+) or inhibitory (-) effect and $R$, $k_{\text{in}}$ and $k_{\text{out}}$ are as before.
The effect function \( (E) \) can again be linear or non-linear and was applied as described in equations 2.15 and 2.14 respectively. If effect was stimulatory \( E_{\text{max}}, EC_{50} \) and \( E_{\text{slope}} \) terms were used or if inhibitory, terms were \( I_{\text{max}}, IC_{50} \) and \( I_{\text{slope}} \).

### 2.2.5.2 Incorporation of baseline

The structural PD models already relate to plasma concentrations in some form. To complete the integrated PKPD models, structural PD models also needed to be related to the baseline models.

Often for direct or effect compartment models, the effect is simply added or subtracted from baseline however, this results in maximum effect or slope parameters with absolute units. Since MBP and HR ranges vary across species and the PD parameters were to be used for predictive purposes, it was decided to apply the effect model to the baseline in a relative manner, thus resulting in \( E_{\text{max}} / E_{\text{slope}} \) (or \( I_{\text{max}} / I_{\text{slope}} \)) with relative units. Incorporation of direct/effect compartment and baseline models is described by equation 2.20:

\[
R = R_0 \cdot (1 \pm E)
\]  
Equation 2.20

\( R \) is response in the presence of drug, \( R_0 \) is the baseline response (determined by either equation 2.8, 2.9, 2.10 or 2.11) and \( E \) is the effect of the drug (determined by either equation 2.14, 2.15, 2.17 or 2.18).

Integration of the indirect response model with baseline was slightly more complex than for direct/effect compartment models. For the indirect response model, when there is no drug present response is determined by equation 2.21:

\[
\frac{dR_0}{dt} = k_{\text{in}} - k_{\text{out}} \cdot R_0
\]  
Equation 2.21

\( \frac{dR_0}{dt} \) is the rate of change in baseline response with time and \( k_{\text{in}}, k_{\text{out}} \) and \( R_0 \) are as described previously.

Typically when indirect response models are used the baseline is assumed to be constant, thus \( \frac{dR_0}{dt} \) is 0, \( R_0 \) is determined by \( k_{\text{in}}/k_{\text{out}} \) and set as the initial condition of the differential equation. However, as already explained, baseline responses were not constant for the cardiovascular measures studied in this project and were described by specific baseline models. Incorporation of the baseline models therefore required
reparameterisation of the indirect response model. It was assumed that the production of response was responsible for the variation in baseline. Thus equation 2.21 was rearranged to reparameterise $k_{in}$ in terms of the baseline response models, as described in equation 2.22:

$$k_{in} = \frac{dR_0}{dt} + k_{out} \cdot R_0$$  \hspace{1cm} \text{Equation 2.22}$$

Where $R_0$ was determined by the baseline model required for each study (equations 2.8 - 2.11) and $dR_0/dt$ was determined by the differentiation of each baseline model, as described in equations 2.23 – 2.11.

For the Sallstrom model (equation 2.8):

$$\frac{dR_0}{dt} = R_{av} \cdot R_{amp} \cdot \frac{dB1}{dt}$$  \hspace{1cm} \text{Equation 2.23}$$

thus

$$\frac{dR_0}{dt} = R_{av} \cdot R_{amp} \cdot \left(y \cdot (B1 - B2) - B1^3 + g(t) \right)$$  \hspace{1cm} \text{Equation 2.24}$$

For the single cosine model (equation 2.9):

$$\frac{dR_0}{dt} = -R_{amp} \cdot \frac{2\pi}{24} \cdot \sin \left( \frac{2\pi}{24} \cdot (T - T_z) \right)$$  \hspace{1cm} \text{Equation 2.25}$$

For the dual cosine model (equation 2.10):

$$\frac{dR_0}{dt} = -R_{amp24} \cdot \frac{2\pi}{24} \cdot \sin \left( \frac{2\pi}{24} \cdot (T - T_{z24}) \right) - R_{amp12} \cdot \frac{2\pi}{12} \cdot \sin \left( \frac{2\pi}{12} \cdot (T - T_{z12}) \right)$$  \hspace{1cm} \text{Equation 2.26}$$

For the linear model (equation 2.11):

$$\frac{dR_0}{dt} = R_{slope}$$  \hspace{1cm} \text{Equation 2.27}$$

The reparameterisation of $k_{in}$ was then incorporated into the indirect model, resulting in equation 2.28:
\[
\frac{dR}{dt} = \left( \frac{dR_0}{dt} + k_{out} \cdot R_0 \right) \cdot (I \pm E) - k_{out} \cdot R \quad \text{Equation 2.28}
\]

Where \( E \) is defined by either equation 2.14 or 2.15, \( R_0 \) is defined by either equation 2.8, 2.9, 2.10 or 2.11 and \( dR_0/dt \) is defined by either equation 2.24, 2.25, 2.26 or 2.27.

### 2.2.5.3 Incorporation of administration effect

The transient increase in MBP/HR was also present after dosing dog and rats with active compound. The administration effect was therefore applied to drug response in the same way as to baseline (equation 2.29) to allow the parameters derived from the baseline response to be fixed if required.

\[
R_{All} = R \cdot (I + R_{Adm}) \quad \text{Equation 2.29}
\]

\( R_{All} \) is the total response, incorporating the response in the presence of drug (\( R \)) and the administration effect (\( R_{Adm} \)).

All integrated PKPD models were written and implemented in Monolix using the MLXTRAN compiler.

### 2.2.6 Initial estimates and conditions

Initial estimates of the structural PK parameters were obtained through a combination of curve stripping or non-compartment methods, literature values and visual inspection. Monolix has a useful feature that allows the user to check visually the fit that the initial estimates give to the data. Initial estimates obtained through other means were therefore adjusted to give the best visual fit.

Initial values for the Sallstrom model parameters \( \gamma, \delta, \) and \( d \) were set as the parameter estimates derived in the Sallstrom study. Initial values for all other baseline model parameters and for PD model parameters were estimated via visual inspection of the data and the Monolix “check initial estimates” feature.

Initial estimates for the variance of the structural parameters (inter-individual variability) were set as 0.25 in all cases. Initial estimates of the residual error variance were left as the default values of 0.3 for proportional error and 1 for additional error.

Initial conditions for most differential equations were left as the default value of 0. The data file was used to set the initial conditions for the appropriate dosing compartment in
the PK models. In addition the data file was also used for the indirect response differential equation (equation 2.28), where the response at time 0 was set as the initial condition for each individual. Initial conditions for the Sallstrom model differentials (equations 2.6 and 2.7) were determined via simulation in MATLAB using the parameter estimates from the Sallstrom study. Simulation of different initial conditions indicated that values of -0.5 and 1 (for equations 2.6 and 2.7 respectively) were required to recover the profiles seen in the Sallstrom study.

2.3 Prediction of human cardiovascular response

The prediction of human MBP/HR responses to the 3 study compounds were performed via simulation in MATLAB version 7.12.0 (R2011a). Scripts for each compound’s predictions were based on the PKPD models derived from the fitting of each species’ safety pharmacology data. Parameter values for PK and baseline were determined via fitting of human data. Values for the PD parameters were set as the population estimates for each species in turn. Where parameters were not specifically related to the drug but to the cardiovascular response (i.e. $k_{out}$), the animal data were scaled to human equivalents using the single species scaling approach (see section 1.2.1). All study design features (i.e. dosing, route of administration etc.) were set according to the human study. Further details of the predictions for each compound are given in sections 3.5, 4.5 and 5.5.
Chapter 3

L-NAME
3 N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME)

3.1 Introduction

L-NAME is a nitric oxide synthase (NOS) inhibitor. NOS is the enzyme that catalyses the oxidation of L-arginine to citrulline and nitric oxide (NO). It exists in three isoforms, one of which (eNOS) is present in vascular endothelium. NO released from vascular endothelial cells acts as a vasodilator in the regulation of blood pressure (Griffith et al., 1996). The mechanism for this is via stimulation of soluble guanylyl cyclase (sGC) and consequent accumulation of cyclic GMP (Pfeiffer et al., 1996). Cyclic GMP (cGMP) is involved in smooth muscle relaxation via multiple mechanisms believed to be: stimulation of a protein kinase that activates myosin light chain phosphatase (MLCP), activation of potassium (K\textsuperscript{+}) channels and inhibition of calcium entry into the cell. Inhibition of NOS disrupts this sequence of events and consequently the normal regulation of blood pressure. Thus, L-NAME causes vasoconstriction and leads to an increase in blood pressure. This in turn activates baroreceptor reflexes, which leads to a decrease in heart rate mediated by the autonomic nervous system.

There are no therapeutic uses for L-NAME, but it has been investigated as a treatment for septic shock, which is often characterised by extensive systemic vasodilation due to overproduction of NO (Avontuur et al., 1998).

*In vitro* studies have shown that L-NAME inhibits NO synthesis by eNOS (Rees et al., 1990). It was subsequently discovered that the inhibitory effects were actually due to its metabolite, N\textsuperscript{G}-nitro-L-arginine (L-NOARG; Pfeiffer et al., 1996). L-NAME is metabolised via hydrolysis to form L-NOARG and methanol (Brouillet et al., 1995). The hydrolysis occurs rapidly in plasma, but has also been shown to occur slowly in buffer (Pfeiffer et al., 1996), which indicates the process is mediated both via plasma esterases and non-enzymatically.

Even though the effects of L-NAME are known to be mediated by L-NOARG, it is still commonly studied as it is far more soluble (Griffith et al., 1996). *In vivo* studies of both compounds have been performed predominantly in rats, where PD studies have shown significant increases in mean arterial blood pressure (MBP) and smaller decreases in heart rate (HR) following administration of L-NAME or L-NOARG (Gardiner et al., 1990; Wang et al., 1995; Wang et al., 1991; Wang et al., 1993). Decreases in cardiac output (CO), stroke volume (SV) and ventricular contractility have also been observed.
(Gardiner et al., 1990). Following administration of L-NAME in humans, an increase in MBP was observed in patients with septic shock (Avontuur et al., 1998) and an increase in blood pressure (BP) and decrease in HR were observed in healthy volunteers (Frandsen et al., 2001).

The PK of L-NOARG has been investigated in a number of rat studies (Piotrovskij et al., 1993; Tabrizi-Fard et al., 1996; Tabrizi-Fard et al., 1994). Results appear consistent, profiles are biphasic and terminal half-life ($t_{1/2}$) is long due to both a low clearance and extensive distribution. Distribution to muscle, liver and kidney appears particularly significant (Tabrizi-Fard et al., 1996) but there is no indication of how the drug is eliminated. PK analysis of L-NOARG concentrations in patients with septic shock has produced values consistent with rat studies (Avontuur et al., 1998).

### 3.2 Pharmacokinetic modelling of L-NAME

#### 3.2.1 Rat study

3.2.1.1 PK model development and refinement

Visual inspection of the individual L-NOARG plasma concentration-time profiles indicated that either a 1 or 2 compartment model could be a suitable fit. Initially, 1 and 2 compartment models with 1st order absorption were investigated, followed by the 2 compartment model modified for the conversion of L-NAME to L-NOARG. No significant drop in the log-likelihood was seen for either 2-compartment model (table 3.1). Model refinement continued with the 1 compartment model due to extremely poor precision of the parameter estimates (RSE=$3\times10^4$-$1\times10^7\%$). Addition of inter-individual variability (IIV) on all 3 structural parameters increased the precision of the estimates but did not reduce the -2LL value significantly. Poor precision and high shrinkage were observed for the CL/F and V/F omega, thus a model with IIV on $k_a$ only was assessed. No significant drop in log-likelihood was observed for this model either and the precision for $k_a$ was still reasonably poor (RSE=$65\%$). Use of a combined residual error model as opposed to proportional residual did not improve the fit as the additional error term was close to zero. Since $k_a$ could not be accurately predicted with any of the 1st order absorption models, a zero-order absorption model was selected. The basic zero-order model with no IIV did not show any improvement compared with the 1st order model. However, inclusion of IIV on absorption duration ($T_{k0}$) produced a significant drop in log-likelihood (-4.69, 1df) and acceptable precision for all parameters.
Table 3.1: Overview of model selection for the rat L-NAME PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt; 1abs</td>
<td>221.59</td>
<td>229.59</td>
<td>-</td>
<td>Very high SE all</td>
</tr>
<tr>
<td>2cpt; 1abs</td>
<td>221.33</td>
<td>233.33</td>
<td>N</td>
<td>Very high SE all</td>
</tr>
<tr>
<td>2cpt; modified</td>
<td>221.20</td>
<td>235.20</td>
<td>N</td>
<td>High SE $k_a$, $k_{\text{met}}$</td>
</tr>
<tr>
<td>1cpt; 1abs; $\eta$ all</td>
<td>219.34</td>
<td>233.34</td>
<td>N</td>
<td>High SE $k_a$; very high SE &amp; shrinkage $\omega_{\text{CL/F, V/F}}$</td>
</tr>
<tr>
<td>1cpt; 1abs; $k_a$</td>
<td>218.47</td>
<td>228.47</td>
<td>N</td>
<td>High SE $k_a$</td>
</tr>
<tr>
<td>1cpt; 1abs; Comb err</td>
<td>221.59</td>
<td>231.59</td>
<td>N</td>
<td>Warning on matrix; implausible $k_a$; very high SE $k_a$</td>
</tr>
<tr>
<td>1cpt; 0abs</td>
<td>221.59</td>
<td>229.59</td>
<td>-</td>
<td>Same AIC 1abs; very high SE all</td>
</tr>
<tr>
<td>2cpt; 0abs</td>
<td>226.82</td>
<td>238.82</td>
<td>N</td>
<td>Very high SE all</td>
</tr>
<tr>
<td>1cpt; 0abs; $\eta$ all</td>
<td>218.07</td>
<td>232.07</td>
<td>N</td>
<td>Very high SE &amp; shrinkage $\omega_{\text{CL/F, V/F}}$</td>
</tr>
<tr>
<td>1cpt; 0abs; $T_{k0}$</td>
<td><strong>216.90</strong></td>
<td><strong>226.9</strong></td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

* Significant drop in -2LL compared to previously best nested model; Y = Yes, N = No

3.2.1.2 Final PK model fit

The final PK model selected for the rat L-NAME PK data was a 1-compartment, zero-order absorption model with inter-individual variability on $T_{k0}$ and proportional residual error. Parameter estimates for the final model are presented in table 3.2. Structural parameters were predicted with good to moderate precision (RSE=9-34%). Inter-individual variability in $T_{k0}$ was high at 82%. Additive residual error was also reasonably high but this was common across models assessed.

Table 3.2: Parameter estimates for the final rat L-NAME PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{k0}$ (h)</td>
<td>0.678</td>
<td>34</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>0.281</td>
<td>9</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>3.89</td>
<td>13</td>
</tr>
<tr>
<td>Inter-individual variability in $T_{k0}$ (%)^b</td>
<td>82</td>
<td>61</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>42.3</td>
<td>14</td>
</tr>
</tbody>
</table>

^a Relative standard error (RSE), expressed as a percentage is calculated as SE divided by the parameter estimate x 100

^b Inter-individual variability is taken as an approximate CV (%), calculated as the square root of variance x 100
Chapter 3: L-NAME

Figure 3.1: Observed versus predicted plasma concentrations for the final rat L-NAME PK model using population (A) or individual (B) parameters.

Figure 3.2: Population weighted residuals (PWRES) versus time (A) or predicted concentration (B) for the final rat L-NAME PK model.

Diagnostic plots (figures 3.1 and 3.2) for the final model do show some tendency for over-prediction at higher concentrations. In the case of the population model, this was the case for all models analysed. Individual plots of predicted concentration-time profiles (Figure 3.3) appear to show the largest over-predictions occur for the 4h time point for the top doses (individuals 7&9). These individual predictions could be improved with the use of a 2 compartment model with IIV and the trend for over-prediction removed. If the rats used to derive plasma concentrations had been the same animals used for determination of effect this model may have been considered. However, since these concentrations were derived from a satellite group of animals, the population estimates were used in the subsequent PKPD models so the simpler model was selected. Weighted residuals show an even distribution around zero with time but the under-prediction of higher doses can be noted again with predicted concentration. Despite this, the majority of residuals fell within ±2 standard deviations.
3.2.2 Dog study

3.2.2.1 PK model development and refinement

The observed L-NOARG concentrations displayed a biphasic profile for all 4 animals, suggesting a 2-compartment model would be the most suitable fit to the data. To confirm this, 1- and 2-compartment models with 1st order absorption were initially analysed with a proportional error model. In both cases Monolix produced a warning regarding the variance matrix indicating that results may not be accurate. The warning was removed with the use of a combined residual error model which was used in subsequent modelling. A significant drop in the log-likelihood (-24.61, 2df) confirmed that the 2-compartment model was superior to the 1-compartment model, but was not further improved by the modified model with an additional central compartment (table 3.3). The $k_a$ value produced by the 2-compartment was deemed an implausible value ($4 \times 10^{47}$) and was also highly imprecise (RSE=$3 \times 10^{14}$%). The addition of inter-individual variability to all structural parameters produced a significant drop in the -2LL value (-16.83, 5df) and did improve the estimate to a more reasonable value (28.8) but
was still very imprecise (RSE=3x10^3%). Removal of etas from all parameters except Q/F improved the model further but the precision of $k_a$ was still deemed much too poor (RSE=938%).

Table 3.3: Overview of model selection for the dog L-NAME PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt; 1abs; Prop err</td>
<td>1432.7</td>
<td>1440.7</td>
<td>-</td>
<td>Warning on matrix; implausible estimates; very high SE $k_a$, CL/F</td>
</tr>
<tr>
<td>1cpt; 1abs; Comb err</td>
<td>372.65</td>
<td>382.65</td>
<td>Y</td>
<td>Implausible $k_a$; very high SE all</td>
</tr>
<tr>
<td>2cpt; 1abs; Comb err</td>
<td>348.04</td>
<td>362.04</td>
<td>Y</td>
<td>Implausible $k_a$; very high SE $k_a$</td>
</tr>
<tr>
<td>2cpt; modified; C err</td>
<td>350.04</td>
<td>366.04</td>
<td>N</td>
<td>Implausible $k_a$ &amp; $k_{met}$</td>
</tr>
<tr>
<td>2cpt; 1abs; C err; $\eta$ all</td>
<td>331.21</td>
<td>355.21</td>
<td>Y</td>
<td>Very high SE $k_a$; very high SE &amp; shrinkage $\omega$ CL/F, $V_1/F$, $V_2/F$</td>
</tr>
<tr>
<td>2cpt; 1abs; C err; $\eta$ Q/F</td>
<td>330.97</td>
<td>346.97</td>
<td>Y</td>
<td>Very high SE $k_a$</td>
</tr>
<tr>
<td>2cpt; 0abs; Comb err</td>
<td>342.18</td>
<td>356.18</td>
<td>-</td>
<td>Lower AIC than 1abs</td>
</tr>
<tr>
<td>2cpt; 0abs; C err; $\eta$ all</td>
<td>331.09</td>
<td>355.09</td>
<td>Y</td>
<td>Very high SE $T_{k0}$; very high SE &amp; shrinkage $\omega$ CL/F, $V_1/F$, $V_2/F$</td>
</tr>
<tr>
<td><strong>2cpt; 0abs; C err; $\eta$ Q/F</strong></td>
<td><strong>331.01</strong></td>
<td><strong>347.01</strong></td>
<td>Y</td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

As for the rat data, a zero-order absorption model was analysed. This resulted in a better fit as deemed by the Aikake Information Criterion (AIC) and had plausible and precise estimates for all parameters. Refinement of this model with the addition of etas for all structural parameters, resulted in a significant drop in log-likelihood (-11.09, 5df) but greatly reduced the precision of the $T_{k0}$ estimate (RSE=10^3%). Removal of etas for all structural parameters except Q/F resulted in a model which was still significantly better in terms of log-likelihood (-11.17, 1df) and had acceptable precision for all parameters, thus was selected as the final model.

3.2.2.2 Final PK model fit

The final PK model selected for the dog L-NAME PK data was a 2-compartment, zero-order absorption model with inter-individual variability on Q/F and combined residual error. Parameter estimates for the final model are presented in table 3.4 and the individual estimates of Q/F presented in table 3.5. Most structural parameters were predicted with good precision (RSE=9-11%). The population estimate of Q/F was predicted with moderate precision (RSE=45%) and inter-individual variability in Q/F was high at 80%. Residual errors could be considered relatively low.
Table 3.4: Parameter estimates for the final dog L-NAME PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{k0}$ (h)</td>
<td>1.00</td>
<td>11</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>0.0455</td>
<td>5</td>
</tr>
<tr>
<td>$V_1/F$ (L/kg)</td>
<td>0.816</td>
<td>11</td>
</tr>
<tr>
<td>Q/F (L/h/kg)</td>
<td>0.149</td>
<td>45</td>
</tr>
<tr>
<td>$V_2/F$ (L/kg)</td>
<td>1.37</td>
<td>11</td>
</tr>
<tr>
<td>Inter-individual variability in Q/F (%)</td>
<td>80</td>
<td>78</td>
</tr>
<tr>
<td>Additive residual error (µM)</td>
<td>1.23</td>
<td>21</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>17.9</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 3.4: Observed versus predicted plasma concentrations for the final dog L-NAME PK model using population (A) or individual (B) parameters.

Table 3.5: Individual Q/F estimates for the final dog L-NAME PK model

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q/F (L/h/kg)</td>
<td>0.089</td>
<td>0.138</td>
<td>0.0879</td>
</tr>
</tbody>
</table>

The diagnostic plots for this model (figures 3.4 and 3.5) show a good fit to the data for both the population and individual models. The scatter plots of predicted against observed concentrations display an even distribution around the line of unity with little deviation. Weighted residuals are evenly distributed around zero, with no apparent pattern and the majority within ±2 standard deviations.
Figure 3.5: Population weighted residuals (PWRES) versus time (A) or predicted concentration (B) for the final dog L-NAME PK model.

Plots of individual predicted concentration-time profiles give a good description of the observed data for both the 10mg/kg and 40mg/kg doses (figure 3.6).

Figure 3.6: Individual plots of observed (circles) and predicted (line) L-NOARG concentrations with time following a 10 (LD) or 40 (HD) mg/kg dose of L-NAME.

### 3.2.3 Guinea-pig study

3.2.3.1 PK model development and refinement

With the design of the guinea-pig study, only 1 concentration per dose was measured. Since these were the same timings for each animal, there was not enough information to describe a full PK profile of L-NOARG. In this scenario a 1-compartment model had to be assumed. A 1-compartment model with proportional error was able to describe the data sufficiently but the structural parameters were estimated with very poor precision (RSE=3x10^{-5}-1x10^{-7}%). Although the addition of inter-individual variability on both

80
structural parameters resulted in a significant reduction in the -2LL value (-15.55, 2df; table 3.6) and precise structural parameter estimates, the omega for CL was small, imprecise and displayed high shrinkage. Removal of the eta for CL still produced a significant drop in log-likelihood from the basic model (-16.29, 1df) and had no issues.

Table 3.6: Overview of model selection for the guinea-pig L-NAME PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt</td>
<td>225.92</td>
<td>231.92</td>
<td></td>
<td>Very high SE CL &amp; V</td>
</tr>
<tr>
<td>1cpt; η all</td>
<td>210.37</td>
<td>220.37</td>
<td>Y</td>
<td>High SE &amp; shrinkage η CL</td>
</tr>
<tr>
<td>1cpt; η V</td>
<td>209.63</td>
<td>217.63</td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

3.2.3.2 Final PK model fit

The final PK model selected for the guinea-pig L-NAME PK data was a 1-compartment model with inter-individual variability on V and proportional residual error. Parameter estimates for the final model are presented in table 3.7 and the individual estimates of V presented in table 3.8. The structural parameters were predicted with good to moderate precision (RSE=9-27%) and inter-individual variability in V was moderately high at 60%. Proportional residual error could be considered relatively low.

Table 3.7: Parameter estimates for the final guinea-pig L-NAME PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/min/kg)</td>
<td>0.0371</td>
<td>11</td>
</tr>
<tr>
<td>V (L/kg)</td>
<td>0.744</td>
<td>27</td>
</tr>
<tr>
<td>Inter-individual variability in V (%)</td>
<td>60</td>
<td>62</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>18.4</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 3.8: Individual estimates of V for the final guinea-pig L-NAME PK model

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP1</th>
<th>GP2</th>
<th>GP3</th>
<th>GP4</th>
<th>GP5</th>
<th>GP6</th>
<th>GP7</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (L/kg)</td>
<td>1.00</td>
<td>0.461</td>
<td>1.01</td>
<td>0.952</td>
<td>0.291</td>
<td>0.875</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Plots of individual predicted concentration-time profiles give a good description of the observed data for all animals (figure 3.7).
Figure 3.7: Individual plots of observed (grey circles) and predicted (dashed line) L-NOARG concentrations with time following multiple doses of L-NAME.

The diagnostic plots for this model (figures 3.8 and 3.9) show a good fit to the data for both the population and individual models. The scatter plots of predicted against observed concentrations display an even distribution around the line of unity with some deviation for the population predicted values but little for the individual values.

Figure 3.8: Observed versus predicted plasma concentrations for the final guinea-pig L-NAME PK model using population (A) or individual (B) parameters.

Weighted residuals are evenly distributed around zero, with no apparent pattern and all except 1 within ±2 standard deviations.
3.3 Modelling of baseline cardiovascular response

3.3.1 Rat study

Mean blood pressure and heart rate data for the rat displayed an apparent circadian rhythm over the 24 hour period of measurement. More specifically, the profiles exhibited lower values during daytime and higher values during the night with sharp increases/decreases at time points coinciding with changes in lighting. These were features consistent with that of the biorhythm model developed by Sallstrom et al. (2005), in rats kept under similar conditions to those studied as part of this project.

For the L-NAME rat study vehicle data was measured in different animals to those dosed with active compound. Visual inspection of the data appeared to show inconsistencies with respect to the time frame over which the measurements had been taken for vehicle treated animals versus dosed animals. Due to the lack of a report for this study, no information was available about any potential differences in timing, with the files simply indicating a dosing time of 7.30am. Vehicle data from the L-NAME rat study was compared with that from the studies of the other 2 compounds, which had administration times of 9am and 10am. This showed that vehicle data from the L-NAME study appeared to be consistent with an administration time of 10am, since the increase in HR/MBP associated with the lights going off was approximately 8 hours after administration, which was known to be 6pm. The dosing time of 7.30am appeared to be valid for the L-NAME dosed rats, since the increases in HR/MBP did not occur until approximately 10.5 hours after administration.
Another feature that appeared to be lacking in the L-NAME study vehicle treated rats, but present in those from the other studies and the L-NAME dosed rats, was a spike in MBP/HR associated with administration. It was felt it was important to characterise this effect as part of the baseline to ensure it didn’t confound the parameter estimates for the pharmacodynamic model.

Due to the uncertainty surrounding the vehicle data for the L-NAME study and to the small numbers of animals used, it was decided to model the vehicle data from all 3 studies together. The Sallstrom model was selected for this as it described the rat profile well and had the advantage of taking into account the timings at which lighting conditions were changed. This meant population estimates for the baseline parameters could then be used in the L-NAME PD model. Dosing times were set to the relevant values for each study (and individual where available) and the administration effect modelled with an appropriate function (see section 2.2.4.2).

### 3.3.1.1 Mean Blood Pressure

#### 3.3.1.1.1 Model selection

The changes in rat mean blood pressure baseline data with time was suitably described by the Sallstrom model with the addition of an administration effect and additive residual error. Initial fitting of the model without inter-individual variability resulted in poor precision (RSE>50%) for most of the parameters. Addition of inter-individual variability to all structural parameters produced a significant drop in log-likelihood (-2247.79, 7df; table 3.9) and improved the precision to acceptable values. However the precision of the variability was poor (RSE=65-94%) and shrinkage high (63-82%) for the parameters D, R<sub>amp</sub> and k<sub>Adm</sub>.

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sallstrom + Adm, no η</td>
<td>12995.31</td>
<td>13011.31</td>
<td>-</td>
<td>High SE for γ, δ, D, P</td>
</tr>
<tr>
<td>Sallstrom + Adm, all η</td>
<td>10747.52</td>
<td>10777.52</td>
<td>Y</td>
<td>High SE &amp; shrinkage ω D, R&lt;sub&gt;amp&lt;/sub&gt;, k&lt;sub&gt;Adm&lt;/sub&gt;</td>
</tr>
<tr>
<td>Sallstrom + Adm, no η D</td>
<td>10745.5</td>
<td>10773.5</td>
<td>Y</td>
<td>High SE &amp; shrinkage ω P, R&lt;sub&gt;amp&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>Sallstrom + Adm, no η D, R&lt;sub&gt;amp&lt;/sub&gt;</strong></td>
<td><strong>10745.86</strong></td>
<td><strong>10771.86</strong></td>
<td><strong>Y</strong></td>
<td>High SE &amp; shrinkage ω P, <strong>FINAL MODEL</strong></td>
</tr>
<tr>
<td>Sallstrom + Adm, no η D, R&lt;sub&gt;amp&lt;/sub&gt;, P</td>
<td>10792.44</td>
<td>10816.44</td>
<td>Y</td>
<td>Estimate for P too large</td>
</tr>
</tbody>
</table>
Removal of the eta with the poorest precision and highest shrinkage (parameter D) resulted in an improved model with respect to the fit statistics and the precision of $k_{Adm}$ eta. However the shrinkage for the $R_{amp}$ eta increased and a very poor precision (RSE=345%) and high level of shrinkage (96%) was seen for the eta on parameter P. Removal of the $R_{amp}$ improved the model further with a similar -2LL value but 1 less parameter, but did not improve the precision and shrinkage observed on P. Removal of the inter-individual variability for P resulted in an over-prediction of the administration effect for all individuals. In addition, the fit statistics still showed the model with eta on P to be significantly better (-46.58, 1d.f.), thus was chosen as the final model.

3.3.1.1.2 Final baseline model fit

The final rat MBP baseline model was selected as the Sallstrom model with administration effect, inter-individual variability on $R_{av}$, gamma, delta and P, and additive residual error. Parameter estimates (table 3.10) were predicted with good precision (RSE=2-31%). Inter-individual variability was low to moderate for most parameters (8-67%) but high for gamma (114%). Additive residual error was low indicating the majority of the observed variability had been accounted for.

Table 3.10: Parameter estimates for the final rat MBP baseline model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (mmHg)</td>
<td>105</td>
<td>2</td>
</tr>
<tr>
<td>$R_{amp}$</td>
<td>0.109</td>
<td>6</td>
</tr>
<tr>
<td>$\gamma$ (min^{-1})</td>
<td>0.0521</td>
<td>31</td>
</tr>
<tr>
<td>$\delta$ (min^{-1})</td>
<td>0.0023</td>
<td>18</td>
</tr>
<tr>
<td>D</td>
<td>0.0418</td>
<td>19</td>
</tr>
<tr>
<td>P</td>
<td>0.509</td>
<td>9</td>
</tr>
<tr>
<td>$k_{Adm}$ (min^{-1})</td>
<td>0.118</td>
<td>18</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{av}$ (%)</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>Inter-individual variability in $\gamma$ (%)</td>
<td>114</td>
<td>39</td>
</tr>
<tr>
<td>Inter-individual variability in $\delta$ (%)</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>Inter-individual variability in P (%)</td>
<td>12</td>
<td>243*</td>
</tr>
<tr>
<td>Inter-individual variability in $k_{Adm}$ (%)</td>
<td>67</td>
<td>41</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>5.17</td>
<td>2</td>
</tr>
</tbody>
</table>

\*High SE but still significantly better model and fits with IIV in P included
The diagnostic plots for this model (figures 3.10 and 3.11) show a good fit to the data. The scatter plots of predicted against observed mean blood pressure display an even distribution around the line of unity. Weighted residuals are evenly distributed around zero. The majority of points fall within ±2 standard deviations, although it appears that more fall outside this range after 500 minutes. This can be explained by the higher level of variability observed during the rats “active phase” i.e. when the lights were off.

Figure 3.10: Observed versus predicted mean blood pressure for the final rat MBP baseline model using population (A) or individual (B) parameters.

![Observed versus predicted mean blood pressure](image)

Figure 3.11: Population weighted residuals (PWRES) versus time (A) or predicted mean blood pressure (B) for the final rat MBP baseline model.

![Population weighted residuals](image)

Plots of individual predicted mean blood pressure-time profiles for the 5 animals administered vehicle as part of the L-NAME study are shown in figure 3.12. The model gives a good description of the observed data for these animals, although it can be noted that the majority do not display the administration effect seen in other rats. This was not an issue since population parameter estimates of the baseline were taken forward to the PD modelling of L-NAME effect on MBP.
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3.3.1.2 Heart Rate

3.3.1.2.1 Model selection

As for mean blood pressure, the changes in rat heart rate baseline data with time was suitably described by the Sallstrom model with the addition of an administration effect. The model was initially fitted without inter-individual variability and additive residual error. Addition of inter-individual variability to all structural parameters produced a significant drop in log-likelihood (-1350.6, 7d.f.) but poor precision (RSE=77%) and high shrinkage (87%) was observed for the $R_{\text{amp}}$ variability. Removal of the eta on $R_{\text{amp}}$ resulted in a virtually identical log-likelihood value (table 3.11) for a model with one less parameter, thus indicating a better model. Removal of any additional etas did not improve the model further.

Table 3.11: Overview of model selection for the rat HR baseline data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sallstrom + Adm, no $\eta$</td>
<td>17649.82</td>
<td>17665.82</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sallstrom + Adm, all $\eta$</td>
<td>16299.22</td>
<td>16329.22</td>
<td>Y</td>
<td>High SE &amp; shrinkage $\omega R_{\text{amp}}$</td>
</tr>
<tr>
<td><strong>Sallstrom + Adm, no $\eta$</strong> $R_{\text{amp}}$</td>
<td><strong>16299.31</strong></td>
<td><strong>16327.31</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

3.3.1.2.2 Final baseline model fit
The final rat HR baseline model was selected as the Sallstrom model with administration effect, inter-individual variability on $R_{av}$, gamma, delta, D, P and $k_{Adm}$, and additive residual error. Parameter estimates (table 3.12) were predicted with very good precision (RSE=2-22%). Inter-individual variability was low for most parameters (7-30%) but moderate to high for P and D (59-82%). This indicated the extent of the increase in heart rate due to administration and to darkness varied the greatest between individual animals. The additive residual error of 24.2bpm was low when compared with average HR (6.5%).

Table 3.12: Parameter estimates for the final rat HR baseline model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (bpm)</td>
<td>370</td>
<td>2</td>
</tr>
<tr>
<td>$R_{amp}$</td>
<td>0.266</td>
<td>2</td>
</tr>
<tr>
<td>$\gamma$ (min$^{-1}$)</td>
<td>0.126</td>
<td>8</td>
</tr>
<tr>
<td>$\delta$ (min$^{-1}$)</td>
<td>0.0032</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>0.0215</td>
<td>22</td>
</tr>
<tr>
<td>P</td>
<td>0.733</td>
<td>16</td>
</tr>
<tr>
<td>$k_{Adm}$ (min$^{-1}$)</td>
<td>0.0743</td>
<td>10</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{av}$ (%)</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>Inter-individual variability in $\gamma$ (%)</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td>Inter-individual variability in $\delta$ (%)</td>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td>Inter-individual variability in D (%)</td>
<td>82</td>
<td>41</td>
</tr>
<tr>
<td>Inter-individual variability in P (%)</td>
<td>59</td>
<td>41</td>
</tr>
<tr>
<td>Inter-individual variability in $k_{Adm}$ (%)</td>
<td>30</td>
<td>56</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>24.2</td>
<td>2</td>
</tr>
</tbody>
</table>

The diagnostic plots for this model (figures 3.13 and 3.15) show a good fit to the data. The scatter plots of predicted against observed heart rate display an even distribution around the line of unity, with slightly more deviation than observed for MBP. Weighted residuals are evenly distributed around zero and a large proportion of the points fall within ±2 standard deviations. As with MBP though, it appears that more fall outside this range after 500 minutes and at the higher beats per minute, again likely due to the higher level of variability in activity during the dark period.
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Figure 3.13: Observed versus predicted heart rate for the final rat HR baseline model using population (A) or individual (B) parameters.

Plots of individual predicted heart rate-time profiles for the 5 animals administered vehicle as part of the L-NAME study are shown in figure 3.14. The model gives a good description of the observed data for these animals, although again the administration effect is generally much lower than seen in other rats, including those dosed with L-NAME. Population parameter estimates of the baseline were taken through to the PD modelling of L-NAME effect on HR.

Figure 3.14: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time following administration of vehicle to rats as part of the L-NAME study.
3.3.2 Dog study

The mean blood pressure and heart rate data for vehicle treated dogs also displayed circadian rhythms over the 24 hours period of data collection. Unlike the rat data there were no sudden jumps in the profile associated with the artificial lighting schedule, instead there was a much more gradual rise and fall over time. In this case a cosine function model appeared much more appropriate to describe the shape of the profiles.

Like the rat data there appeared to be a spike in MBP/HR at the time of administration in most cases. Therefore, an attempt to model this was performed by applying the administration effect function.

Due to an even smaller number of animals used for the dog studies, the vehicle data from all 3 studies were again modelled together to help get the best individual model fits and provide enough information to estimate the inter-individual variability. This was particularly important for the L-NAME study since one animal had to be excluded from the analysis due to an artificially high heart rate during the first half of the study.

All the dogs treated with vehicle were also dosed with active compound during the same study; therefore, individual baseline parameters estimated as part of this model could be fixed during the PD modelling.

3.3.2.1 Mean blood pressure

3.3.2.1.1 Model selection

The changes in dog mean blood pressure baseline data with time were suitably described by a single cosine function with a period of 24 hours and additive residual
error. The addition of an administration effect to the model resulted in a significant drop in log-likelihood (-9.78, 2df; table 3.13) but the associated parameters P and $k_{Adm}$ were imprecisely estimated (RSE=125-268%). Inclusion of inter-individual variability on all structural parameters resulted in a further significant drop in log-likelihood (-1096.56, 5df) but only a slight improvement in the precision of P and $k_{Adm}$ (RSE=80-222%). In addition theetas for these parameters were themselves imprecisely estimated (RSE=681-2140%) with high levels of shrinkage (62-90%). Removal of the variability on the administration effect parameters resulted in a similar -2LL value, indicating the possibility of a better model. However, P was estimated as an implausibly high value ($4 \times 10^5$) with extremely poor precision (RSE=9x10^8%). Since the administration effect could not be precisely estimated, the basic cosine model was selected instead. Inclusion of inter-individual variability on the structural parameters resulted in a significant drop in -2LL value compared to the base cosine model (-1060.59, 3df) and thus selected as the final model.

Table 3.13: Overview of model selection for the dog MBP baseline data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>COS 24h, no $\eta$</td>
<td>7432.33</td>
<td>7440.33</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>COS 24h + Adm, no $\eta$</td>
<td>7422.55</td>
<td>7434.55</td>
<td>Y</td>
<td>High SE P, $k_{Adm}$</td>
</tr>
<tr>
<td>COS 24h + Adm, all $\eta$</td>
<td>6325.99</td>
<td>6347.99</td>
<td>Y</td>
<td>High SE P, $k_{Adm}$; very high SE &amp; shrinkage $\omega$ P, $k_{Adm}$</td>
</tr>
<tr>
<td>COS 24h + Adm, no $\eta$ P, $k_{Adm}$</td>
<td>6326.52</td>
<td>6344.52</td>
<td>Y</td>
<td>Implausible estimate P, extremely high SE P, $k_{Adm}$</td>
</tr>
<tr>
<td>COS 24h, all $\eta$</td>
<td>6361.96</td>
<td>6375.96</td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

### 3.3.2.1.2 Final baseline model fit

The final dog MBP baseline model was selected as a single cosine function model with 24 hour period, inter-individual variability on $R_{av}$, $R_{amp}$ and $T_z$, and additive residual error. Parameter estimates (table 3.14) were predicted with very good precision (RSE=2-24%). Inter-individual variability was low for $R_{av}$ (8%) but moderate to high for $R_{amp}$ and $T_z$ (55-72%). The amplitude varied across all dogs and can be seen clearly in figure 3.20 for the dogs used in the L-NAME study. The main reason for the high variability seen in $T_z$ was due to 1 animal from the milrinone study (see section 4.3.2.1) whose blood pressure peaked during the night time (approximately 3am) as opposed to the majority that peaked around midday (approximately 11am-1pm). Additive residual error was low at 5.61mmHg.
Table 3.14: Parameter estimates for the final dog MBP baseline model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (mmHg)</td>
<td>106</td>
<td>2</td>
</tr>
<tr>
<td>$R_{amp}$ (mmHg)</td>
<td>3.42</td>
<td>18</td>
</tr>
<tr>
<td>$T_z$ (h)</td>
<td>3.65</td>
<td>24</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{av}$ (%)</td>
<td>8</td>
<td>43</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{amp}$ (%)</td>
<td>55</td>
<td>51</td>
</tr>
<tr>
<td>Inter-individual variability in $T_z$ (%)</td>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>5.61</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 3.16: Observed versus predicted mean blood pressure for the final dog MBP baseline model using population (A) or individual (B) parameters.

Figure 3.17: Population weighted residuals (PWRES) versus time (A) or predicted mean blood pressure (B) for the final dog MBP baseline model.

The diagnostic plots for this model show an acceptable fit to the data. The plots of predicted against observed heart rate (figures 3.16) display an even scatter around the
line of unity, although the scatter is quite wide due to the degree of variability between consecutive points. Weighted residuals (figure 3.17) are evenly distributed around zero with no apparent patterns to suggest model misspecification. Despite the variability, a large proportion of the points also fall within ±2 standard deviations.

Individual values of the model parameters for the dogs used in the L-NAME study are shown in table 3.15. Overall the individual parameters for all of the dogs from all 3 studies showed very little difference to those estimated with the administration effect included. In the case of the dogs from the L-NAME study no clear administration effect was seen on administration of vehicle (figure 3.18) or L-NAME (see section 3.4.2.1.2, figure 3.35) anyway and so the model without an administration effect was sufficient. In the cases where an administration effect was observed, estimation of the administration effect model parameters was attempted as part of the PD modelling (see sections 4.4.2.1 and 5.4.2.1).

Table 3.15: Individual estimates of the final dog MBP baseline model parameters for dogs used in the L-NAME study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (mmHg)</td>
<td>107</td>
<td>115</td>
<td>96.0</td>
</tr>
<tr>
<td>$R_{amp}$ (mmHg)</td>
<td>7.94</td>
<td>2.19</td>
<td>2.95</td>
</tr>
<tr>
<td>$T_z$ (h)</td>
<td>2.36</td>
<td>1.71</td>
<td>4.24</td>
</tr>
</tbody>
</table>

Figure 3.18: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time following administration of vehicle to dogs as part of the L-NAME study.

3.3.2.2 Heart rate

3.3.2.2.1 Model selection

The changes in dog heart rate baseline data with time were adequately described by a single cosine function with a period of 24 hours for most animals. However, the profile
was not as clear for some individual animals. Therefore, a dual cosine model with periods of 24 and 12 hours was explored. A fit of the dual cosine model with no inter-individual variability and additive error resulted in a significant drop in log-likelihood (-19.97, 2df; table 3.16) when compared with the single cosine model. In addition it appeared that an administration effect was present in the majority of animals. Inclusion of an administration effect model to the dual cosine function reduced the log-likelihood further (-45.05, 2df), although there was some imprecision of $P$ (RSE=52%) and a high correlation between $P$ and $k_{Adm}$ (0.95). Inclusion of inter-individual variability on all structural parameters again produced a significant reduction in log-likelihood (-442.55, 7df) but also produced a value greater than 24 hours for $T_{z12}$ (31.1h). In addition the precision of the omegas for $R_{amp12}$ and $P$ was poor (RSE=121-354%) and there was high shrinkage for the $T_{z12}$ and $P$ variabilities (89-93%). Removal of the eta for $T_{z12}$ reduced the -2LL value in addition to reducing the parameter estimate for $T_{z12}$ to within 24 hours (23.6h). The poor precision and high shrinkage for inter-individual variability in $P$ also reduced to acceptable levels, however, precision for $R_{amp12}$ and $k_{Adm}$ became worse (RSE=251-670%) along with high shrinkage (81-90%). Removal of the etas for these 2 parameters resulted in a model with similar log-likelihood and no major issues.

Table 3.16: Overview of model selection for the dog HR baseline data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>COS 24h, no $\eta$</td>
<td>8288.36</td>
<td>8296.36</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>COS 24h &amp; 12h, no $\eta$</td>
<td>8268.39</td>
<td>8280.39</td>
<td>Y</td>
<td>Slightly high SE $P$; Cor $P$ &amp; $k_{Adm}$ = 0.95</td>
</tr>
<tr>
<td>COS 24h&amp;12h + Adm, no $\eta$</td>
<td>8223.34</td>
<td>8239.34</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>COS 24h&amp;12h + Adm, all $\eta$</td>
<td>7780.79</td>
<td>7810.79</td>
<td>Y</td>
<td>Inplausible value $T_{z12}$, high shrinkage $\omega$ $T_{z12}$</td>
</tr>
<tr>
<td>COS 24h&amp;12h + Adm, no $\eta$ $T_{z12}$</td>
<td>7775.92</td>
<td>7803.92</td>
<td>Y</td>
<td>High SE &amp; shrinkage $\omega$ $R_{amp12}$, $k_{Adm}$</td>
</tr>
<tr>
<td>COS 24h&amp;12h + Adm, no $\eta$ $R_{amp12}$, $T_{z12}$, $k_{Adm}$</td>
<td><strong>7776.68</strong></td>
<td><strong>7780.6</strong></td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

**3.3.2.2.2 Final baseline model fit**

The final dog HR baseline model was selected as a dual cosine function model with 24 and 12 hour periods, inter-individual variability on $R_{av}$, $R_{amp24}$, $T_{z24}$, and $P$, and additive residual error. Parameter estimates (table 3.17) were predicted with good to moderate precision (RSE=3-36%). Inter-individual variability was low for $T_{z24}$ and $R_{av}$ (9-10%) but high for $P$ and $R_{amp24}$ (71-108%). As with MBP, variability in the 24 hour amplitude
varied across all dogs. For the dogs used in the L-NAME study, it was generally much higher than the population estimate (table 3.18) and effectively masked the 12 hour period (figure 3.21). Much of the requirement for the dual cosine model actually came from the profiles of dogs used in the doxazosin study (see section 5.3.2.2). The extent of the administration effect also varied across all animals but the lowest values were observed from 2 of the animals in the L-NAME study (table 3.18). The lack of administration effect can be seen in the individual plots of heart rate with time (figure 3.21). Additive residual error was higher than the other baseline models when considered relative to $R_{av}$ (14.6%).

Table 3.17: Parameter estimates for the final dog HR baseline model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (bpm)</td>
<td>78.9</td>
<td>3</td>
</tr>
<tr>
<td>$R_{amp24}$ (bpm)</td>
<td>4.97</td>
<td>36</td>
</tr>
<tr>
<td>$T_{z24}$ (h)</td>
<td>12.2</td>
<td>4</td>
</tr>
<tr>
<td>$R_{amp12}$ (bpm)</td>
<td>2.39</td>
<td>22</td>
</tr>
<tr>
<td>$T_{z12}$ (h)</td>
<td>23.6</td>
<td>2</td>
</tr>
<tr>
<td>$P$</td>
<td>1.48</td>
<td>36</td>
</tr>
<tr>
<td>$k_{Adm}$ (h$^{-1}$)</td>
<td>9.14</td>
<td>19</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{av}$ (%)</td>
<td>10</td>
<td>44</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{amp24}$ (%)</td>
<td>108</td>
<td>50</td>
</tr>
<tr>
<td>Inter-individual variability in $T_{z24}$ (%)</td>
<td>9</td>
<td>70</td>
</tr>
<tr>
<td>Inter-individual variability in $P$ (%)</td>
<td>71</td>
<td>58</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>11.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.18: Individual estimates of the final dog HR baseline model parameters for dogs used in the L-NAME study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (bpm)</td>
<td>85.5</td>
<td>94.3</td>
<td>85.6</td>
</tr>
<tr>
<td>$R_{amp24}$ (bpm)</td>
<td>14.3</td>
<td>18.8</td>
<td>5.83</td>
</tr>
<tr>
<td>$T_{z24}$ (h)</td>
<td>12.0</td>
<td>13.1</td>
<td>12.2</td>
</tr>
<tr>
<td>$P$</td>
<td>0.861</td>
<td>0.642</td>
<td>1.86</td>
</tr>
</tbody>
</table>

Diagnostic plots for this model show an acceptable fit to the data (figures 3.19 and 3.20). An even distribution of points around the line of unity can be seen for the scatter plots of predicted against observed heart rate. Weighted residuals are evenly
distributed around zero with no apparent patterns to suggest model misspecification and a large proportion of the points also fall within ±2 standard deviations.

Figure 3.19: Observed versus predicted heart rate for the final dog HR baseline model using population (A) or individual (B) parameters.

Figure 3.20: Population weighted residuals (PWRES) versus time (A) or predicted heart rate (B) for the final dog HR baseline model.

Figure 3.21: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time following administration of vehicle to dogs as part of the L-NAME study.
3.3.3 Guinea-pig study

A vehicle leg in different individual animals was performed as part of the L-NAME guinea-pig study and although referred to in the report, was not available as part of the data files. Mean blood pressure and heart rate data for the vehicle legs of the milrinone and doxazosin studies were provided though. Visual inspection of these profiles appeared to show changes in the data with time but due to the short time frame of the studies (<90 minutes), it was not possible to see any full circadian rhythms. In this case an empirical model was required to describe any changes seen.

In addition to the vehicle treated animals, there was additional pre-dose data available for each of the animals administered active compound. Once again due to the low numbers of animals plus the lack of vehicle data for the L-NAME study, it was decided to model all the vehicle and pre-dose data together. Then during the PD modelling, baseline values for the dosed animals could then be fixed from the respective pre-dose data.

3.3.3.1 Mean blood pressure

3.3.3.1.1 Model selection

Visual inspection of the vehicle and pre-dose guinea-pig mean blood pressure data indicated that for the majority of cases the MBP-time profile was approximately linear. Some of the vehicle profiles appeared to show some fluctuations or more curved shapes, although there was no consistency in this with respect to time. Since the pre-dose data provided an indication of the baseline for each specific dosed animal, the assumption had to be made that the baseline pattern would continue in the same manner if dosing had not occurred. Therefore, with no regularity in any of the fluctuating patterns, the most complex model that could be considered was a linear one. Since overall there was generally only a slight change in MBP with time a constant baseline model was considered in the first instance.

Comparison of the model fit for a linear model with inter-individual variability on the intercept (R_int) to that of a individual constant baseline showed significant drop in log-likelihood (-5.18, 1df; table 3.16) and an overall better fit to the data. Addition of inter-individual variability to the slope parameter produced a further significant drop in -2LL value (-171.95, 1df) and the best individual fits to both vehicle and pre-dose data.
Table 3.19: Overview of model selection for the GP MBP baseline data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant, $\eta$</td>
<td>4766.39</td>
<td>4772.39</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Linear, $R_{\text{int}} \eta$</td>
<td>4761.21</td>
<td>4769.21</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td><strong>Linear, $R_{\text{int}}$ &amp; $R_{\text{slope}} \eta$</strong></td>
<td><strong>4589.26</strong></td>
<td><strong>4599.26</strong></td>
<td>Y</td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

3.3.3.1.2 Final baseline model fit

The final guinea-pig MBP baseline model was selected as a linear model with inter-individual variability on $R_{\text{int}}$ and $R_{\text{slope}}$ and additive residual error. The parameter estimate for $R_{\text{int}}$ was predicted with excellent precision (RSE=2%), however that for $R_{\text{slope}}$ was predicted with quite poor precision (RSE=63%; table 3.20). Since the population estimates were not used for any further fitting or prediction purposes, this was not considered to be an issue. Good individual estimates were most important for correct fitting of the pharmacodynamic model. The fact that the range of individual slopes encompassed both positive and negative values, may have contributed to the poor precision of the population estimate. Despite the variation of both positive and negative values, inter-individual variability was low for both parameters (10-14%). Additive residual error was also low.

Table 3.20: Parameter estimates for the final GP MBP baseline model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{int}}$ (mmHg)</td>
<td>40.9</td>
<td>2</td>
</tr>
<tr>
<td>$R_{\text{slope}}$ (mmHg/min)</td>
<td>-0.0293</td>
<td>63</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{\text{int}}$ (%)</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{\text{slope}}$ (%)</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>1.75</td>
<td>2</td>
</tr>
</tbody>
</table>

Diagnostic plots for the final model show a good fit to the data (figures 3.22 and 3.23). An even distribution of points around the line of unity can be seen for the scatter plots of predicated against observed mean blood pressure and little deviation from the line in the case of the individual predictions. Weighted residuals are evenly distributed around zero with no apparent patterns and once again a large proportion of the points fall within ±2 standard deviations.
Figure 3.22: Observed versus predicted mean blood pressure for the final guinea-pig MBP baseline model using population (A) or individual (B) parameters.

Figure 3.23: Population weighted residuals (PWRES) versus time (A) or predicted mean blood pressure (B) for the final guinea-pig MBP baseline model.

Individual parameter values for the pre-dose MBP data of guinea-pigs dosed with L-NAME are shown in table 3.21 and plots of the individual mean blood pressure time profiles shown in figure 3.24.

Table 3.21: Individual estimates of the final MBP baseline model parameters for guinea-pigs used in the L-NAME study

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP 1</th>
<th>GP 2</th>
<th>GP 4</th>
<th>GP 5</th>
<th>GP 6</th>
<th>GP 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>R\textsubscript{Int} (mmHg)</td>
<td>55.5</td>
<td>40.8</td>
<td>38.4</td>
<td>47.2</td>
<td>45.6</td>
<td>45.5</td>
</tr>
<tr>
<td>R\textsubscript{Slope} (mmHg/min)</td>
<td>-0.346</td>
<td>0.127</td>
<td>0.160</td>
<td>0.092</td>
<td>-0.177</td>
<td>0.034</td>
</tr>
</tbody>
</table>

A range of initial MBP values can be seen for these animals, although most are higher than the population estimate. As was seen overall, values for R\textsubscript{Slope} span both positive and negative ranges. For the majority of these individuals though, MBP increased with
time and no association between initial MBP and increase or decrease with time appeared to be present.

Figure 3.24: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time prior to administration of L-NAME in guinea-pigs.

3.3.3.2 Heart rate

3.3.3.2.1 Model selection

As for the MBP data, changes in guinea-pig heart rate with time appeared to be approximately linear in nature. Again there were some profiles that appeared to show fluctuating patterns but with little consistency. The majority of individuals presented with a clearly decreasing profile over the course of the study and thus a linear model with negative slope was expected to give the best fit.

The need for a linear model was confirmed via a significant drop in log-likelihood (-749.47, 1df) when compared to a constant baseline model (table 3.22). Addition of inter-individual variability on the slope parameter produced a further significant drop in -2LL value and the best fits to the data.

Table 3.22: Overview of model selection for the GP HR baseline data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant, ( \eta )</td>
<td>7649.47</td>
<td>7655.47</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Linear, ( R_{\text{int}} \eta )</td>
<td>6900.00</td>
<td>6908.00</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Linear, ( R_{\text{int}} ) &amp; ( R_{\text{slope}} \eta )</td>
<td>6281.94</td>
<td>6291.94</td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>
3.3.3.2 Final model fit

The final guinea-pig HR baseline model was selected as a linear model with inter-individual variability on $R_{\text{Int}}$ and $R_{\text{slope}}$ and additive residual error. Parameters were estimated with good precision (RSE=1-18%; table 3.23). Inter-individual variability for $R_{\text{Int}}$ was low (8%) but for $R_{\text{slope}}$ was moderate (32%), indicating that even though the majority of slopes were negative there was still variation in the extent of the HR decrease with time. Additive error was low once again.

Table 3.23: Parameter estimates for the final GP HR baseline model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{Int}}$ (bpm)</td>
<td>251</td>
<td>1</td>
</tr>
<tr>
<td>$R_{\text{slope}}$ (bpm/min)</td>
<td>-0.324</td>
<td>18</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{\text{Int}}$ (%)</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Inter-individual variability in $R_{\text{slope}}$ (%)</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>3.93</td>
<td>2</td>
</tr>
</tbody>
</table>

Diagnostic plots for the final model once again show a good fit to the data (figures 3.25 and 3.26). The scatter plots of predicated against observed heart rate displayed an even distribution of points around the line of unity and little deviation from the line in the case of the individual predictions. Weighted residuals are evenly distributed around zero with the majority of the points falling within ±2 standard deviations.

Figure 3.25: Observed versus predicted heart rate for the final guinea-pig HR baseline model using population (A) or individual (B) parameters.
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Figure 3.26: Population weighted residuals (PWRES) versus time (A) or predicted heart rate (B) for the final guinea-pig HR baseline model.

Figure 3.27: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time prior to administration of L-NAME in guinea-pigs.

Individual parameter values for the pre-dose heart rate data of guinea-pigs dosed with L-NAME are shown in table 3.24 and plots of the individual heart rate-time profiles shown in figure 3.27.

Table 3.24: Individual estimates of the final HR baseline model parameters for guinea-pigs used in the L-NAME study

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP 1</th>
<th>GP 2</th>
<th>GP 4</th>
<th>GP 5</th>
<th>GP 6</th>
<th>GP 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>R_{Int} (mmHg)</td>
<td>317</td>
<td>250</td>
<td>238</td>
<td>275</td>
<td>268</td>
<td>275</td>
</tr>
<tr>
<td>R_{Slope} (mmHg/min)</td>
<td>-0.561</td>
<td>-0.380</td>
<td>-0.035</td>
<td>-0.203</td>
<td>-0.551</td>
<td>-0.437</td>
</tr>
</tbody>
</table>

The values generally show a higher initial heart rate for these animals compared with the population estimate, although there is quite a large range across the individuals. All
the animals show a decrease in heart rate with time, although to different degrees. Although not true in all cases, the higher initial heart rates appear to be associated with a larger decrease with time and vice versa.

### 3.4 Pharmacodynamic modelling of the cardiovascular response to L-NAME

As expected from the evidence in the literature (see section 3.1) the cardiovascular response to L-NAME in all three animal models was an increase in mean blood pressure and a decrease in heart rate. The responses were therefore modelled using appropriate stimulatory and inhibitory effect models respectively, as part of an integrated PKPD model with fixed PK and baseline parameters.

#### 3.4.1 Rat study

Since PK and baseline data were obtained from different individual animals to those for which PD data were collected, the population parameters estimates for the PK and baseline models were fixed in the integrated PKPD model. However, to allow for differences in the typical MBP/HR values for these individual rats the baseline parameter $R_{\text{av}}$ and associated variability were estimated rather than fixed. In addition due to its absence in some cases, the administration effect was also estimated for each individual.

##### 3.4.1.1 Mean blood pressure

#### 3.4.1.1.1 PD model selection

Comparison of the time courses of the rat L-NAME PK and MBP PD data showed a clear delay in the response profile in relation to that of the concentration profile. Therefore the appropriate PD model would describe this delay either by the use of an effect compartment model or an indirect response model with stimulation of the production of response ($k_{\text{in}}$). It was felt the latter would be more suitable; however, both models were fit using a stimulatory $E_{\text{max}}$ function and compared. Both models gave a good fit to the data but comparison of the fit statistics indicated that the indirect model was superior (table 3.25). To further refine the fit, inter-individual variability was applied to the structural parameters, which produced a significant drop in log-likelihood (-106.95, 3df) but imprecision of the $EC_{50}$ estimate (RSE=72%). Removal of the eta for $EC_{50}$ did increase the -2LL value by 15.88 points but improved the precision of the $EC_{50}$ to acceptable levels (RSE=33%).
Table 3.25: Overview of model selection for the rat L-NAME MBP PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect cpt; ( E_{\text{max}} ); no ( \eta )</td>
<td>16052.37</td>
<td>16072.37</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Indirect; ( E_{\text{max}} ); no ( \eta )</td>
<td>15947.61</td>
<td>15967.61</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td>Indirect; ( E_{\text{max}} ); all ( \eta )</td>
<td>15840.66</td>
<td>15866.66</td>
<td>Y</td>
<td>High SE ( EC_{50} )</td>
</tr>
<tr>
<td><strong>Indirect; ( E_{\text{max}} ) model; no ( \eta ); ( EC_{50} ) ( \text{final} )</strong></td>
<td>15856.54</td>
<td>15880.54</td>
<td>Y</td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

3.4.1.1.2 Fit PD model fit

The final L-NAME rat mean blood pressure PD model was selected as an indirect response model with stimulatory effect on the production of response described via an \( E_{\text{max}} \) model, with inter-individual variability on \( E_{\text{max}} \) and \( k_{\text{out}} \), and additive residual error. Structural parameters were estimated with good to moderate precision (RSE=15-42%; table 3.26) and additive residual error was low.

Table 3.26: Parameter estimates for the final rat L-NAME MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_{\text{max}} )</td>
<td>0.357</td>
<td>42</td>
</tr>
<tr>
<td>( EC_{50} ) (( \mu \text{M} ))</td>
<td>44.8</td>
<td>33</td>
</tr>
<tr>
<td>( k_{\text{out}} ) (( \text{min}^{-1} ))</td>
<td>0.0153</td>
<td>15</td>
</tr>
<tr>
<td>Inter-individual variability in ( E_{\text{max}} ) (%)</td>
<td>140</td>
<td>40</td>
</tr>
<tr>
<td>Inter-individual variability in ( k_{\text{out}} ) (%)</td>
<td>46</td>
<td>53</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>6.6</td>
<td>1</td>
</tr>
</tbody>
</table>

A high level of inter-individual variability was estimated for \( E_{\text{max}} \) (140%) indicating extensive differences in the extent of the response between individual animals. In addition there was also a moderate level of variability for \( k_{\text{out}} \) (46%), suggesting varied turnover and hence time course of the response too. Both of these characteristics can be observed in the individual mean blood pressure time plots (figure 3.28).

Diagnostic plots show a good fit to the data (figures 3.29 and 3.30). The scatter plots of predicted against observed mean blood pressure display an even distribution of points around the line of unity and not too much deviation from the line in the case of the individual predictions. Weighted residuals are evenly distributed around zero with no observable pattern indicative of model misspecification. A good proportion of the points also fall within ±2 standard deviations.
Figure 3.28: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time in rats following administration of 10 (ID=1-6), 30 (ID = 7-12) or 100 (ID = 13-18) mg/kg L-NAME.
3.4.1.2 Heart rate

3.4.1.2.1 PD model selection

Comparison of the time courses of the rat L-NAME PK and HR PD data indicated a delay in the response profile in relation to that of the concentration profile, although it was not as obvious as that for the MBP. The fit of a direct $I_{\text{max}}$ model was therefore compared with an effect compartment model and an indirect response model with inhibition of $k_{\text{in}}$, to see which best described the profiles. Although none of the 3 models gave an adequate fit to the data it was clear that the direct model did predict the peak effect too early. Comparison of the fit statistics indicated that the indirect model was superior (table 3.27) to that of the effect compartment model and so was selected for further refinement. Addition of inter-individual variability to all parameters produced a significant drop in log-likelihood (-186.22, 3df) but also resulted in extremely poor
precision of the $k_{\text{out}}$ estimate (RSE=246%) and did not improve the problems with the overall fit. It appeared that the fixed baseline parameters may be causing the issue; therefore, etas were estimated for the 3 fixed parameters that had inter-individual variability applied as part of the baseline model ($\gamma$, $\delta$, $D$). This allowed individual values of these parameters to be estimated and better describe the individual profiles. Addition of these estimated etas to the indirect model without variability on the PD parameters resulted in a significant drop in -2LL compared to the basic indirect model (-297.15, 3df) and vastly improved the fit to the data. The $k_{\text{out}}$ parameter was estimated with much improved but still slightly poor precision (RSE=66%). Further refinement was made via the addition of inter-individual variability on all 3 PD parameters, which produced a further significant drop in -2LL (-51.38, 3df). No improvement was made on the precision of $k_{\text{out}}$ and there was also poor precision (RSE=95%) for the $IC_{50}$ eta. Moderate shrinkage was also observed for both the $IC_{50}$ and $k_{\text{out}}$ etas (66-67%). Removal of the eta for $k_{\text{out}}$ increased the -2LL slightly and improved the precision of the $k_{\text{out}}$ parameter estimate to acceptable levels, however, the precision of the $IC_{50}$ estimate worsened (RSE=77%). Removal of $IC_{50}$ eta again increased the -2LL value but resulted in precise estimates for all parameters.

Table 3.27: Overview of model selection for the data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $I_{\text{max}}$; no PD $\eta$</td>
<td>22185.75</td>
<td>22203.75</td>
<td>-</td>
<td>Poor fit</td>
</tr>
<tr>
<td>Effect cpt; $I_{\text{max}}$; no PD $\eta$</td>
<td>22186.64</td>
<td>22206.64</td>
<td>-</td>
<td>Poor fit</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no PD $\eta$</td>
<td>22180.98</td>
<td>22200.98</td>
<td>-</td>
<td>Lowest AIC; Poor fit</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; all PD $\eta$</td>
<td>21994.76</td>
<td>22020.76</td>
<td>Y</td>
<td>Poor fit; v. high SE $k_{\text{out}}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no PD $\eta$; fit $\eta$ BL $\gamma$, $\delta$, $D$</td>
<td>21883.83</td>
<td>21909.84</td>
<td>Y</td>
<td>Better fit; high SE $k_{\text{out}}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; all PD $\eta$; fit $\eta$ BL $\gamma$, $\delta$, $D$</td>
<td>21832.45</td>
<td>21864.45</td>
<td>Y</td>
<td>High SE $k_{\text{out}}$, Mod shrinkage $\omega$ $IC_{50}$, $k_{\text{out}}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no $k_{\text{out}}$; fit $\eta$ BL $\gamma$, $\delta$, $D$</td>
<td>21836.01</td>
<td>21866.01</td>
<td>Y</td>
<td>High SE $IC_{50}$, Mod shrinkage $\omega$ $IC_{50}$</td>
</tr>
<tr>
<td><strong>Indirect; $I_{\text{max}}$; no $\eta$ $IC_{50}$ &amp; $k_{\text{out}}$; fit $\eta$ BL $\gamma$, $\delta$, $D$</strong></td>
<td><strong>21840.82</strong></td>
<td><strong>21868.82</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

3.4.1.2.2 Final PD model fit

The final L-NAME heart rate PD model was selected as an indirect response model with an inhibitory effect on the production of response described via an $I_{\text{max}}$ model, with inter-individual variability on $I_{\text{max}}$ and additive residual error. Structural parameters
were estimated with good to moderate precision (RSE=22-38%; table 3.28) and additive residual error was low. The inter-individual variability estimated for $I_{\text{max}}$ was high at 126%, once again indicating extensive differences in the extent of the response between individual animals. Individual plots of heart rate with time (figure 3.33) show a reasonable fit. The only issue may be that allowing the baseline values $\gamma$ and $\delta$ to vary has produced extra or early oscillations in some individual animals (IDs 14 & 17).

Table 3.28: Parameter estimates for the final rat HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{max}}$</td>
<td>0.145</td>
<td>38</td>
</tr>
<tr>
<td>$IC_{50}$ ($\mu$M)</td>
<td>41</td>
<td>22</td>
</tr>
<tr>
<td>$k_{\text{out}}$ ($\text{min}^{-1}$)</td>
<td>0.0484</td>
<td>33</td>
</tr>
<tr>
<td>Inter-individual variability in $I_{\text{max}}$ (%)</td>
<td>126</td>
<td>45</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>24</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 3.31: Observed versus predicted heart rate for the final rat L-NAME HR model using population (A) or individual (B) parameters.

Diagnostic plots show a good fit to the data (figures 3.31 and 3.33). The scatter plots of predicted against observed heart rate display an even distribution of points around the line of unity. Slightly greater deviation from the line of unity can be seen for the higher values with the individual predictions. This is likely due to the higher variability seen during the rats’ active phase, as with the baseline model. This can also be seen in the weighted residual plots with more residual spread for the higher values and later time points. Overall though residuals are evenly distributed around zero and a large proportion of the points fall within ±2 standard deviations.
Figure 3.32: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time in rats following administration of 10 (ID=1-6), 30 (ID = 7-12) or 100 (ID = 13-18) mg/kg L-NAME.
3.4.2 Dog study

Since all of the data (PK, baseline and PD) were obtained from the same set of animals, it was the individual estimates for the PK and baseline parameters that were fixed in the integrated PKPD model. The study design used had allowed a washout of a week between doses, yet L-NOARG concentrations were still detected in some of the pre-dose samples. Due to this fact, the data were modelled as the study had been performed, i.e. as a continuous timeframe with the specific order of dosing defined for each different animal.

3.4.2.1 Mean blood pressure

3.4.2.1.1 PD model selection

As for the rat, a comparison of the PK and PD profiles clearly showed a delay in peak response for MBP in the dog. Therefore, once more using a stimulatory $E_{\text{max}}$ function a comparison was made between an effect compartment model and an indirect response model with stimulation of the production of response. Unlike for the rat, in this case the goodness of fit statistics were not much help in selecting the best model. The log-likelihood and AIC values for both models were exactly the same and both models gave a reasonable fit to the data. This meant the decision of which of the models to select was based on which one had shown the best fit for the rat and which made more sense in physiological terms. In both of these cases this was the indirect response model. To further refine the fit, inter-individual variability was applied to the structural parameters. This appeared to cause an issue as the log-likelihood value actually increased, which is indicative of the model having converged to a local minima. To try and resolve this various sets of different initial estimates were tested for both the full (with inter-
individual variability) and reduced (no inter-individual variability) models. For all sets of estimates, the fit statistics did not change at all for the base model and only slightly for the full model. In all cases the $E_{\text{max}}$ and $EC_{50}$ estimates converged to approximately the same values and for the full model $k_{\text{out}}$ values converged to approximately the same too. However for the reduced model, $k_{\text{out}}$ estimates failed to change virtually at all from their initial values. It was therefore concluded that for this scenario (possibly due to such low number of individuals), the fit statistics were not reliable and would be ignored. The best model would therefore be chosen on the other criteria i.e. individual fits, diagnostic plots, parameter precision. The full model appeared to produce a slightly better fit (and more reliable estimates for $k_{\text{out}}$) than the reduced model However, the inter-individual variability values for $E_{\text{max}}$ and $k_{\text{out}}$ were extremely small, imprecise (RSE=83-617%) and had considerable shrinkage (96-100%). Etas for these 2 parameters were therefore removed, resulting in a model with variability on $EC_{50}$. This model still produced a reasonable fit with precise parameter estimates that were very similar to those of the full model.

3.4.2.1.2 Final PD model fit

The final L-NAME dog mean blood pressure PD model was selected as an indirect response model with stimulatory effect on the production of response described via an $E_{\text{max}}$ model, with inter-individual variability on $EC_{50}$, and additive residual error. Structural parameters were estimated with good to moderate precision (RSE=1-37%; table 3.29). A moderate level of inter-individual variability was estimated for $EC_{50}$ (60%) due to the compound having a much lower potency in one of the three dogs (~50µM) compared to the other two (~15µM).

Table 3.29: Parameter estimates for the final dog MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.195</td>
<td>4</td>
</tr>
<tr>
<td>$EC_{50}$ (µM)</td>
<td>21.6</td>
<td>37</td>
</tr>
<tr>
<td>$k_{\text{out}}$ (h$^{-1}$)</td>
<td>1.33</td>
<td>1</td>
</tr>
<tr>
<td>Inter-individual variability in $EC_{50}$ (%)</td>
<td>61</td>
<td>86</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>10.3</td>
<td>2</td>
</tr>
</tbody>
</table>

Additive residual error was higher than observed for the dog MBP baseline model. This is most likely due to the underestimation of the effect of the 20mg/kg L-NAME dose in dog 3. The reason for the underestimation is the higher effect seen at this dose compared
with the 40mg/kg dose. This can be observed in the individual mean blood pressure time plots (figure 3.36).

Diagnostic plots show an acceptable fit to the data (figures 3.34 and 3.35). The scatter plots of predicted against observed mean blood pressure display an even distribution of points around the line of unity, with a possible slight tendency for under-prediction at the higher values. Weighted residuals are evenly distributed around zero with no indication of model misspecification. The distribution is much more widely spread than observed previously with many more points greater than ±2 standard deviations. This is likely due to the high level of within-subject variability observed.

Figure 3.34: Observed versus predicted mean blood pressure for the final dog L-NAME MBP model using population (A) or individual (B) parameters.

Figure 3.35: Population weighted residuals (PWRES) versus time (A) or predicted mean blood pressure (B) for the final dog L-NAME MBP model.
Figure 3.36: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time in dogs following administration of 10 (LD), 20 (MD) or 40 (HD) mg/kg L-NAME.

3.4.2.2 Heart rate

3.4.2.2.1 PD model selection

Once again, comparison of the PK & PD time courses indicated a delay in response, although as for rat HR it was not as obvious as that for the MBP. The fit of a direct $I_{\text{max}}$ model was again compared with an effect compartment model and an indirect response model with inhibition of $k_{\text{in}}$, to see which best described the profiles. This confirmed that modelling the delay was required since the direct model predicted the peak effect too early. In addition there were once again issues with the fit statistics, with all 3 models predicting exactly the same log-likelihood value (and exactly the same value as MBP). Once again the indirect model was selected since it was the best model for the rat and made most sense physiologically. Also as with MBP the log-likelihood increased on the addition of inter-individual variability and again a number of different initial estimates were used to check the model wasn’t stuck in a local minimum. An additional issue arose with the estimation $k_{\text{out}}$. Without any inter-individual variability applied the estimate was too low to predict the correct time course of effect, but when
an eta was included, the precision of the population estimate was very poor (RSE=115%). The problem appeared to be due to one individual in particular, for which the shape of the profile could not be defined correctly. To overcome this issue, as for the rat HR, the baseline parameters were allowed to vary. This solved the problem and the final model required an eta on I$_{max}$ only to adequately describe the individual profiles and give precise population estimates.

3.4.2.2.2 Final PD model fit

The final L-NAME dog heart rate PD model was selected as an indirect response model with inhibitory effect on the production of response described via an I$_{max}$ model, with inter-individual variability on I$_{max}$, and additive residual error. Structural parameters were estimated with good precision (RSE=3-19%; table 3.30) and a moderate level of inter-individual variability was estimated for I$_{max}$ (56%). The variability again was associated with one of the dogs for which L-NAME had a lower magnitude of effect (~0.25) compared to the other two (~0.5). This can be observed in the individual heart rate time plots (figure 3.37), where the extent of inhibition for dog 1 is less than for 2 or 3. Additive residual error was similar to that predicted for the dog HR baseline model.

Table 3.30: Parameter estimates for the final dog HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I$_{max}$</td>
<td>0.422</td>
<td>19</td>
</tr>
<tr>
<td>IC$_{50}$ (µM)</td>
<td>3.33</td>
<td>11</td>
</tr>
<tr>
<td>k$_{out}$ (h$^{-1}$)</td>
<td>1.94</td>
<td>3</td>
</tr>
<tr>
<td>Inter-individual variability in I$_{max}$ (%)</td>
<td>56</td>
<td>82</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>12.3</td>
<td>2</td>
</tr>
</tbody>
</table>

Diagnostic plots once again show an acceptable fit to the data (figures 3.38 and 3.39). The scatter plots of predicted against observed heart rate display an even distribution of points around the line of unity. Weighted residuals are evenly distributed around zero with no indication of any patterns to suggest model misspecification. As for the dog MBP model, the distribution is widely spread with many points greater than ±2 standard deviations. Again, this is likely due to the high level of within-subject variability observed.
Figure 3.37: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time in dogs following administration of 10 (LD), 20 (MD) or 40 (HD) mg/kg L-NAME.

Figure 3.38: Observed versus predicted heart rate for the final dog L-NAME HR model using population (A) or individual (B) parameters.
3.4.3 Guinea-pig study

PK and PD data had been collected in the same individual animals therefore individual PK estimates were fixed in the integrated PKPD model. Although the vehicle data were obtained from different animals, the pre-dose responses allowed individual estimated of the baseline to be derived (see section 3.3.3) hence these values were also fixed in the PKPD model for each individual separately.

3.4.3.1 Mean blood pressure

3.4.3.1.1 PD model selection

Due to the nature of the guinea-pig study design with multiple infusions and few concentration time points, it was not obvious as to whether there was any delay between concentration and effect by comparing the time profiles. An $E_{\text{max}}$ model with direct effect was therefore compared to one with an effect compartment and to an indirect model with stimulation of production of response. The goodness of fit statistics indicated the effect compartment to be the best model (table 3.31) although parameters were estimated with very poor precision (RSE=2000%). Addition of inter-individual variability produced a significant drop in log-likelihood (-480.04, 3df) but the precision was still outside acceptable limits (RSE=112-157%). The indirect model was tested as an alternative, even though its parameters were also poorly estimated, it was not to the same degree (RSE=60-90%). Addition of etas to the indirect model parameters again resulted in a significant drop in -2LL value (-484.89, 3df) but little improvement was seen for the parameter precision. Since parameters could not be precisely estimated for the 2 models encompassing a delay, the direct model was then selected. Inclusion of inter-individual variability, resulted in a significant drop in log-likelihood (-464.94, 2df).
and acceptable parameter estimate precision. A comparison of the fits for the 3 models showed very little difference and so indicated that a delay was probably not required and over-parameterisation could be the reason for poor precision.

Table 3.31: Overview of model selection for the data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $E_{\text{max}}$; no $\eta$</td>
<td>2344.02</td>
<td>2350.02</td>
<td></td>
<td>Lowest AIC; Very high SE $\text{EC}<em>{50}$, $k</em>{e0}$; Cor = 1</td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; no $\eta$</td>
<td>2322.64</td>
<td>2330.64</td>
<td></td>
<td>High SE $\text{EC}<em>{50}$, $k</em>{e0}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; no $\eta$</td>
<td>2326.36</td>
<td>2334.36</td>
<td></td>
<td>High SE $\text{EC}<em>{50}$, $k</em>{e0}$</td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; all $\eta$</td>
<td>1842.60</td>
<td>1856.60</td>
<td>Y</td>
<td>High SE $\text{EC}<em>{50}$, $k</em>{e0}$; High SE $\omega\text{EC}<em>{50}$, $k</em>{e0}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; all $\eta$</td>
<td>1841.47</td>
<td>1855.47</td>
<td>Y</td>
<td>High SE $E_{\text{max}}$, $\text{EC}<em>{50}$; High SE $\omega\text{EC}</em>{50}$, $k_{out}$</td>
</tr>
<tr>
<td>Direct; $E_{\text{max}}$; all $\eta$</td>
<td>1879.08</td>
<td>1889.08</td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

3.4.3.1.2 Final PD model fit

The final L-NAME guinea-pig mean blood pressure PD model was selected as a direct $E_{\text{max}}$ model, with inter-individual variability on $E_{\text{max}}$ and $\text{EC}_{50}$, and additive residual error. Precision of the structural parameter estimates was just on the limit of acceptable (RSE=49-50%; table 3.32) and inter-individual variability was high for both $E_{\text{max}}$ and $\text{EC}_{50}$ (111-118%). Part of the reason for the high inter-individual variability in $E_{\text{max}}$ was potentially due to the differing slopes for the baselines. Additive residual error was reasonably low.

Table 3.32: Parameter estimates for the final guinea-pig MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.719</td>
<td>49</td>
</tr>
<tr>
<td>$\text{EC}_{50}$ (µM)</td>
<td>105</td>
<td>50</td>
</tr>
<tr>
<td>Inter-individual variability in $E_{\text{max}}$ (%)</td>
<td>118</td>
<td>60</td>
</tr>
<tr>
<td>Inter-individual variability in $\text{EC}_{50}$ (%)</td>
<td>111</td>
<td>68</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>3.6</td>
<td>4</td>
</tr>
</tbody>
</table>

Diagnostic plots show an acceptable fit to the data (figures 3.40 and 3.42). The scatter plots of population model predicated against observed mean blood pressure shows some bias towards over-prediction for the high values but an even distribution of points around the line of unity is seen for the individual model. Weighted residuals are evenly
distributed around zero with a good proportion of points within ±2 standard deviations. Residuals are less scattered during the first 20 minutes due to this being pre-dose and so fixed to individual values.

Figure 3.40: Observed versus predicted mean blood pressure for the final guinea-pig L-NAME MBP model using population (A) or individual (B) parameters.

Plots of individual predicted mean blood pressure-time profiles give a good description of the observed data (figure 3.41).

Figure 3.41: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time in guinea-pigs following administration of L-NAME.
3.4.3.2 Heart rate

3.4.3.2.1 PD model selection

As was the case for MBP, it was difficult to determine the possibility of a delay due to the lack of PK data. Therefore, a direct $I_{\text{max}}$ model was compared to an effect compartment model and an indirect model with inhibition of $k_{\text{in}}$. The indirect model was the best fit according to the fit statistics (table 3.33) but showed very poor precision for the $IC_{50}$ estimate (RSE=147%).

Table 3.33: Overview of model selection for the data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $I_{\text{max}}$; no $\eta$</td>
<td>2716.76</td>
<td>2722.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $I_{\text{max}}$; no $\eta$</td>
<td>2687.97</td>
<td>2695.97</td>
<td></td>
<td>Very high SE $IC_{50}$, $k_{\text{e0}}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no $\eta$</td>
<td>2679.75</td>
<td>2687.75</td>
<td></td>
<td>Lowest AIC; High SE $IC_{50}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; all $\eta$</td>
<td>2631.98</td>
<td>2645.98</td>
<td>Y</td>
<td>High SE $IC_{50}$, $k_{\text{out}}$; high SE &amp; shrinkage $\omega$ $k_{\text{out}}$</td>
</tr>
<tr>
<td>Direct; $I_{\text{max}}$; all $\eta$</td>
<td>2669.32</td>
<td>2679.32</td>
<td>Y</td>
<td>High SE $\omega$ $IC_{50}$</td>
</tr>
<tr>
<td>Direct; $I_{\text{max}}$; no $IC_{50}$ $\eta$</td>
<td><strong>2669.27</strong></td>
<td><strong>2677.27</strong></td>
<td>Y</td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

Addition of inter-individual variability resulted in a drop in log-likelihood (-46.99, 3df) but was not able to improve the precision, in fact precision was actually poorer for $k_{\text{out}}$ too (RSE=62%). Introducing variability to any combination of the parameters was not able to improve the precision to acceptable levels. This issue with precision meant the selection of direct model and refinement by the introduction of variability. Addition of etas to both structural parameters produced a significant drop in log-likelihood (-47.44, 2df), however the precision for the $IC_{50}$ omega was poor (RSE=148%). Removal of this
eta resulted in a model with a virtually identical -2LL value and no issues. Once again the model fits were very similar between models and it was likely the models introducing a delay were over-parameterised, leading to the poor precision of standard error.

3.4.3.2.2 Final PD model fit

The final L-NAME guinea-pig heart rate PD model was selected as a direct $I_{\text{max}}$ model, with inter-individual variability on $I_{\text{max}}$, and additive residual error. Precision of the structural parameter estimates was reasonably good (RSE=21-28%; table 3.34) and inter-individual variability for $I_{\text{max}}$ was moderate (50%). Additive residual error was low.

Table 3.34: Parameter estimates for the final rat HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{max}}$</td>
<td>0.0882</td>
<td>21</td>
</tr>
<tr>
<td>$IC_{50}$ (µM)</td>
<td>22.2</td>
<td>28</td>
</tr>
<tr>
<td>Inter-individual variability in $I_{\text{max}}$ (%)</td>
<td>50</td>
<td>65</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>12.1</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 3.43: Observed versus predicted heart rate for the final guinea-pig L-NAME HR model using population (A) or individual (B) parameters.

Diagnostic plots show an acceptable fit to the data (figures 3.43 and 3.44). The majority of points on the scatter plots of predicted against observed heart rate are evenly distributed around zero but a few low points deviate from the line substantially. These points are mostly for one individual animal where the heart dropped suddenly over a few minutes at the start of the higher infusions (see figure 3.45). The points deviate
from the general pattern possibly due to the pacing protocol carried out just prior to them. Weighted residuals are evenly distributed around zero with most within ±2 standard deviations, except for the deviating point mentioned above. Residuals are again less scattered during the first 20 minutes due to fixed individual values for pre-dose.

Figure 3.44: Population weighted residuals (PWRES) versus time (A) or predicted heart rate (B) for the final guinea-pig L-NAME HR model.

Figure 3.45: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time in guinea-pigs following administration of L-NAME.

3.5 Prediction of human cardiovascular response to L-NAME

3.5.1 Human PK & PD data

3.5.1.1 Literature search

A literature search for L-NAME studies in human subjects revealed a number of articles. In a few of these, L-NAME had been studied as a potential treatment for the severe vasodilation associated with septic shock e.g. Avontuur et al., (1998). Others had
been performed in healthy volunteers but had other confounding issues such as the effect of exercise or of a meal. There were still a number of studies available in healthy volunteers and no confounding issues, although for most of these, single time points for blood pressure and heart rate response, before and after administration of L-NAME were the only data available. There was however one study (Wecht et al., 2008) that had actually measured the timeframe of response to L-NAME and to placebo.

The Wecht 2008 study had a single ascending dose design with placebo arm. On each of 5 occasions the study subjects received either placebo (30ml saline) or a dose of 0.5, 1, 2, or 4mg/kg L-NAME via intravenous infusion over 60 minutes. The study subjects were either healthy volunteers (n=7) or individuals with severe spinal cord injury, the data for the latter was ignored. Subjects had rested for 20 minutes before the initiation of the infusion and remained in the supine position for the period of data collection (4 hours). Mean blood pressure and heart rate were measured at the start of the infusion and then at 30, 60, 120, 180 and 240 minutes.

The one downside to this study was that although blood samples had been taken from the subjects at corresponding times to the cardiovascular measurements, these were not used to measure drug concentrations but noradrenaline concentrations instead. This meant that pharmacokinetic data had to be obtained from elsewhere.

Pharmacokinetic data for healthy volunteers was difficult to find. There were no papers in the literature with actual PK parameters, except for the Avontuur 1998 paper, which was a study in severely ill patients with septic shock. Eventually a study was located where L-NOARG concentrations had been measured in healthy volunteers after a 60 minute intravenous infusion of L-NAME (Frandsen et al., 2001). The dose that had been administered was 4mg/kg, the same as the highest dose in the Wecht study, and concentrations were measured for 165 minutes. The Avontuur study measured plasma concentrations for a number of days and showed clearly that the L-NOARG profile in humans was biphasic, with a long terminal half-life, the same as seen in pre-clinical species. The short time frame of measurements in the Frandsen study would therefore only give information about the alpha phase of the profile. This would be sufficient for simulation of L-NOARG human plasma concentrations for the 4 hours over which the MBP and HR had been monitored in the Wecht study but would not be suitable for describing the profile beyond this time or for comparison of PK parameters between species.
3.5.1.2 Modelling of PK and baseline data

In both the Frandsen and Wecht studies, the data were reported graphically. Thus, to obtain the concentration/MBP/HR points each graph was digitized.

A 1 compartment model with proportional error was fitted to the concentration-time points from the Frandsen paper. The estimated parameter values were 0.381L/h/kg for CL and 1.67l/kg for V. These were precise estimates (RSE=3-8%) and proportional error was low at 5.25%. The fit of the data is shown in figure 3.46.

![Plot of observed (black circles) and predicted (solid line) plasma L-NOARG concentrations with time in healthy volunteers following administration of L-NAME. Data from Frandsen et al., (2001).](image)

Since MBP and HR measurements were only taken over a 4 hour time frame, this was not a long enough period of time to fit a circadian rhythm model. The placebo response data were therefore fitted using empirical polynomial functions, with the simple aim of describing the overall shape of the profiles.

The fits of the human baseline data are shown in figure 3.47. For the MBP data a 5th order polynomial produced the best fit and for HR a 3rd order polynomial was found to be sufficient. Parameters were estimated with excellent precision for MBP (RSE=1-4%) and with good to moderate precision for HR (RSE=21-34%). Additive residual errors were low in both cases (0.0069mmHg and 0.658bpm).
3.5.2 Predictions

Integrated PKPD models for prediction of human cardiovascular response to L-NAME were formed based on the models derived for each of the 3 pre-clinical species. PK parameters were fixed to those derived from the Frandsen study and baseline parameters were fixed to those derived from the modelling of placebo data. The only parameter that was altered was the value of MBP/HR at time 0, which was reset as the observed value for each dose.

Since unbound concentrations were necessary for the PKPD model, the predicted human L-NOARG plasma concentrations required conversion using the fraction unbound in plasma. Unfortunately this value does not appear to have been measured in human plasma. Fortunately L-NOARG is not a highly bound compound and values were reasonably consistent across species, therefore a mean value of the 3 species was used, which was 0.78.

3.5.2.1 Mean blood pressure

The prediction of L-NAME effect on human mean blood pressure was either described via an indirect model with stimulation of the production of response using an \( E_{\text{max}} \) function or via a direct \( E_{\text{max}} \) model. The former was used with PD parameters derived from either the rat or the dog study and the latter with values derived from the guinea-pig study. The \( k_{\text{out}} \) values were converted to equivalent human values using the single species scaling technique. These values are summarised in table 3.35.
Table 3.35: Summary of final MBP PD model parameters for all 3 species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Dog</th>
<th>Guinea-pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.357</td>
<td>0.195</td>
<td>0.719</td>
</tr>
<tr>
<td>EC$_{50}$ (µM)</td>
<td>44.8</td>
<td>21.6</td>
<td>105</td>
</tr>
<tr>
<td>$k_{\text{out}}$ (h$^{-1}$)</td>
<td>0.244</td>
<td>0.905</td>
<td>-</td>
</tr>
</tbody>
</table>

It can be seen from the table that there is no consistency in the values across the 3 species in addition to the difference in PD model required.

Figure 3.48: Prediction of observed human mean blood pressure (coloured circles) following administration of 0.5 (A), 1 (B), 2 (C) or 4 mg/kg (D) L-NAME, using the PD model and parameter estimates for rat (solid line), dog (dashed line) or guinea-pig (dotted-dashed line).

The predictions are shown in figure 3.48. As can be seen for the lower two doses (0.5 and 1mg/kg) there is actually little effect of L-NAME and so the predictions appear good. However, for the higher two doses, a large increase in mean blood pressure is observed, which is not captured by any of the three animal models. The guinea-pig appears to provide the best prediction at least with respect to the time course of the response, which appears to be direct in humans. This may be related to the route of
administration, which was intravenous infusion in both cases. It also produces the highest increase of the three models but still severely under-predicts the effect.

3.5.2.2 Heart rate

The prediction of L-NAME effect on human heart rate was either described via an indirect model with inhibition of the production of response using an $I_{\text{max}}$ function or via a direct $I_{\text{max}}$ model. The former was used with PD parameters derived from either the rat or the dog study and the latter with values derived from the guinea-pig study. The $k_{\text{out}}$ values were converted to equivalent human values using the single species scaling technique. These values are summarised in table 3.36.

Table 3.36: Final HR PD model parameters for all species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Dog</th>
<th>Guinea-pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{max}}$</td>
<td>0.145</td>
<td>0.422</td>
<td>0.0882</td>
</tr>
<tr>
<td>$IC_{50}$ (µM)</td>
<td>41</td>
<td>3.33</td>
<td>22.2</td>
</tr>
<tr>
<td>$k_{\text{out}}$ (h$^{-1}$)</td>
<td>0.77</td>
<td>1.32</td>
<td>-</td>
</tr>
</tbody>
</table>

As can be seen from the table there is no consistency in the values across the three species and in the case of the dog and guinea-pig, no consistency with the MBP values, although they are both lower. However, the potency values for the rat appear similar for the two effects (44.8 and 41µM).

The predictions are shown in figure 3.49. The model for rat and guinea-pig do not appear to show any real change from baseline for these doses. However the dog model appears to be able to predict the extent of human effect, if not the correct time course. It appears that once again in human L-NAME has a direct effect on heart rate, which is not captured by the dog model, however if the parameter value were used within a direct $I_{\text{max}}$ model, a good prediction could be achieved.
Figure 3.49: Prediction of observed human heart rate (coloured circles) following administration of 0.5 (A), 1 (B), 2 (C) or 4 mg/kg (D) L-NAME, using the PD model and parameter estimates for rat (solid line), dog (dashed line) or guinea-pig (dotted-dashed line).
Chapter 4
Milrinone
4 Milrinone

4.1 Introduction

Milrinone is a phosphodiesterase (PDE) III inhibitor, used in the treatment of congestive heart failure (CHF). Milrinone competes with the second messenger cyclic adenosine monophosphate (cAMP) for the PDE III enzyme and thus decreases its degradation (Honerjager, 1991). This results in an increased level of cAMP, which has a number of actions on the heart and vascular system. In cardiac muscle, cAMP activates protein kinase A (PKA), which in turn phosphorylates and activates L-type calcium channels and enhances release of calcium by the sarcoplasmic reticulum. This leads to an increased level of calcium entering the cell to activate the contractile proteins, thus increasing the contractile state of the myocardium. Increased levels of calcium also increase the diastolic depolarisation of sinoatrial pacemaker cells, which increases cardiac automaticity and thus heart rate. In the vascular smooth muscle, cAMP inhibits myosin light chain kinase (MLCK) the enzyme responsible for phosphorylating myosin, which causes contraction. Thus, an increase in cAMP has an opposing effect to that of cardiac muscle initiating relaxation of the muscle, leading to vasodilation and a subsequent decrease in blood pressure.

The PD effects of milrinone have been studied in patients with chronic heart failure (CHF) and in healthy subjects. The main effect observed in patients was an increase in cardiac output (CO; Benotti et al., 1985). Increases in heart rate and decreases in blood pressure were observed in healthy subjects (Larsson et al., 1986) and for higher doses in patients (Benotti et al., 1985). PD effects reported in dogs were increases in CO, HR and rate of rise of left ventricular pressure (a measure of contractility) and a decrease in BP (Alousi et al., 1986). In rats, an increase in HR and decrease in BP were observed (Verrijk et al., 1990).

In terms of PK characteristics, in healthy individuals volume and clearance are both low, resulting in a short terminal half life (Larsson et al., 1986; Stroshane et al., 1984). In CHF patients, clearance is markedly decreased leading to an increased t1/2 (Benotti et al., 1985; Edelson et al., 1986). Since milrinone is mainly excreted unchanged in the urine, a decreased renal blood flow due to severe cardiac impairment would result in a reduced renal clearance. Clearance and volume are also low for rats (Brocks et al., 2005) and dogs (Edelson et al., 1983).
4.2 Pharmacokinetic modelling of milrinone

4.2.1 Rat study

4.2.1.1 PK model development and refinement

Observed milrinone plasma concentrations with time displayed biphasic profiles for all animals, suggesting a 2-compartment model would be the most suitable fit to the data. This was confirmed via a drop in log-likelihood when compared to a 1-compartment model (-39.24, 2df, table 4.1), both with proportional error. Precision was poor for the structural parameters (RSE = 407-1x10⁶%), therefore further refinement was needed. Addition of inter-individual variability to all parameters did not result in a significant drop in the -2LL value but did improve the precision of all structural parameters except \( k_a \) to acceptable levels (RSE<50%). Poor precision and high shrinkage were also observed for the \( k_a \), CL/F and \( V_1/F \) omegas, therefore a model with variability only on \( Q/F \) and \( V_2/F \) was attempted. The drop in log-likelihood was significant for this model (-9.20, 2df), however precision for \( k_a \) and \( V_1/F \) was still unacceptable (RSE=66-88%).

Table 4.1: Overview of model selection for the rat milrinone PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt; 1abs; Prop err</td>
<td>876.45</td>
<td>884.45</td>
<td>-</td>
<td>Very high SE all</td>
</tr>
<tr>
<td>2cpt; 1abs; Prop err</td>
<td>837.21</td>
<td>849.21</td>
<td>Y</td>
<td>Very high SE all</td>
</tr>
<tr>
<td>2cpt; 1abs; P err; all ( \eta )</td>
<td>831.32</td>
<td>853.32</td>
<td>N</td>
<td>High SE ( k_a ); high SE &amp; shrinkage ( \omega ) ( k_a ) CL/F ( V_1/F )</td>
</tr>
<tr>
<td>2cpt; 1abs; P err; ( \eta )</td>
<td>828.01</td>
<td>844.01</td>
<td>Y</td>
<td>High SE ( k_a ), ( V_1/F )</td>
</tr>
<tr>
<td>2cpt; 1abs; Comb err</td>
<td>834.11</td>
<td>848.11</td>
<td>N</td>
<td>V. high SE all</td>
</tr>
<tr>
<td>2cpt; 1abs; C err; all ( \eta )</td>
<td>831.13</td>
<td>855.13</td>
<td>N</td>
<td>High SE ( k_a ), ( a ); high SE &amp; shrinkage ( \omega ) ( k_a ) CL/F ( V_1/F )</td>
</tr>
<tr>
<td>2cpt; 1abs; Add err</td>
<td>995.38</td>
<td>1007.38</td>
<td>-</td>
<td>Implausible ( k_a ); v. high SE all</td>
</tr>
<tr>
<td>2cpt; 1abs; A err; all ( \eta )</td>
<td>913.49</td>
<td>935.49</td>
<td>Y</td>
<td>High SE &amp; shrinkage ( \omega ) CL/F ( V_1/F )</td>
</tr>
<tr>
<td>2cpt; 1abs; ( \eta ) ( k_a ) Q/F ( V_2/F )</td>
<td><strong>914.07</strong></td>
<td><strong>932.07</strong></td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

* Significant drop in -2LL compared to previously best nested model

Use of a combined residual error did not improve the model according to the fit statistics. In addition, once IIV was introduced, the additive part of the error model fell to a very small value with poor precision, effectively reducing the model to that with proportional error. Additive residual error was then applied in an attempt to improve the
model. The fit statistics did not show this to be a better model and without inter-individual variability the $k_a$ value was implausible (12200h$^{-1}$) and precision for all parameters extremely poor (RSE=$5\times10^4$-$7\times10^8\%$). However, the addition of variability for all structural parameters resulted in precise population estimates and a significant drop in the -2LL value (-81.89, 5df). The omegas for CL/F and $V_1/F$ were however imprecise (RSE=$203$-$645\%$) and had very high shrinkage (83-99\%). Removal of the etas for these two parameters resulted in a similar log-likelihood value and no other issues.

4.2.1.2 Final PK model fit

The final PK model selected for the rat milrinone PK data was a 2-compartment, first-order absorption model with inter-individual variability on $k_a$, Q/F and $V_2/F$, and additive residual error. Parameter estimates for the final model are presented in table 4.2. Structural parameters were predicted with good to moderate precision (RSE=4-30\%). Inter-individual variability for Q/F was high at 71\% but moderate for $k_a$ and $V_2/F$ (27-37\%).

Table 4.2: Parameter estimates for the final rat milrinone PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>9.54</td>
<td>29</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>1.12</td>
<td>4</td>
</tr>
<tr>
<td>$V_1/F$ (L/kg)</td>
<td>1.62</td>
<td>12</td>
</tr>
<tr>
<td>Q/F (L/h/kg)</td>
<td>4.83</td>
<td>30</td>
</tr>
<tr>
<td>$V_2/F$ (L/kg)</td>
<td>3.28</td>
<td>14</td>
</tr>
<tr>
<td>Inter-individual variability in $k_a$ (%)$^b$</td>
<td>37</td>
<td>89</td>
</tr>
<tr>
<td>Inter-individual variability in Q/F (%)</td>
<td>71</td>
<td>59</td>
</tr>
<tr>
<td>Inter-individual variability in $V_2/F$ (%)</td>
<td>27</td>
<td>73</td>
</tr>
<tr>
<td>Additive residual error (nM)</td>
<td>338</td>
<td>11</td>
</tr>
</tbody>
</table>

$^a$ Relative standard error (RSE), expressed as a percentage is calculated as SE divided by the parameter estimate x 100

$^b$ Inter-individual variability is taken as an approximate CV (%), calculated as the square root of variance x 100

The diagnostic plots for this model (figures 4.2 and 4.3) show a good fit to the data for both the population and individual models. The scatter plots of predicted against observed concentrations display an even distribution around the line of unity with very little deviation in the case of the individual model. Weighted residuals are evenly distributed around zero, with the majority falling within ±2 standard deviations. Plots of
individual predicted concentration-time profiles give a good description of the observed data for all 3 doses (figure 4.1).

Figure 4.1: Individual plots of observed (grey circles) and predicted (dashed line) plasma concentrations with time in rats following doses of 0.35 (ID 1-3), 3.5 (ID 4-6) or 10.5mg/kg (ID 7-9) milrinone.

Figure 4.2: Observed versus predicted plasma concentrations for the final rat milrinone PK model using population (A) or individual (B) parameters.
Chapter 4: Milrinone

4.2.2 Dog study

4.2.2.1 PK model selection

The mono-phasic nature of the individual plasma concentration-time profiles indicated that a 1-compartment model would be sufficient for a good fit. To confirm this, 1- and 2-compartment models with 1st order absorption were analysed with a proportional error model. The 2-compartment model did not produce a significant drop in log-likelihood (-0.14, 2df; table 4.3), confirming the 1-compartment model was satisfactory. To further refine the model, inter-individual variability was applied to all structural parameters. This again did not result in a significant drop in -2LL value (-5.45, 3df) but the eta for V/F displayed very high shrinkage (96%) and was estimated poorly (RSE=1300%). Removal of this eta improved the model but was still not quite significant with respect to the log-likelihood (-5.48, 2df). Poor precision (RSE=123%) and moderate shrinkage (47%) was associated with the $k_a$ variability, therefore this eta was removed in a final attempt to improve the model. A model with inter-individual variability only on clearance produced a significant drop in log-likelihood when compared to the base model (-4.99, 1df) and no issues with parameter precision.

Table 4.3: Overview of model selection for the dog milrinone PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt; 1abs</td>
<td>-11.61</td>
<td>-3.61</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2cpt; 1abs</td>
<td>-11.75</td>
<td>0.25</td>
<td>N</td>
<td>High SE all</td>
</tr>
<tr>
<td>1cpt; 1abs; all $\eta$</td>
<td>-17.06</td>
<td>-3.06</td>
<td>N</td>
<td>V. high SE &amp; shrinkage $\omega$ V/F</td>
</tr>
<tr>
<td>1cpt; 1abs; $\eta$ $k_a$ CL/F</td>
<td>-17.09</td>
<td>-5.09</td>
<td>N</td>
<td>Moderate SE &amp; shrinkage $\omega$ $k_a$</td>
</tr>
<tr>
<td>1cpt; 1abs; $\eta$ CL/F</td>
<td><strong>-16.60</strong></td>
<td><strong>-6.60</strong></td>
<td><strong>Y</strong></td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>
4.2.2.2 Final PK model fit

The final PK model selected for the dog milrinone PK data was a 1-compartment, first-order absorption model with inter-individual variability on CL/F, and proportional residual error. Parameter estimates for the final model are presented in table 4.4 and the individual estimates of CL/F presented in table 4.5. Structural parameters were predicted with good precision (RSE=7-26%). Inter-individual variability for CL/F was moderate at 30% and proportional residual error reasonably low at 16.6%.

Table 4.4: Parameter estimates for the final dog milrinone PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>6.14</td>
<td>26</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>0.267</td>
<td>17</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>0.829</td>
<td>7</td>
</tr>
<tr>
<td>Inter-individual variability in CL/F (%)</td>
<td>30</td>
<td>84</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>16.6</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 4.5: Individual CL/F estimates for the final dog milrinone PK model

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (L/h/kg)</td>
<td>0.223</td>
<td>0.204</td>
<td>0.352</td>
<td>0.371</td>
</tr>
</tbody>
</table>

Figure 4.4: Observed versus predicted plasma concentrations for the final dog milrinone PK model using population (A) or individual (B) parameters.

The diagnostic plots for this model (figures 4.4 and 4.5) show an acceptable fit to the data for both the population and individual models. The scatter plots of predicted against observed concentrations display an even distribution around the line of unity. Weighted residuals are evenly distributed around zero, with the majority falling within
±2 standard deviations. Plots of individual predicted concentration-time profiles give a good description of the observed data (figure 4.6).

**Figure 4.5**: Population weighted residuals (PWRES) versus time (A) or predicted concentration (B) for the final dog milrinone PK model.

**Figure 4.6**: Individual plots of observed (grey circles) and predicted (dashed line) plasma concentrations with time in dogs following a dose of 0.1mg/kg milrinone.

### 4.2.3 Guinea-pig study

#### 4.2.3.1 PK model selection

As for the L-NAME study, only 1 concentration per dose was measured at the same time point for each animal. Since there was not enough information to describe a full PK profile a 1-compartment model had to be assumed. A 1-compartment model with proportional error described the data sufficiently. In an attempt to improve the fit, inter-individual variability on both structural parameters was added. This resulted in a
significant reduction in the -2LL value (-14.3, 2df, table 4.6) however the omega for V was imprecise (RSE=234%) and displayed moderate shrinkage (62%). Removal of the eta for V produced a better model with respect to the fit statistics and had no issues with respect to parameter precision.

Table 4.6: Overview of model selection for the guinea-pig milrinone PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt</td>
<td>373.72</td>
<td>379.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1cpt; all η</td>
<td>359.42</td>
<td>369.42</td>
<td>Y</td>
<td>High SE &amp; mod shrinkage ω V</td>
</tr>
<tr>
<td>1cpt; η CL</td>
<td>359.52</td>
<td>367.52</td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

4.2.3.2 Final PK model fit

The final PK model selected for the guinea-pig milrinone PK data was a 1-compartment model with inter-individual variability on CL and proportional residual error. Parameter estimates for the final model are presented in table 4.7 and the individual estimates of CL presented in table 4.8. The structural parameters were predicted with good precision (RSE=12-20%) and inter-individual variability in CL was fairly low at 17%. Proportional residual error was also relatively low at 9.5%.

Table 4.7: Parameter estimates for the final guinea-pig milrinone PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/min/kg)</td>
<td>0.0197</td>
<td>12</td>
</tr>
<tr>
<td>V (L/kg)</td>
<td>0.217</td>
<td>20</td>
</tr>
<tr>
<td>Inter-individual variability in CL (%)</td>
<td>17</td>
<td>58</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>9.51</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 4.8: Individual estimates of CL for the final guinea-pig milrinone PK model

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP1</th>
<th>GP2</th>
<th>GP3</th>
<th>GP4</th>
<th>GP5</th>
<th>GP6</th>
<th>GP7</th>
<th>GP8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/min/kg)</td>
<td>0.0189</td>
<td>0.0150</td>
<td>0.0219</td>
<td>0.00192</td>
<td>0.0257</td>
<td>0.0186</td>
<td>0.0177</td>
<td>0.0239</td>
</tr>
</tbody>
</table>

The diagnostic plots (figures 4.7 and 4.9) show an acceptable fit to the data. The scatter plots of predicated against observed concentrations overall display an even distribution around the line of unity, however there does appear to be some bias towards over-prediction for the higher concentrations. This can also be observed in the weighted residual plots, but despite this virtually all points fall within ±2 standard deviations.
Individual predicted concentration-time profiles give a good overall description of the observed data (figure 4.8).

Figure 4.7: Observed versus predicted plasma concentrations for the final guinea-pig milrinone PK model using population (A) or individual (B) parameters.

Figure 4.8: Individual plots of observed (grey circles) and predicted (dashed line) plasma concentrations with time in guinea-pigs administered multiple doses of milrinone.
4.3 Modelling of baseline cardiovascular response

Baseline models for each species were developed as a joint analysis using all vehicle data collected during the studies for all three compounds. Therefore, all details of model development are described in section 3.3 and only details of the relevant individual animals’ parameter values and fits are described in this section.

4.3.1 Rat study

4.3.1.1 Mean blood pressure

As described in section 3.3.1.1.2, the best fit to the rat mean blood pressure baseline data was given by the Sallstrom model with administration effect and inter-individual variability on $R_{av}$, gamma, delta, P and $k_{Adm}$. Individual parameter values for the rats used in the milrinone study are given in table 4.9. Parameter values for $R_{amp}$ and D were the joint analysis population estimates of 0.109 and 0.0418 respectively.

Table 4.9: Individual estimates of the final mean blood pressure baseline model parameters for rats used in the milrinone study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
<th>Rat 5</th>
<th>Rat 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (mmHg)</td>
<td>101</td>
<td>97.7</td>
<td>111</td>
<td>100</td>
<td>123</td>
<td>107</td>
</tr>
<tr>
<td>$\gamma$ (min$^{-1}$)</td>
<td>0.0407</td>
<td>0.0267</td>
<td>0.0697</td>
<td>0.0174</td>
<td>0.0104</td>
<td>0.0415</td>
</tr>
<tr>
<td>$\delta$ (min$^{-1}$)</td>
<td>0.0024</td>
<td>0.00216</td>
<td>0.00533</td>
<td>0.00205</td>
<td>0.00210</td>
<td>0.00213</td>
</tr>
<tr>
<td>P</td>
<td>0.514</td>
<td>0.500</td>
<td>0.503</td>
<td>0.542</td>
<td>0.531</td>
<td>0.534</td>
</tr>
<tr>
<td>$k_{Adm}$ (min$^{-1}$)</td>
<td>0.0872</td>
<td>0.0819</td>
<td>0.0904</td>
<td>0.0655</td>
<td>0.128</td>
<td>0.0868</td>
</tr>
</tbody>
</table>
The majority of the parameter estimates for these animals fall in line with the joint analysis population estimates and associated variability, although the high level of variability in gamma is not seen for this subset of individuals. The majority of $k_{Adm}$ values are also lower than the joint analysis population estimate, indicating a slower onset/offset of the administration effect than on average.

Individual predicted mean blood pressure-time profiles for the milrinone study rats are shown in figure 4.10. Overall they give a good description of the observed data.

![Figure 4.10: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time following administration of vehicle to rats as part of the milrinone study.](image)

4.3.1.2 Heart rate

As described in section 3.3.1.2.2, the best fit to the rat heart rate baseline data was given by the Sallstrom model with administration effect and inter-individual variability on $R_{av}$, gamma, delta, D, P and $k_{Adm}$. Individual parameter values for the rats used in the milrinone study are given in table 4.10. The $R_{amp}$ joint analysis population estimate of 0.266 was fixed for all animals. In general the values for the animals used in the milrinone study are in agreement with the joint analysis population estimates and their variabilities. The only exception is that the values for P are all higher than the joint analysis population estimate, indicating that the administration effect is more pronounced in these individual animals. This large effect can be observed in the individual predicted heart rate-time profiles (figure 4.11), which give a good overall description of the observed data for these animals.
Table 4.10: Individual estimates of the final rat heart rate baseline model parameters for rats used in the milrinone study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
<th>Rat 5</th>
<th>Rat 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (bpm)</td>
<td>354</td>
<td>371</td>
<td>376</td>
<td>374</td>
<td>343</td>
<td>345</td>
</tr>
<tr>
<td>$\gamma$ (min$^{-1}$)</td>
<td>0.167</td>
<td>0.119</td>
<td>0.167</td>
<td>0.0783</td>
<td>0.155</td>
<td>0.176</td>
</tr>
<tr>
<td>$\delta$ (min$^{-1}$)</td>
<td>0.00313</td>
<td>0.00271</td>
<td>0.00404</td>
<td>0.00288</td>
<td>0.00417</td>
<td>0.00396</td>
</tr>
<tr>
<td>D</td>
<td>0.0641</td>
<td>0.0478</td>
<td>0.0233</td>
<td>0.00793</td>
<td>0.0322</td>
<td>0.0156</td>
</tr>
<tr>
<td>P</td>
<td>0.874</td>
<td>0.856</td>
<td>1.24</td>
<td>1.2</td>
<td>1.05</td>
<td>1.58</td>
</tr>
<tr>
<td>$k_{Adm}$ (min$^{-1}$)</td>
<td>0.0644</td>
<td>0.0582</td>
<td>0.0777</td>
<td>0.0564</td>
<td>0.0937</td>
<td>0.0679</td>
</tr>
</tbody>
</table>

Figure 4.11: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time following administration of vehicle to rats as part of the milrinone study.

4.3.2 Dog study

4.3.2.1 Mean blood pressure

As described in section 3.3.2.1.2, the best fit to the dog mean blood pressure baseline data was given by a single cosine function model with 24 hour period and inter-individual variability on all three structural parameters ($R_{av}$, $R_{amp}$ and $T_z$). Individual parameter values for the dogs used in the milrinone study are given in table 4.11. The values for $R_{av}$ fall into line with that of the joint analysis population estimate and its variability. $R_{amp}$ values are all higher than the joint analysis population estimate, indicating that for these animals a higher degree of change in mean blood pressure was observed over the 24 hour period. $T_z$ values for 3 of the individuals agree well with the joint analysis population estimate but for dog 2 the value was much higher. The profile
for this individual dog (see figure 4.13, ID=6) displayed a completely different profile to the others used in the 3 different studies, with an almost opposite circadian rhythm (higher during the night as opposed to the day). The huge difference in timing of peak blood pressure for this individual explains most of the high variability associated with $T_z$ in the model.

Table 4.11: Individual estimates of the final dog mean blood pressure baseline model parameters for dogs used in the milrinone study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (mmHg)</td>
<td>91.5</td>
<td>104</td>
<td>99.6</td>
<td>108</td>
</tr>
<tr>
<td>$R_{amp}$ (mmHg)</td>
<td>4.32</td>
<td>4.75</td>
<td>5.02</td>
<td>4.44</td>
</tr>
<tr>
<td>$T_z$ (h)</td>
<td>2.95</td>
<td>18.1</td>
<td>4.46</td>
<td>3.86</td>
</tr>
</tbody>
</table>

Individual predicted mean blood pressure-time profiles (figure 4.12) give a good overall description of the observed data for these animals.

Figure 4.12: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time following administration of vehicle to dogs as part of the milrinone study.

4.3.2.2 Heart rate

As described in section 3.3.2.2.2, the best fit to the dog heart rate baseline data was given by a dual cosine function model with 24 and 12 hour periods and administration effect and inter-individual variability on $R_{av}$, $R_{amp24}$, $T_{z24}$ and $P$. Individual parameter
values for the dogs used in the milrinone study are given in Table 4.12. Parameter values for $R_{\text{amp12}}$, $T_{z12}$ and $k_{\text{Adm}}$ were the joint analysis population estimates of 2.39, 23.6h and 9.14h$^{-1}$ respectively.

Table 4.12: Individual estimates of the final dog heart baseline model parameters for dogs used in the milrinone study.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{av}}$ (bpm)</td>
<td>70.8</td>
<td>67.1</td>
<td>66.9</td>
<td>76.3</td>
</tr>
<tr>
<td>$R_{\text{amp24}}$ (bpm)</td>
<td>5.35</td>
<td>12.0</td>
<td>6.96</td>
<td>14.6</td>
</tr>
<tr>
<td>$T_{z24}$ (h)</td>
<td>11.0</td>
<td>11.4</td>
<td>11.6</td>
<td>13.1</td>
</tr>
<tr>
<td>$P$</td>
<td>1.24</td>
<td>3.67</td>
<td>1.67</td>
<td>2.46</td>
</tr>
</tbody>
</table>

Figure 4.13: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time following administration of vehicle to dogs as part of the milrinone study.

In general the values for the animals used in the milrinone study are in agreement with the joint analysis population estimates and their variabilities. However, $R_{\text{av}}$ values were all lower than the joint analysis population value, indicating in general the heart rate for these animals was lower than the typical dog. Also $R_{\text{amp24}}$ values for these animals were all higher than the joint analysis population estimate, indicating the degree of change in heart rate observed over the 24 hour period was greater for these dogs. This was particularly the case for dogs 2 & 4, which had values at least twice the joint analysis population estimate. Variability for this parameter was particularly high though and similar values were observed for the dogs used in the L-NAME study. The same two
individual dogs also had values for P that were much higher than the joint analysis population estimate, indicating that the administration effect is more pronounced in these individual animals. This large effect can be observed in the individual predicted heart rate-time profiles (figure 4.13, ID6 & 8), which give a good overall description of the observed data for these animals.

### 4.3.3 Guinea-pig study

#### 4.3.3.1 Mean blood pressure

As described in section 3.3.3.1.2, the best fit to the guinea-pig mean blood pressure baseline data was given by a linear model with inter-individual variability on both $R_{\text{Int}}$ and $R_{\text{slope}}$. Data from both vehicle treated animals and pre-dose data for dosed animals was used in the joint analysis. Individual parameter values for the guinea-pigs dosed with milrinone (derived from the pre-dose data) are given in table 4.13.

Table 4.13: Individual estimates of the final MBP baseline model parameters for guinea-pigs used in the milrinone study

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP 1</th>
<th>GP 2</th>
<th>GP 3</th>
<th>GP 4</th>
<th>GP 5</th>
<th>GP 6</th>
<th>GP 7</th>
<th>GP 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{Int}}$ (mmHg)</td>
<td>40.3</td>
<td>35.9</td>
<td>41.9</td>
<td>52.1</td>
<td>39.8</td>
<td>35.1</td>
<td>53.2</td>
<td>47.2</td>
</tr>
<tr>
<td>$R_{\text{slope}}$ (mmHg/min)</td>
<td>-0.025</td>
<td>-0.047</td>
<td>-0.079</td>
<td>-0.113</td>
<td>-0.023</td>
<td>-0.072</td>
<td>-0.058</td>
<td>-0.115</td>
</tr>
</tbody>
</table>

Figure 4.14: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time prior to administration of milrinone in guinea-pigs.

$R_{\text{Int}}$ values were consistent with the joint analysis population estimate and its variability. $R_{\text{slope}}$ values were all negative for this set of animals. This was unlike all the other groups, where the change in mean blood pressure increased with time in some animals.
and decreased with time in others. Individual predicted mean blood pressure-time profiles (figure 4.14) give a good overall description of the observed data for these animals and it can be observed that the decrease with time is minimal in most cases.

Predicted mean blood pressure-time profiles for the vehicle treated animals used in the milrinone study are shown in figure 4.15. The linear pattern with time can be clearly seen for most individuals, except possibly individual V2, where there is some inconsistency of the middle time points. Profiles both increase and decrease with time and to different extents as seen across the individual animals used in all three studies.

![Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time following administration of vehicle to guinea-pigs as part of the milrinone study.](image)

Figure 4.15: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time following administration of vehicle to guinea-pigs as part of the milrinone study.

### 4.3.3.2 Heart rate

As described in section 3.3.3.2.2, the best fit to the guinea-pig heart rate baseline data was given by a linear model with inter-individual variability on both $R_{\text{int}}$ and $R_{\text{slope}}$. Once again, data from both vehicle treated animals and the pre-dose data for dosed animals was used in the joint analysis. Individual parameter values for the guinea-pigs dosed with milrinone (derived from the pre-dose data) are given in table 4.14. Both $R_{\text{int}}$ and $R_{\text{slope}}$ values were consistent with the joint analysis population estimates, although less variability was seen for the $R_{\text{slope}}$ values. As was observed with these individuals a decrease in heart rate with time was observed for the majority of guinea-pigs studied. Individual predicted heart rate-time profiles (figure 4.16) give a good overall description of the observed data for these animals.
Table 4.14: Individual estimates of the final HR baseline model parameters for guinea-pigs used in the milrinone study

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP 1</th>
<th>GP 2</th>
<th>GP 3</th>
<th>GP 4</th>
<th>GP 5</th>
<th>GP 6</th>
<th>GP 7</th>
<th>GP 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{Int}}$ (bpm)</td>
<td>240</td>
<td>218</td>
<td>248</td>
<td>274</td>
<td>256</td>
<td>241</td>
<td>240</td>
<td>261</td>
</tr>
<tr>
<td>$R_{\text{Slope}}$ (bpm/min)</td>
<td>-0.213</td>
<td>-0.386</td>
<td>-0.359</td>
<td>-0.205</td>
<td>-0.277</td>
<td>-0.577</td>
<td>-0.635</td>
<td>-0.715</td>
</tr>
</tbody>
</table>

Figure 4.16: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time prior to administration of milrinone in guinea-pigs.

Figure 4.17: Individual plots of observed (circles) and predicted (line) heart rate with time following vehicle administration to guinea-pigs as part of the milrinone study.

Predicted heart rate-time profiles for the vehicle treated animals used in the milrinone study are shown in figure 4.17. The linear pattern with time can once again be seen for most individuals, except individuals V2 and V4, where heart rate appears to fluctuate over time. Profiles all decrease with time but to very different extents, for a couple of
individuals (V4 and V7) the slope is so slight it appears almost constant ($R_{\text{slope}} = -0.003$ and -0.004 respectively).

### 4.4 Pharmacodynamic modelling of the cardiovascular response to milrinone

The cardiovascular responses to milrinone in all three animal models were a decrease in mean blood pressure and an associated increase in heart rate. This is in agreement with what is known about the mechanism of action of milrinone (see section 4.1). Integrated PKPD models with fixed PK and baseline parameters and appropriate inhibitory or stimulatory effect functions were used to model the data.

#### 4.4.1 Rat study

Milrinone plasma concentrations were collected from different individual animals to those in which cardiovascular responses were measured. This meant that the population parameters from the PK model were fixed for all individuals in the PKPD model. Cardiovascular responses following the administration of vehicle however had been measured in the same individual animals as those used to measure response in the presence of milrinone. Therefore individual baseline parameters were fixed for each rat. It was however noted that on average heart rate and mean blood pressure could vary between the different doses, despite the actual effect. In this scenario, usually an interoccasion variability could be applied to a parameter to take account for this. Unfortunately, this caused issues for Monolix. To get around the problem, each dosing occasion was treated as a different individual, all with individual baseline parameters still fixed. The $R_{\text{av}}$ parameter was then allowed to vary for each individual. The resulting eta would thus be a combination of inter-individual and inter-occasion variability.

#### 4.4.1.1 Mean blood pressure

##### 4.4.1.1.1 PD model selection

Comparison of the time courses of the rat milrinone PK and MBP PD data indicated a slight delay in the response profile in relation to that of the concentration profile. To confirm this, the fit of a direct $I_{\text{max}}$ model was compared with an effect compartment model and an indirect response model with inhibition of $k_{\text{in}}$, to see which best described the profiles. It was clear from this that was the direct model did predict the peak effect too early. Comparison of the fit statistics (table 4.15) indicated that the indirect model was superior to that of the effect compartment model and thus was selected for further
refinement. Addition of inter-individual variability to all parameters produced a significant drop in log-likelihood (-39.2, 3df). However the omega for $I_{\text{max}}$ was estimated with poor precision (RSE=116%) and had high shrinkage (94%). Removal of the eta for $I_{\text{max}}$ improved the model with a very similar -2LL value. However, the estimate for $I_{\text{max}}$ was 1, which would indicate the maximum effect of the drug would result in a blood pressure of zero. Since the actual effect seen for the doses was small and the fact that this degree of effect is also physiologically improbable (due to feedback mechanisms), it seemed likely that a linear function would be more appropriate. An indirect model with a linear function resulted in an improved fit according to the goodness of fit statistics. To further improve the model, inter-individual variability was applied to both structural parameters, which resulted in a significant drop in log-likelihood (-40.25, 2df).

Table 4.15: Overview of model selection for the rat milrinone MBP PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $I_{\text{max}}$; no $\eta$</td>
<td>12558.15</td>
<td>12567.15</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $I_{\text{max}}$; no $\eta$</td>
<td>12556.70</td>
<td>12568.70</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no $\eta$</td>
<td>12477.01</td>
<td>12489.01</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; all $\eta$</td>
<td>12437.81</td>
<td>12455.81</td>
<td>Y</td>
<td>High SE &amp; shrinkage $\omega I_{\text{max}}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$ model; no $\eta I_{\text{max}}$</td>
<td>12437.05</td>
<td>12453.05</td>
<td>Y</td>
<td>$I_{\text{max}} = 1$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{slope}}$; no $\eta$</td>
<td>12475.60</td>
<td>12485.60</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td><strong>Indirect; $I_{\text{slope}}$; all $\eta$</strong></td>
<td><strong>12435.35</strong></td>
<td><strong>12449.35</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

4.4.1.1.2 Final PD model fit

The final milrinone rat mean blood pressure PD model was selected as an indirect response model with inhibitory effect on the production of response described via a linear model, with inter-individual variability on $I_{\text{slope}}$ and $k_{\text{out}}$, and additive residual error. Structural parameters were estimated with good precision (RSE=12-24%; table 4.16) and additive residual error was low. Variability for $I_{\text{slope}}$ was relatively low but for $k_{\text{out}}$ was moderately high. This suggests a varied turnover and hence time course of the response. Although some of the variability is real, it must be noted that it may be inflated due to the approach taken where each dose is treated as a separate individual. Changes from baseline unrelated to drug effect may be captured by the $k_{\text{out}}$ parameter, leading to over-estimation of variability.
Table 4.16: Parameter estimates for the final rat milrinone MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{slope}}$ ($\mu\text{M}^{-1}$)</td>
<td>0.0478</td>
<td>12</td>
</tr>
<tr>
<td>$k_{\text{out}}$ (min$^{-1}$)</td>
<td>0.0359</td>
<td>24</td>
</tr>
<tr>
<td>Inter-individual variability in $I_{\text{slope}}$ (%)</td>
<td>33</td>
<td>58</td>
</tr>
<tr>
<td>Inter-individual variability in $k_{\text{out}}$ (%)</td>
<td>73</td>
<td>53</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>5.06</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 4.18: Observed versus predicted mean blood pressure for the final rat milrinone MBP PD model using population (A) or individual (B) parameters.

Figure 4.19: Population weighted residuals (PWRES) versus time (A) or mean blood pressure (B) for the final rat milrinone MBP PD model.

Diagnostic plots show a good fit to the data (figures 4.18 and 4.19). The scatter plots of predicted against observed mean blood pressure display an even distribution of points around the line of unity and not too much deviation from the line in the case of the individual predictions.
Figure 4.20: Individual plots of observed (circles) and predicted (line) mean blood pressure with time in rats following administration of 0.35(LD), 3.5(MD) or 10.5(HD) mg/kg milrinone.
Weighted residuals are evenly distributed around zero with no observable pattern indicative of model misspecification. A good proportion of the points also fall within ±2 standard deviations. Individual predicted mean blood pressure-time profiles give a good overall description of the observed data and the drug effect (figure 4.20).

4.4.1.2 Heart rate

4.4.1.2.1 PD model selection

Due to the large spike in HR caused by the administration effect, it was difficult to tell whether there was any delay in the response profile in relation to that of the concentration profile. To investigate the best model, the fit of a direct $E_{\text{max}}$ model was compared with an effect compartment model and an indirect response model with stimulation of $k_{in}$. The direct model did not describe the time course well, indicating that one of the other models would be required. Comparison of the fit statistics (table 4.17) indicated that the indirect model was superior to that of the effect compartment model and thus was selected for further refinement.

Table 4.17: Overview of model selection for the rat milrinone HR PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $E_{\text{max}}$; no $\eta$</td>
<td>18806.23</td>
<td>18816.23</td>
<td>-</td>
<td>Implausible estimates; very high SE</td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; no $\eta$</td>
<td>18712.79</td>
<td>18724.79</td>
<td>-</td>
<td>Lower AIC; Implausible estimates; very high SE</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; no $\eta$</td>
<td>18689.73</td>
<td>18701.73</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; all $\eta$</td>
<td>18644.16</td>
<td>18662.16</td>
<td>Y</td>
<td>High SE &amp; shrinkage $\omega$ $E_{\text{max}}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$ model; no $\eta E_{\text{max}}$</td>
<td>18645.37</td>
<td>18661.37</td>
<td>Y</td>
<td>Implausible $E_{\text{max}}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{slope}}$; no $\eta$</td>
<td>18689.77</td>
<td>18699.77</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td><strong>Indirect; $E_{\text{slope}}$; all $\eta$</strong></td>
<td><strong>18635.89</strong></td>
<td><strong>18649.89</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

Addition of inter-individual variability to all parameters produced a significant drop in log-likelihood (-45.57, 3df). However once again the omega for $E_{\text{max}}$ was estimated with poor precision (RSE=247%) and had high shrinkage (93%). Removal of the eta for $E_{\text{max}}$ improved the model with only a small increase in log-likelihood. However, once again there were issues with the maximum effect value, which was estimated at 2.09. This value is implausible since it would result in heart rate values in excess of 1000bpm, beyond the physiological limit for maximum heart rate in the rat. There was actually only a very small effect seen for the dose range studied and therefore likely to
be in the linear portion of the concentration effect curve. Consequently, it would appear that the model was trying to fit efficacy and potency values that would result in this slope and a linear function would be more appropriate. A fit of the indirect model with a linear function resulted in an improved model with a fall in AIC value. Addition of inter-individual variability improved the model further, with a significant reduction in log-likelihood (-53.88, 2df).

4.4.1.2.2 Final PD model fit

The final milrinone rat heart rate PD model was selected as an indirect response model with stimulatory effect on the production of response described via a linear model, with inter-individual variability on $E_{\text{slope}}$ and $k_{\text{out}}$, and additive residual error. Structural parameters were estimated with good to moderate precision (RSE=15-41%; table 4.18) and additive residual error was low.

Table 4.18: Parameter estimates for the final rat milrinone HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{slope}}$ (µM$^{-1}$)</td>
<td>0.113</td>
<td>15</td>
</tr>
<tr>
<td>$k_{\text{out}}$ (min$^{-1}$)</td>
<td>0.0145</td>
<td>41</td>
</tr>
<tr>
<td>Inter-individual variability in $E_{\text{slope}}$ (%)</td>
<td>44</td>
<td>51</td>
</tr>
<tr>
<td>Inter-individual variability in $k_{\text{out}}$ (%)</td>
<td>140</td>
<td>46</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>23.7</td>
<td>2</td>
</tr>
</tbody>
</table>

Variability for $E_{\text{slope}}$ was again relatively low but for $k_{\text{out}}$ was high, again indicating a varied time course for the response. As for MBP, some of the changes unrelated to drug effect could be captured by the $k_{\text{out}}$ parameter. It appears from the individual plots (figure 4.21) that the fit for a few of the individuals is not ideal. It is possible that the fixed baseline parameters are preventing a good fit and if allowed to vary could improve it. However, this may cause further fitting issues as well due to the complexity of the baseline model parameters.

Diagnostic plots show an acceptable fit to the data (figures 4.22 and 4.23). The scatter plots of predicted against observed mean blood pressure display an even distribution of points around the line of unity. Weighted residuals are evenly distributed around zero and a good proportion of the points also fall within ±2 standard deviations.
Figure 4.21: Individual plots of observed (circles) and predicted (line) heart rate with time in rats following administration of 0.35(LD), 3.5(MD) or 10.5(HD) mg/kg milrinone.
4.4.2 Dog study

The same individual animals were used to collect the data on milrinone plasma concentrations and the cardiovascular response to both vehicle and milrinone. Therefore individual estimates of baseline and PK parameters were fixed in the integrated PKPD model. The study design used had allowed a washout of 72 hours between doses, which was sufficient. The different doses could therefore be modelled as different occasions, as opposed to the continuous timeframe used for L-NAME. Unfortunately due to the issues with modelling different occasions in Monolix, each dose had to be treated as a different individual. The PK and baseline parameters were kept fixed to each individual’s values, however to account for any differences in average blood pressure or heart rate the $R_{av}$ values were once again allowed to vary.
4.4.2.1 Mean blood pressure

4.4.2.1.1 PD model selection

As for the rat, comparison of the milrinone plasma concentration and response time courses indicated there was a slight delay in mean blood pressure response. This was confirmed through comparison of the fit of a direct $I_{\text{max}}$ model to an effect compartment model and an indirect response model with inhibition of $k_{\text{in}}$. Peak mean blood pressure response predicted by the direct model appeared too early. An administration effect was also observed for some of the animals but a function was not included as the associated parameters $P$ and $k_{\text{Adm}}$ could not be precisely estimated. This is possibly due to the lack of time points in the short time frame over which it occurred (less than 0.5 hours).

Table 4.19: Overview of model selection for the dog milrinone MBP PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $I_{\text{max}}$, no $\eta$</td>
<td>8819.29</td>
<td>8829.29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $I_{\text{max}}$, no $\eta$</td>
<td>8772.36</td>
<td>8784.36</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$, no $\eta$</td>
<td>8706.50</td>
<td>8718.50</td>
<td>- Lower AIC</td>
<td></td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$, all $\eta$</td>
<td>8686.06</td>
<td>8704.06</td>
<td>Y High SE &amp; shrinkage $\omega$ $I_{\text{max}}, k_{\text{out}}$</td>
<td></td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$ model; no $\eta$ $I_{\text{max}}, k_{\text{out}}$</td>
<td>8685.54</td>
<td>8699.65</td>
<td>Y $I_{\text{max}} = 1$</td>
<td></td>
</tr>
<tr>
<td>Indirect; $I_{\text{slope}}$, no $\eta$</td>
<td>8702.69</td>
<td>8712.69</td>
<td>- Lower AIC</td>
<td></td>
</tr>
<tr>
<td>Indirect; $I_{\text{slope}}$, all $\eta$</td>
<td>8684.42</td>
<td>8698.42</td>
<td>Y High SE &amp; shrinkage $\omega$ $k_{\text{out}}$</td>
<td></td>
</tr>
<tr>
<td><strong>Indirect; $I_{\text{slope}}$, no $\eta$ $k_{\text{out}}$</strong></td>
<td><strong>8684.31</strong></td>
<td><strong>8696.31</strong></td>
<td><strong>Y</strong> <strong>FINAL MODEL</strong></td>
<td></td>
</tr>
</tbody>
</table>

Fit statistics for the effect compartment and indirect models (table 4.19) indicated once again that the indirect model was superior and thus was chosen for further refinement. Addition of inter-individual variability to all parameters produced a significant drop in log-likelihood (-20.4, 3df). However the omegas for $I_{\text{max}}$ and $k_{\text{out}}$ were estimated with very poor precision (RSE=491-572%) and had very high shrinkage (94-100%). Removal of the etas for these two parameters improved the model with a slightly reduced -2LL value. However, the estimate for $I_{\text{max}}$ was again 1, indicating a maximum effect of zero blood pressure. Once again the actual observed effect for the dose range used was very much less than this predicted maximum. Due to this and the physiological improbability of the value a linear function was deemed more appropriate to fit this data. Incorporation of a linear function into the indirect model resulted in an improved fit according to the goodness of fit statistics. Addition of inter-individual
variability to both structural parameters, resulted in a significant drop in log-likelihood (-18.27, 2df) but the omega for $k_{out}$ was imprecisely predicted (RSE=312%) and displayed high shrinkage (89%). A model with only variability on the slope parameter resulted in a very similar log-likelihood value and no further issues.

### 4.4.2.1.2 Final PD model fit

The final milrinone dog mean blood pressure PD model was selected as an indirect response model with inhibitory effect on the production of response described via a linear model, with inter-individual variability on $I_{\text{slope}}$ and additive residual error. Structural parameters were estimated with good precision (RSE=17-19%; table 4.20) and additive residual error was low. Variability for $I_{\text{slope}}$ was moderate although this is possibly slightly over-estimated due to the nature of the modelling procedure applied in this case.

Table 4.20: Parameter estimates for the final dog milrinone MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{slope}}$ ($\mu$M$^{-1}$)</td>
<td>0.712</td>
<td>19</td>
</tr>
<tr>
<td>$k_{out}$ ($h^{-1}$)</td>
<td>3.13</td>
<td>17</td>
</tr>
<tr>
<td>Inter-individual variability in $I_{\text{slope}}$ (%)</td>
<td>53</td>
<td>58</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>4.93</td>
<td>2</td>
</tr>
</tbody>
</table>

![Observed versus predicted mean blood pressure for the final dog milrinone PK model using population (A) or individual (B) parameters.](image)

Figure 4.24: Observed versus predicted mean blood pressure for the final dog milrinone PK model using population (A) or individual (B) parameters.

Diagnostic plots show an acceptable fit to the data (figures 4.24 and 4.26). The scatter plots of predicated against observed mean blood pressure display an even distribution of points around the line of unity and not too much deviation from the line in the case of
the individual predictions. Weighted residuals are evenly distributed around zero with no observable pattern indicative of model misspecification. A good proportion of the points also fall within ±2 standard deviations.

Individual predicted mean blood pressure-time profiles give a good overall description of the observed data and the drug effect (figure 4.25).

Figure 4.25: Individual plots of observed (circles) and predicted (line) mean blood pressure with time in dogs administered doses of 0.03(LD), 0.1(MD) or 0.3(HD) mg/kg milrinone.
4.4.2.2 Heart rate

4.4.2.2.1 PD model selection

As for the rat, it was not immediately obvious from comparison of the concentration and response time course whether or not a delay in response was present due to the administration effect. The fit of a direct $E_{\text{max}}$ model was again compared with an effect compartment model and an indirect response model with stimulation of $k_{\text{in}}$, to see which best described the profiles. This confirmed that modelling the delay was required since the direct model did not adequately describe the profiles. The indirect model once again proved superior to the effect compartment model via comparison of the fit statistics (table 4.21). Addition of inter-individual variability to all parameters produced a significant drop in log-likelihood ($-23.59$, 3df). However once again there were issues with poor precision for the $E_{\text{max}}$ and $EC_{50}$ omegas (RSE=128-153%) and high eta shrinkage (84-93%). Removal of these etas improved the model with only a small increase in log-likelihood. However, yet again the maximum effect value was deemed an implausible estimate. The $E_{\text{max}}$ value (2.04) was very similar to that predicted for rat and as in that case, much higher than any observed effect for the dose range studied. At high enough concentrations, it would also result in a predicted heart rate greater than the actual maximum heart rate for the dog. A linear function was once again modelled as part of an indirect model and resulted in an improved fit according to the AIC values. Addition of inter-individual variability improved the model further, with a significant reduction in log-likelihood ($-24.85$, 2df) but also resulted in reasonably poor precision for the $E_{\text{slope}}$ omega (RSE=79%) and moderately high eta shrinkage (76%). Removal of
the $E_{\text{slope}}$ eta resulted in an improved model with respect to the fit statistics and no precision issues.

Table 4.21: Overview of model selection for the dog milrinone HR PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $E_{\text{max}}$; no η</td>
<td>11050.13</td>
<td>11060.13</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; no η</td>
<td>10920.85</td>
<td>10932.85</td>
<td>-</td>
<td>High SE EC$_{50}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; no η</td>
<td>10893.23</td>
<td>10905.23</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$, all η</td>
<td>10869.64</td>
<td>10887.64</td>
<td>Y</td>
<td>High SE &amp; shrinkage $\omega E_{\text{max}}, EC_{50}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$, $E_{\text{max}}$, EC$_{50}$</td>
<td>10871.14</td>
<td>10885.14</td>
<td>Y</td>
<td>Implausible $E_{\text{max}}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{slope}}$; no η</td>
<td>10894.75</td>
<td>10904.75</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td>Indirect; $E_{\text{slope}}$, all η</td>
<td>10869.90</td>
<td>10883.90</td>
<td>Y</td>
<td>Moderate SE &amp; shrinkage $\omega E_{\text{slope}}$</td>
</tr>
<tr>
<td><strong>Indirect; $E_{\text{slope}}$; no η</strong></td>
<td><strong>10871.08</strong></td>
<td><strong>10883.08</strong></td>
<td>Y</td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

4.4.2.2.2 Final PD model fit

The final milrinone dog heart rate PD model was selected as an indirect response model with stimulatory effect on the production of response described via a linear model, with inter-individual variability on $k_{\text{out}}$ and additive residual error. Structural parameters were estimated with good to moderate precision (RSE=13-40%; table 4.22) and additive residual error was acceptable. Variability for $k_{\text{out}}$ was once again high at 129%. From inspection of individual values, this was the case both across individuals and between doses. Since there was no real effect at the two lower doses, it was likely that this variability was artificially high due to an attempt to fit what is actually intra-individual variability unrelated to drug effect. Once again this is as a consequence of the modelling approach taken.

Table 4.22: Parameter estimates for the final dog milrinone HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{slope}}$ (µM$^{-1}$)</td>
<td>1.55</td>
<td>13</td>
</tr>
<tr>
<td>$k_{\text{out}}$ (h$^{-1}$)</td>
<td>0.524</td>
<td>40</td>
</tr>
<tr>
<td>Inter-individual variability in $k_{\text{out}}$ (%)</td>
<td>129</td>
<td>49</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>10.8</td>
<td>2</td>
</tr>
</tbody>
</table>
Diagnostic plots (figures 4.27 and 4.28) once again show an acceptable fit to the data. The scatter plots of predicted against observed heart rate display an even distribution of points around the line of unity and although there is more deviation from the line than observed in other cases. Weighted residuals are evenly distributed around zero with a reasonable proportion of the points falling within ±2 standard deviations.

Figure 4.27: Observed versus predicted heart rate for the final dog milrinone HR PD model using population (A) or individual (B) parameters.

Figure 4.28: Population weighted residuals (PWRES) versus time (A) or predicted heart rate (B) for the final dog milrinone HR PD model.
4.4.3 Guinea-pig study

Both milrinone plasma concentrations and cardiovascular responses had been collected in the same individual animals meaning individual PK estimates were fixed in the integrated PKPD model. In addition the baseline parameters were derived from the pre-dose data for each animal (see section 4.3.3) so were also fixed to individual values.

4.4.3.1 Mean blood pressure

4.4.3.1.1 PD model selection

Since the study design was the same for milrinone as for L-NAME, it was again difficult to determine whether there was any delay between concentration and effect. The three different models (direct, effect compartment and indirect with inhibition of
\( k_{in} \) with an \( I_{\text{max}} \) function were once again compared for goodness of fit. The lowest AIC value (table 4.23) resulted from the fit of the indirect response model, which was in agreement with the models for rat and dog. The model was improved by the addition of inter-individual variability on all structural parameters, with a significant drop in log-likelihood observed (-65.8, 3df). The omega for \( k_{out} \) was estimated with very poor precision (RSE=1370\%) and very high shrinkage (98\%). In addition the precision for the \( I_{\text{max}} \) omega was also quite poor (RSE=80\%) with moderate shrinkage (45\%). Removal of the \( k_{out} \) eta resulted in an improved model with slightly lower \(-2\text{LL}\) value but the \( I_{\text{max}} \) omega became more imprecisely estimated (RSE=209\%) and shrinkage higher (80\%). Therefore this eta was also removed resulting in a virtually identical log-likelihood and no other issues.

| Table 4.23: Overview of model selection for the guinea-pig milrinone MBP PD data |
|---------------------------------|---------|----|-----------------|
| Model                          | -2LL    | AIC | Sig  | Comments                     |
| Direct; \( I_{\text{max}} \); no \( \eta \) | 1877.96 | 1883.96 | -    |                              |
| Effect cpt; \( I_{\text{max}} \); no \( \eta \) | 1862.81 | 1870.81 | -    |                              |
| Indirect; \( I_{\text{max}} \); no \( \eta \) | 1860.28 | 1868.28 | -    | Lower AIC                    |
| Indirect; \( I_{\text{max}} \); all \( \eta \) | 1794.48 | 1808.48 | Y    | High SE & shrinkage \( \omega \) \( k_{out} \) |
| Indirect; \( I_{\text{max}} \); no \( \eta \) \( k_{out} \) | 1793.51 | 1805.51 | Y    | High SE & shrinkage \( \omega \) \( I_{\text{max}} \) |
| **Indirect; \( I_{\text{max}} \) model; no \( \eta \) \( I_{\text{max}}, k_{out} \)** | **1793.49** | **1803.49** | **Y** | **FINAL MODEL** |

4.4.3.1.2 Final PD model fit

The final milrinone guinea-pig mean blood pressure PD model was selected as an indirect response model with inhibitory effect on the production of response described via an \( I_{\text{max}} \) model, with inter-individual variability on IC\(_{50}\), and additive residual error. Precision of the structural parameter estimates was good (RSE=9-29\%; table 4.24) and inter-individual variability was moderate for IC\(_{50}\) at 48\%. Additive error was low.

| Table 4.24: Parameter estimates for the final guinea-pig milrinone MBP PD model |
|----------------------------|--------|-----|
| Parameter                  | Estimate | RSE(%) |
| \( I_{\text{max}} \)       | 0.46    | 9    |
| IC\(_{50}\) (\( \mu \)M)  | 0.494   | 23   |
| \( k_{out} \) (min\(^{-1}\)) | 0.546   | 29   |
| Inter-individual variability in IC\(_{50}\) (%) | 48 | 55 |
| Additive residual error (mmHg) | 1.96 | 3 |
Individual predicted mean blood pressure-time profiles give a good overall description of the observed data and the drug effect (figure 4.30).

Figure 4.30: Individual plots of observed (circles) and predicted (line) mean blood pressure with time in guinea-pigs administered multiple doses of milrinone.

Diagnostic plots (figures 4.31 and 4.32) show good fit to the data for both population and individual models. The scatter plots of predicted against observed mean blood pressure display an even distribution of points around the line of unity and little deviation from the line for both population and individual models. Weighted residuals are evenly distributed around zero with a good proportion of points within ±2 standard deviations.

Figure 4.31: Observed versus predicted mean blood pressure for the final guinea-pig milrinone MBP PD model using population (A) or individual (B) parameters.
4.4.3.2 Heart rate

4.4.3.2.1 PD model selection

As was the case for mean blood pressure, it was difficult to determine the possibility of a delay due to the lack of concentration measurements. A direct $E_{\text{max}}$ model was therefore compared to an effect compartment model and an indirect model with stimulation of $k_{\text{in}}$. The indirect model was the best fit according to the fit statistics (table 4.25), which once again agreed with the model choice for rat and dog. Addition of inter-individual variability resulted in a huge drop in log-likelihood (-791.32, 3df) and parameters were all estimated with acceptable precision and shrinkage.

Table 4.25: Overview of model selection for the guinea-pig milrinone HR PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $E_{\text{max}}$; no $\eta$</td>
<td>3527.77</td>
<td>3533.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; no $\eta$</td>
<td>3521.44</td>
<td>3529.75</td>
<td></td>
<td>High SE $k_{\text{e0}}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; no $\eta$</td>
<td>3520.26</td>
<td>3528.26</td>
<td></td>
<td>Lower AIC</td>
</tr>
<tr>
<td><strong>Indirect; $E_{\text{max}}$; all $\eta$</strong></td>
<td><strong>2728.94</strong></td>
<td><strong>2742.94</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

4.4.3.2.2 Final PD model fit

The final milrinone guinea-pig heart rate PD model was selected as an indirect response model with stimulatory effect on the production of response described via an $E_{\text{max}}$ model, with inter-individual variability on $E_{\text{max}}, EC_{50}$ and $k_{\text{out}},$ and additive residual error. Precision of the structural parameter estimates was within acceptable limits (RSE=12-48%; table 4.26). Inter-individual variability was reasonable low for $E_{\text{max}}$ (31%) but high for $EC_{50}$ and $k_{\text{out}}$ (115-132%). The high $k_{\text{out}}$ values observed for some
individuals indicate that actually the effect may be more or less direct in these animals. However, it is difficult to know for sure due to possible inaccuracies with predicted concentration profiles resulting from so few data.

Table 4.26: Parameter estimates for the final guinea-pig milrinone HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.357</td>
<td>12</td>
</tr>
<tr>
<td>$EC_{50}$ ($\mu$M)</td>
<td>0.126</td>
<td>48</td>
</tr>
<tr>
<td>$k_{\text{out}}$ ($h^{-1}$)</td>
<td>0.848</td>
<td>46</td>
</tr>
<tr>
<td>Inter-individual variability in $E_{\text{max}}$ (%)</td>
<td>31</td>
<td>58</td>
</tr>
<tr>
<td>Inter-individual variability in $EC_{50}$ (%)</td>
<td>132</td>
<td>52</td>
</tr>
<tr>
<td>Inter-individual variability in $k_{\text{out}}$ (%)</td>
<td>115</td>
<td>62</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>5.58</td>
<td>4</td>
</tr>
</tbody>
</table>

Individual predicted heart rate-time profiles give a good overall description of the observed data and the drug effect (figure 4.33).

Figure 4.33: Individual plots of observed (circles) and predicted (line) heart rate with time in guinea-pigs administered multiple doses of milrinone.

Diagnostic plots show an acceptable fit to the data (figures 4.34 and 4.35). The scatter plots of population model predicated against observed heart rate shows some bias towards over-prediction for the high values but an even distribution of points around the line of unity is seen for the individual model. Weighted residuals are evenly distributed around zero with a good proportion of points within $\pm 2$ standard deviations.
4.5 Prediction of human cardiovascular response to milrinone

4.5.1 Human PK & PD data

4.5.1.1 Literature search

A literature search for milrinone studies in human subjects revealed a number of articles, however the majority of these had been performed in patients with cardiac failure (e.g. Benotti et al., 1985; Edelson et al., 1986) or undergoing cardiac surgery (Bailey et al., 1994; Butterworth et al., 1995). Only one study that measured cardiac response to milrinone in healthy volunteers was located (Larsson et al., 1986).

The Larsson study was a comparison of single dose concentration and effect profiles of milrinone in healthy volunteers and patients with renal impairment. The data for the latter was ignored. At 8am on the study day a 5mg oral dose of milrinone was
administered. The subjects had fasted overnight and continued to do so for 2 hours post-dose. Blood samples were taken pre-dose and at 10, 20, 30, 40, 50, 60 minutes and 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours following administration. Systolic and diastolic blood pressures and heart rate were measured with subjects in the supine position and after 2 minutes standing, before dosing and then at the same times as blood sampling. For the first hour after administration, only data in the supine position were measured.

The one major issue with the Larsson study was that it was not placebo controlled. This meant it was difficult to determine how much the observed changes in blood pressure and heart rate were related to drug effect and how much was normal diurnal variation. Since data were reported over a 12 hour period during the day, it was felt it would be wrong to assume a constant baseline. For this reason a literature search for blood pressure and heart rate changes in healthy volunteers was undertaken. The literature on the circadian rhythms of cardiovascular effects is vast. However, much of it is studied in patient populations to see the impact of the condition or disease. A few studies observing healthy volunteers over a 24 hour period were located. From these, 2 studies were selected based on the quality of the data reported.

Mean blood pressure data were reported in a study by Algalarrondo et al., (2012). This was a study comparing the circadian rhythms of blood pressure in patients with familial amyloid neuropathy and healthy control subjects. The data for the former were ignored. A total of 49 control subjects were studied, a reasonably even mixture of both men and women and of an approximately similar age range to the control subjects in the Larsson study. Data were reported as the mean values from all subjects.

Heart rate data were reported in a study by Clarke et al., (1976). This was a study of heart rate purely in healthy volunteers; anyone with cardiovascular irregularities was screened out before the study began. A total of 86 subjects were studied, again approximately even distribution of male and female and an age range spanning the typical adult population (16-65 years). Data were reported as the mean values from male and female subjects separately; therefore an average of the data at each time point was calculated.
4.5.1.2 Modelling of PK and baseline data

In all three studies (Algalarrondo et al., 2012; Clarke et al., 1976; Larsson et al., 1986) the data was reported graphically, so each graph was digitized to obtain the concentration/MBP/HR points.

A 1 compartment, 1st order absorption model with proportional error was fitted to concentration-time points from the average profile of the healthy volunteers in the Larsson study. The estimated parameter values were $3.95h^{-1}$ for $k_a$, $0.261L/h/kg$ for CL/F and $0.403L/kg$ for V/F. The estimates were precise (RSE=4-23%) and proportional error was reasonably low at 13.4%. The fit is shown in figure 4.36.

![Figure 4.36: Plot of observed (black circles) and predicted (solid line) plasma milrinone concentration with time in healthy volunteers following oral administration of 5mg milrinone. Data from Larsson et al., (1986).](image)

Since MBP and HR measurements were taken over a 24 hour time frame, it was possible to fit a circadian rhythm model. However, since the effect data were only reported over a 12 hour period, it was not certain how the data could be corrected for the average MBP/HR in those individuals. In addition although various cosine models were attempted, an adequate fit to the HR data was not achieved. The data for the same 12 hour period as reported in the Larsson study (8am – 8pm) were therefore fitted using empirical polynomial functions, with the simple aim of describing the overall shape of the profiles. These could be easily corrected using the pre-dose MBP/HR value.

For the MBP data a 4th order polynomial produced the best fit and for HR a 6th order polynomial was required. Parameters were estimated with good precision for MBP (RSE=10-14%) and with excellent precision for HR (RSE=1-7%). Additive residual
errors were low in both cases (1.12mmHg and 0.381bpm). The fits of the human baseline data are shown in figure 4.37.

Figure 4.37: Plot of observed (circles) and predicted (solid line) mean blood pressure (A) or heart rate (B) with time in healthy volunteers. Data from Algalarrondo et al., 2012 (A) and Clarke et al., 1976 (B).

### 4.5.2 Predictions

Integrated PKPD models for prediction of human cardiovascular response to milrinone were scripted based on the models derived for each of the 3 pre-clinical species. PK parameters were fixed to those derived from the mean concentration time profile in the Larsson study. Predicted human milrinone plasma concentrations were converted to unbound concentrations using the fraction unbound in plasma value of 0.3 reported in the prescribing information. Baseline parameters were fixed to those derived from the modelling of MBP data from the Algalarrondo study and HR data from the Clarke study. The only parameter that was altered was the value of MBP/HR at time 0, which was reset as the observed value from the Larsson study.

#### 4.5.2.1 Mean blood pressure

The prediction of milrinone effect on human mean blood pressure was described via an indirect model with inhibition of the production of response using either a linear or $I_{\text{max}}$ function. The former was used with PD parameters derived from either the rat or the dog study and the latter with values derived from the guinea-pig study. The $k_{\text{out}}$ values were converted to equivalent human values using the single species scaling technique. These values are summarised in table 4.27.

As can be seen from the table there is no consistency in the values across the 3 species in addition to the difference in PD model required.
Table 4.27: Summary of final milrinone MBP PD model parameters for all 3 species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Dog</th>
<th>Guinea-pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_{slope} (µM^{-1})</td>
<td>0.0478</td>
<td>0.712</td>
<td>-</td>
</tr>
<tr>
<td>I_{max}</td>
<td>-</td>
<td>-</td>
<td>0.46</td>
</tr>
<tr>
<td>IC_{50} (µM)</td>
<td>-</td>
<td>-</td>
<td>0.494</td>
</tr>
<tr>
<td>k_{out} (h^{-1})</td>
<td>0.572</td>
<td>2.13</td>
<td>9.97</td>
</tr>
</tbody>
</table>

The predictions are shown in figure 4.38. As can be observed the data from all 3 animal models hugely under-predict the effect of milrinone on mean blood pressure in humans. The rat model does not appear to change at all from baseline. The dog and guinea-pig models both predict a decrease over the first 6 hours but not to the level observed in humans. The time of maximum decrease is also predicted too early by both the dog and guinea-pig models. Overall the results indicate that the indirect model may be the correct model but a lower k_{out} parameter would be required in addition to a higher degree of inhibition.

Figure 4.38: Prediction of observed human mean blood pressure (black circles) following oral administration of 5mg milrinone, using the PD model and parameter estimates for rat (solid line), dog (dashed line) or guinea-pig (dotted-dashed line).

4.5.2.2 Heart rate

The prediction of milrinone effect on human heart rate was described via an indirect model with stimulation of the production of response using either a linear or E_{max} function. The former was used with PD parameters derived from either the rat or the dog study and the latter with values derived from the guinea-pig study. The k_{out} values
Chapter 4: Milrinone

were converted to equivalent human values using the single species scaling technique. These values are summarised in table 4.28. Once again there is no consistency in the values across the 3 species and no consistency with the MBP value either, except for the rank order of values.

Table 4.28: Summary of final HR PD model parameters for all 3 species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Dog</th>
<th>Guinea-pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{slope}}$ ($\mu$M$^{-1}$)</td>
<td>0.113</td>
<td>1.55</td>
<td>-</td>
</tr>
<tr>
<td>$E_{\max}$</td>
<td>-</td>
<td>-</td>
<td>0.357</td>
</tr>
<tr>
<td>$EC_{50}$ ($\mu$M)</td>
<td>-</td>
<td>-</td>
<td>0.126</td>
</tr>
<tr>
<td>$k_{\text{out}}$ ($h^{-1}$)</td>
<td>0.231</td>
<td>0.357</td>
<td>15.48</td>
</tr>
</tbody>
</table>

Figure 4.39: Prediction of observed human heart rate (black circles) following oral administration of 5mg milrinone, using the PD model and parameter estimates for rat (solid line), dog (dashed line) or guinea-pig (dotted-dashed line).

The predictions are shown in figure 4.39. The rat model does not appear to show any changes from baseline once again. The guinea-pig model actually over-predicts the extent of effect and again also predicts the time-course incorrectly, with maximum effect occurring much too early. The dog model produces the best prediction, accurately predicting the extent of the effect if not the time course. Once more the actual maximum effect is observed later than predicted, indicating that the indirect model may be correct but $k_{\text{out}}$ values should be lower. One other consideration though is whether the baseline accurately reflects that of the dosed individuals.
Chapter 5

Doxazosin
Chapter 5: Doxazosin

5 Doxazosin

5.1 Introduction

Doxazosin is a selective α1-adrenoceptor antagonist used in the treatment of hypertension. α1-adrenoceptors located in vascular smooth muscle are stimulated by the endogenous neurotransmitters adrenaline and noradrenaline as part of the sympathetic nervous system control of blood pressure. Binding of (nor-)adrenaline to α1-adrenoceptors activates the inositol triphosphate (IP$_3$) signal transduction pathway which leads to muscle contraction (vasoconstriction). Thus by blocking the receptor doxazosin causes vasodilation, reduction in peripheral resistance and a fall in blood pressure. Doxazosin has also been used as a treatment for benign prostatic hyperplasia, where it causes relaxation of protastic smooth muscle and improves urinary flow.

The selective inhibiton of α1-adrenoceptors by doxazosin has been shown both in vitro and in vivo in animals (Alabaster et al., 1986). Pre-clinical PD studies have shown doxazosin reduces arterial blood pressure while having little effect on heart rate in dogs (Alabaster et al., 1986) and rabbits (Hamilton et al., 1985). In healthy human volunteers doxazosin has been shown to reduce both systolic and diastolic blood pressures (Elliott et al., 1982; Meredith et al., 1988; Vincent et al., 1983), with a more pronounced effect in the standing as opposed to supine position. Equivalent effects were observed in hypertensive patients (Donnelly et al., 1989; Elliott et al., 1986; Frick et al., 1986). In many of the single dose studies an increase in HR was also observed in both healthy and hypertensive individuals (Donnelly et al., 1989; Elliott et al., 1982; Elliott et al., 1986; Vincent et al., 1983); however on chronic dosing changes were non-significant (Conrad et al., 1988; Donnelly et al., 1989; Elliott et al., 1986; Frick et al., 1986).

PK studies in healthy volunteers (Elliott et al., 1982; Meredith et al., 1988; Vincent et al., 1983) have shown equivalence with those in hypertensive patients (Conrad et al., 1988; Donnelly et al., 1989; Elliott et al., 1986; Frick et al., 1986). Concentration-profiles appear biphasic and most were fitted to 2-compartment models after iv dosing, although models with 1-compartment elimination and 1$^{st}$-order absorption appeared sufficient for oral dosing in most cases (Donnelly et al., 1989; Meredith et al., 1988; Vincent et al., 1983). Clearance values are low and volume is intermediate, resulting in a moderately long half-life (Elliott et al., 1986).
5.2 PK modelling of doxazosin

5.2.1 Rat study

5.2.1.1 PK model development and refinement

Observed doxazosin plasma concentrations with time displayed mono-phasic profiles for all rats, suggesting a 1-compartment model would be the most suitable fit to the data. A comparison of 1- and 2-compartment models, both with 1<sup>st</sup>-order absorption and proportional error confirmed the 1-compartment model was the most appropriate model. There was no significant drop in log-likelihood for the 2-compartment model (-0.09, 2df, table 5.1) and the precision was poor for the majority of its structural parameters (RSE = 137-2x10<sup>4</sup>%). In an attempt to refine the 1-compartment model inter-individual variability was applied to all parameters. Once again this did not result in a significant drop in -2LL value (-0.15, 3df) and all 3 omegas had poor precision (RSE=306-1330%) and high shrinkage (81-96%). Addition of any of the 3 etas in any combination did not improve the model.

Table 5.1: Overview of model selection for the rat doxazosin PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt; 1abs; no η</td>
<td>569.02</td>
<td>577.02</td>
<td></td>
<td>FINAL MODEL</td>
</tr>
<tr>
<td>2cpt; 1abs; no η</td>
<td>568.93</td>
<td>580.93</td>
<td>N</td>
<td>High SE k&lt;sub&gt;a&lt;/sub&gt; V₁/F Q/F V₂/F</td>
</tr>
<tr>
<td>1cpt; 1abs; all η</td>
<td>568.87</td>
<td>582.87</td>
<td>N</td>
<td>High SE &amp; shrinkage all</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant drop in -2LL compared to previously best nested model

5.2.1.2 Final PK model fit

The final PK model selected for the rat doxazosin PK data was a 1-compartment, first-order absorption model with proportional residual error. Parameter estimates for the final model are presented in table 5.2. Structural parameters were predicted with good to moderate precision (RSE=5-16%). Residual error was moderate at 29.7%, however this was the case for all models considered.

The diagnostic plots for this model (figures 5.1 and 5.2) show a good fit to the data. The scatter plot of predicated against observed concentrations displays an even distribution around the line of unity with not too much deviation. Weighted residuals are evenly distributed around zero, with most falling within ±2 standard deviations.
Table 5.2: Parameter estimates for the final rat doxazosin PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (h^{-1})</td>
<td>1.88</td>
<td>16</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>5.08</td>
<td>5</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>18.4</td>
<td>6</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>29.7</td>
<td>10</td>
</tr>
</tbody>
</table>

^a Relative standard error (RSE), expressed as a percentage is calculated as SE divided by the parameter estimate x 100

Figure 5.1: Observed versus predicted plasma concentrations for the final rat doxazosin PK model.

Figure 5.2: Population weighted residuals (PWRES) versus time (A) or predicted concentration (B) for the final rat doxazosin PK model.

Plots of individual predicted concentration-time profiles give a good description of the observed data for all 3 doses (figure 5.3).
5.2.2 Dog study

Plasma-concentration time points were available for 24 hours for all four dogs. However for two of them the 24 hour time point was below the limit of quantification (BLQ). Since the lower limit of quantification (LLOQ) was known for this study and the previous time point was at 6 hours, it was decided to incorporate the LLOQ into the modelling for these individuals to ensure the estimated parameters predicted profiles that were BLQ at 24 hours.

5.2.2.1 PK model development and refinement

The doxazosin plasma concentration-time profiles were once again all mono-phasic in nature and thus indicated a 1- compartment model would be sufficient for a good fit. To confirm this, 1- and 2- compartment models with 1st order absorption were analysed with a proportional error model. The 2-compartment model did not produce a significant drop in log-likelihood (-0.14, 2df; table 5.3), confirming the 1-compartment model was sufficient. To further refine the model, inter-individual variability was
applied to all structural parameters. This resulted in a significant drop in log-likelihood (-22.18, 3df) but the omega for V/F was estimated with poor precision (RSE=195%) and moderate shrinkage (56%). Removal of the eta on V/F resulted in a very similar -2LL value and no further issues.

Table 5.3: Overview of model selection for the dog doxazosin PK data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cpt; 1abs</td>
<td>161.60</td>
<td>169.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2cpt; 1abs</td>
<td>161.81</td>
<td>173.81</td>
<td>N</td>
<td>High SE kₐ V₁/F Q/F V₂/F</td>
</tr>
<tr>
<td>1cpt; 1abs; all η</td>
<td>139.42</td>
<td>153.42</td>
<td>Y</td>
<td>High SE &amp; mod shrinkage ø V/F</td>
</tr>
<tr>
<td>1cpt; 1abs; η kₐ CL/F</td>
<td>139.57</td>
<td>151.57</td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

5.2.2.2 Final PK model fit

The final PK model selected for the dog doxazosin PK data was a 1-compartment, first-order absorption model with inter-individual variability on kₐ and CL/F, and proportional residual error. Parameter estimates for the final model are presented in table 5.4 and the individual estimates of kₐ and CL/F presented in table 5.5. Structural parameters were predicted with good to moderate precision (RSE=4-29%). Inter-individual variability for CL/F was low at 30% and for kₐ moderate at 55%. Proportional residual error was relatively low at 11.5%.

Table 5.4: Parameter estimates for the final dog doxazosin PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>kₐ (h⁻¹)</td>
<td>1.19</td>
<td>29</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>2.51</td>
<td>7</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>23.6</td>
<td>4</td>
</tr>
<tr>
<td>Inter-individual variability in kₐ (%)</td>
<td>55</td>
<td>75</td>
</tr>
<tr>
<td>Inter-individual variability in CL/F (%)</td>
<td>15</td>
<td>93</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>11.5</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 5.5: Individual kₐ and CL/F estimates for the final dog doxazosin PK model

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>kₐ (h⁻¹)</td>
<td>0.712</td>
<td>2.18</td>
<td>0.673</td>
<td>1.67</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>2.20</td>
<td>2.63</td>
<td>2.98</td>
<td>2.26</td>
</tr>
</tbody>
</table>
The diagnostic plots for this model (figures 5.4 and 5.5) show an acceptable fit to the data for both the population and individual models. The scatter plots of predicted against observed concentrations display an even distribution around the line of unity with little deviation in the case of the individual model. Weighted residuals are evenly distributed around zero, with all except one falling within $\pm 2$ standard deviations.

Figure 5.4: Observed versus predicted plasma concentrations for the final dog doxazosin PK model using population (A) or individual (B) parameters. LLOQ data are shown in red.

Figure 5.5: Population weighted residuals (PWRES) versus time (A) or predicted concentration (B) for the final dog doxazosin PK model. LLOQ data are shown in red.

Plots of individual predicted concentration-time profiles give a good description of the observed data and profiles for dogs 2 and 3 fall below the LLOQ at 24 hours (figure 5.6).
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5.2.3 Guinea-pig study

5.2.3.1 PK model development and refinement

As for the other compounds, only 1 concentration per dose was measured at approximately the same time point for each animal. Since there was not enough information to describe a full PK profile a 1-compartment model had to be assumed. A 1-compartment model with proportional error described the data sufficiently but CL was estimated with poor precision (RSE=103%).

In an attempt to improve the fit, inter-individual variability on both structural parameters was added. This resulted in a significant reduction in the -2LL value (-10.84, 2df, table 5.6) although the precision for the CL estimate was still poor (RSE=52%). In addition the omega for CL was imprecise (RSE=477%) and displayed high shrinkage (84%). Removal of the eta for CL produced a better model with respect to the fit.
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statistics but the CL estimate was still imprecise (RSE=62%). To try and improve the precision for the CL estimate, a model with only eta on CL was evaluated. When compared with the model with only eta on V, this model was not an improvement in relation to the fit statistics. It did however still produce a significant drop in log-likelihood from the base model (-8.77, 1df) and had no issues with respect to parameter precision.

5.2.3.2 Final PK model fit

The final PK model selected for the guinea-pig doxazosin PK data was a 1-compartment model with inter-individual variability on CL and proportional residual error. Parameter estimates for the final model are presented in table 5.7 and the individual estimates of CL presented in table 5.8. The structural parameters were predicted with good to moderate precision (RSE=10-37%) and inter-individual variability in CL was moderate at 53%. Proportional residual error was relatively low at 12.8%.

Table 5.7: Parameter estimates for the final guinea-pig doxazosin PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/min/kg)</td>
<td>0.0457</td>
<td>37</td>
</tr>
<tr>
<td>V (L/kg)</td>
<td>1.93</td>
<td>10</td>
</tr>
<tr>
<td>Inter-individual variability in CL (%)</td>
<td>53</td>
<td>64</td>
</tr>
<tr>
<td>Proportional residual error (%)</td>
<td>12.8</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure 5.7: Observed versus predicted plasma concentrations for the final guinea-pig doxazosin PK model using population (A) or individual (B) parameters.

Diagnostic plots (figures 5.7 and 5.8) show an acceptable fit to the data for both the population and individual models. The plots of predicted against observed
concentrations display an even distribution around the line of unity with not too much scatter. Weighted residuals are evenly distributed around zero, with virtually all falling within ±2 standard deviations. Individual predicted concentration-time profiles give a good overall description of the observed data (figure 5.9).

Table 5.8: Individual estimates of CL for the final guinea-pig doxazosin PK model

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP1</th>
<th>GP2</th>
<th>GP3</th>
<th>GP4</th>
<th>GP5</th>
<th>GP6</th>
<th>GP7</th>
<th>GP8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/min/kg)</td>
<td>0.0925</td>
<td>0.0303</td>
<td>0.0487</td>
<td>0.0766</td>
<td>0.0648</td>
<td>0.0211</td>
<td>0.0535</td>
<td>0.0446</td>
</tr>
</tbody>
</table>

Figure 5.8: Population weighted residuals (PWRES) versus time (A) or predicted concentration (B) for the final guinea-pig doxazosin PK model.

Figure 5.9: Individual plots of observed (circles) and predicted (dashed line) plasma concentrations with time in guinea-pigs administered multiple doses of doxazosin.
5.3 **Modelling of baseline cardiovascular response**

Baseline models for each species were developed as a joint analysis using vehicle data collected during the studies for all three compounds. Therefore, details of model development are described in section 3.3 and only details of the relevant individual animals’ parameter values and fits are described in this section.

### 5.3.1 Rat study

#### 5.3.1.1 Mean blood pressure

As described in section 3.3.1.1.2, the best fit to the rat mean blood pressure baseline data was given by the Sallstrom model with administration effect and inter-individual variability on $R_{av}$, gamma, delta, P and $k_{Adm}$. Individual parameter values for the rats used in the doxazosin study are given in table 5.9. Parameter values for $R_{mp}$ and D were the joint analysis population estimates of 0.109 and 0.0418 respectively.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
<th>Rat 5</th>
<th>Rat 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (mmHg)</td>
<td>105</td>
<td>116</td>
<td>115</td>
<td>106</td>
<td>111</td>
<td>117</td>
</tr>
<tr>
<td>$\gamma$ (min$^{-1}$)</td>
<td>0.190</td>
<td>0.142</td>
<td>0.0871</td>
<td>0.0873</td>
<td>0.108</td>
<td>0.225</td>
</tr>
<tr>
<td>$\delta$ (min$^{-1}$)</td>
<td>0.00247</td>
<td>0.00252</td>
<td>0.00162</td>
<td>0.00246</td>
<td>0.00127</td>
<td>0.00326</td>
</tr>
<tr>
<td>P</td>
<td>0.500</td>
<td>0.530</td>
<td>0.510</td>
<td>0.508</td>
<td>0.504</td>
<td>0.494</td>
</tr>
<tr>
<td>$k_{Adm}$ (min$^{-1}$)</td>
<td>0.0585</td>
<td>0.0656</td>
<td>0.166</td>
<td>0.0914</td>
<td>0.0467</td>
<td>0.181</td>
</tr>
</tbody>
</table>

The majority of the parameter estimates for the animals used in the doxazosin study fall in line with the joint analysis population estimates and associated variability. The main exception from this is $R_{av}$ where all the values for the animals from the doxazosin study are higher than the joint analysis population estimate, indicating a higher average mean blood pressure for these individuals. This may be due to the fact that the rats used in the doxazosin study are a different strain to those used for the L-NAME and milrinone studies (Wistar Han as opposed to Sprague Dawley). Individual predicted mean blood pressure-time profiles for doxazosin give a good description of the observed data for these animals (figure 5.10).
Figure 5.10: Individual plots of observed (circles) and predicted (line) blood pressure with time following administration of vehicle to rats as part of the doxazosin study.

5.3.1.2 Heart rate

As described in section 3.3.1.2.2, the best fit to the rat heart rate baseline data was given by the Sallstrom model with administration effect and inter-individual variability on $R_{av}$, gamma, delta, D, P and $k_{Adm}$. Individual parameter values for the rats used in the doxazosin study are given in table 5.10. The $R_{amp}$ joint analysis population estimate of 0.266 was fixed for all animals.

Table 5.10: Individual estimates of the final rat HR baseline model parameters for rats used in the doxazosin study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
<th>Rat 5</th>
<th>Rat 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{av}$ (bpm)</td>
<td>406</td>
<td>398</td>
<td>386</td>
<td>379</td>
<td>412</td>
<td>406</td>
</tr>
<tr>
<td>$\gamma$ (min$^{-1}$)</td>
<td>0.166</td>
<td>0.106</td>
<td>0.0965</td>
<td>0.115</td>
<td>0.103</td>
<td>0.168</td>
</tr>
<tr>
<td>$\delta$ (min$^{-1}$)</td>
<td>0.00316</td>
<td>0.00282</td>
<td>0.00334</td>
<td>0.00314</td>
<td>0.00226</td>
<td>0.00307</td>
</tr>
<tr>
<td>D</td>
<td>0.0114</td>
<td>0.0550</td>
<td>0.0167</td>
<td>0.118</td>
<td>0.0135</td>
<td>0.0125</td>
</tr>
<tr>
<td>P</td>
<td>0.975</td>
<td>1.02</td>
<td>0.561</td>
<td>1.18</td>
<td>1.11</td>
<td>0.499</td>
</tr>
<tr>
<td>$k_{Adm}$ (min$^{-1}$)</td>
<td>0.0555</td>
<td>0.0689</td>
<td>0.0749</td>
<td>0.0644</td>
<td>0.0567</td>
<td>0.0927</td>
</tr>
</tbody>
</table>

In general the values for the animals used in the doxazosin study are in agreement with the joint analysis population estimates and their variabilities. The main exception is again that the values for $R_{av}$ are all higher than the joint analysis population estimate, potentially indicating higher average heart rate in Wistar Han rats compared to Sprague
Dawley rats. The majority of P values are also higher, indicating that the administration effect is more pronounced in these animals. This effect can be observed in the individual predicted heart rate-time profiles (figure 5.11), which give a good overall description of the observed data for these animals.

Figure 5.11: Individual plots of observed (circles) and predicted (line) heart rate with time following administration of vehicle to rats as part of the doxazosin study.

5.3.2 Dog study

5.3.2.1 Mean blood pressure

As described in section 3.3.2.1.2, the best fit to the dog mean blood pressure baseline data was given by a single cosine function model with 24 hour period and inter-individual variability on all three structural parameters (R\textsubscript{av}, R\textsubscript{amp} and T\textsubscript{z}). Individual parameter values for the dogs used in the doxazosin study are given in table 5.11.

Table 5.11: Individual estimates of the final dog MBP baseline model parameters for dogs used in the doxazosin study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>R\textsubscript{av} (mmHg)</td>
<td>117</td>
<td>104</td>
<td>116</td>
<td>113</td>
</tr>
<tr>
<td>R\textsubscript{amp} (mmHg)</td>
<td>3.29</td>
<td>6.35</td>
<td>1.58</td>
<td>2.33</td>
</tr>
<tr>
<td>T\textsubscript{z} (h)</td>
<td>7.51</td>
<td>2.88</td>
<td>2.53</td>
<td>4.21</td>
</tr>
</tbody>
</table>

Apart from dog 2, all animals had higher R\textsubscript{av} values than the joint analysis population estimate, indicating a higher average blood pressure in these individuals. R\textsubscript{amp} values
fall into line with the joint analysis population estimate and its variability highlighting the range of mean blood pressure changes observed over the 24 hour period. $T_z$ values generally agree with the joint analysis population estimate. As mentioned in section 4.3.2.1 much of the high level of $T_z$ variability is attributed to an individual animal from the milrinone study with an unusual profile. Dog 1 from the doxazosin study also has a $T_z$ value later than the majority of dogs, contributing to this variability, although the difference in the profile is not as great as the dog from the milrinone study. Individual predicted mean blood pressure-time profiles (figure 5.12) give a good overall description of the observed data for these animals.

![Figure 5.12](image)

**Figure 5.12**: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time following administration of vehicle to dogs as part of the doxazosin study.

### 5.3.2.2 Heart rate

As described in section 3.3.2.2.2, the best fit to the dog heart rate baseline data was given by a dual cosine function model with 24 and 12 hour periods and administration effect and inter-individual variability on $R_{av}$, $R_{amp24}$, $T_{z24}$ and $P$. Individual parameter values for the dogs used in the doxazosin study are given in table 5.12. Parameter values for $R_{amp12}$, $T_{z12}$ and $k_{Adm}$ were the joint analysis population estimates of 2.39, 23.6h and 9.14h$^{-1}$ respectively. Apart from $T_{z24}$, values for the animals used in the doxazosin study differed considerably from the joint analysis population estimates. Virtually all $R_{av}$ values were higher than the joint analysis population value, indicating in general the
heart rate for these animals was higher than the typical dog. Also with the exception of dog 2, $R_{\text{amp24}}$ values for these animals were all much lower (<40%) than the joint analysis population estimate, indicating the degree of change in heart rate observed over the 24 hour period was much less for these dogs. Variability for $R_{\text{amp24}}$ was particularly high and much of this may be due to these particular individual dogs, since values for all other dogs were much higher. The lack of change over the 24 hours can be observed in the individual predicted heart rate-time profiles (figure 5.13). In addition, a large administration effect for dogs 3&4 can also be observed in the figure. These 2 individual dogs had $P$ values much higher than the joint analysis population estimate. Overall the individual predicted heart rate-time profiles give a good description of the observed data for these animals.

Table 5.12: Individual estimates of the final dog HR baseline model parameters for dogs used in the doxazosin study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{av}}$ (bpm)</td>
<td>79.9</td>
<td>85.3</td>
<td>81.7</td>
<td>78.8</td>
</tr>
<tr>
<td>$R_{\text{amp1}}$ (bpm)</td>
<td>1.98</td>
<td>5.79</td>
<td>1.60</td>
<td>1.12</td>
</tr>
<tr>
<td>$T_z1$ (h)</td>
<td>11.8</td>
<td>14.2</td>
<td>12.2</td>
<td>11.9</td>
</tr>
<tr>
<td>$P$</td>
<td>1.04</td>
<td>1.56</td>
<td>3.86</td>
<td>3.07</td>
</tr>
</tbody>
</table>

Figure 5.13: Individual plots of observed (circles) and predicted (solid line) heart rate with time following administration of vehicle to dogs as part of the doxazosin study.
5.3.3 Guinea-pig study

5.3.3.1 Mean blood pressure

As described in section 3.3.3.1.2, the best fit to the guinea-pig mean blood pressure baseline data was given by a linear model with inter-individual variability on both $R_{\text{Int}}$ and $R_{\text{slope}}$. Data from both vehicle treated animals and the pre-dose data for dosed animals were used in the joint analysis. Individual parameter values for the guinea-pigs dosed with doxazosin (derived from the pre-dose data) are given in table 5.13.

Table 5.13: Individual estimates of the final MBP baseline model parameters for guinea-pigs used in the doxazosin study

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP 1</th>
<th>GP 2</th>
<th>GP 3</th>
<th>GP 4</th>
<th>GP 5</th>
<th>GP 6</th>
<th>GP 7</th>
<th>GP 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{Int}}$ (mmHg)</td>
<td>32.8</td>
<td>42.5</td>
<td>34.7</td>
<td>33.0</td>
<td>47.6</td>
<td>39.5</td>
<td>45.0</td>
<td>37.4</td>
</tr>
<tr>
<td>$R_{\text{slope}}$ (mmHg/min)</td>
<td>0.086</td>
<td>0.014</td>
<td>-0.004</td>
<td>0.007</td>
<td>-0.080</td>
<td>-0.044</td>
<td>-0.094</td>
<td>0.028</td>
</tr>
</tbody>
</table>

For this set of animals $R_{\text{Int}}$ and $R_{\text{slope}}$ values were consistent with the joint analysis population estimates and their variabilities. Individual predicted mean blood pressure-time profiles (figure 5.14) give a good overall description of the observed data for these animals, although for some variability between points was high.

Figure 5.14: Individual plots of observed (grey circles) and predicted (solid line) mean blood pressure with time prior to administration of doxazosin in guinea-pigs.

Predicted mean blood pressure-time profiles for the vehicle treated animals used in the doxazosin study are shown in figure 5.15. The linear pattern with time can be observed for most individuals, although for V10 and V14, the pattern appears slightly different.
for the first 20 minutes compared to the remainder of time when vehicle was administered. Whether this is a true vehicle effect or not cannot be determined with this small number of individuals. Profiles both increase and decrease with time and to different extents as seen across the individual animals used in all three studies.

Figure 5.15: Individual plots of observed (circles) and predicted (line) mean blood pressure with time following administration of vehicle to guinea-pigs as part of the doxazosin study.

5.3.3.2 Heart rate

As described in section 3.3.3.2.2, the best fit to the guinea-pig heart rate baseline data was given by a linear model with inter-individual variability on both $R_{\text{Int}}$ and $R_{\text{slope}}$. Once again, data from both vehicle treated animals and the pre-dose data for dosed animals was used in the joint analysis. Individual parameter values for the guinea-pigs dosed with doxazosin (derived from the pre-dose data) are given in table 5.14.

Table 5.14: Individual estimates of the final HR baseline model parameters for guinea-pigs used in the doxazosin study

<table>
<thead>
<tr>
<th>Animal</th>
<th>GP 1</th>
<th>GP 2</th>
<th>GP 3</th>
<th>GP 4</th>
<th>GP 5</th>
<th>GP 6</th>
<th>GP 7</th>
<th>GP 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{Int}}$ (bpm)</td>
<td>243</td>
<td>279</td>
<td>237</td>
<td>243</td>
<td>258</td>
<td>271</td>
<td>258</td>
<td>230</td>
</tr>
<tr>
<td>$R_{\text{slope}}$ (bpm/min)</td>
<td>0.0619</td>
<td>-0.215</td>
<td>-0.259</td>
<td>-0.829</td>
<td>-0.574</td>
<td>-0.194</td>
<td>-0.419</td>
<td>-0.277</td>
</tr>
</tbody>
</table>

Both $R_{\text{Int}}$ and $R_{\text{slope}}$ values were consistent with the joint analysis population estimates and their variabilities. The majority also displayed a decrease in heart rate with time, as was the case for the animals used in the other studies. There was one exception that had an increase in heart rate with time but not to any great extent. Individual predicted heart
rate-time profiles (figure 5.16) give a good overall description of the observed data for these animals.

Figure 5.16: Individual plots of observed (grey circles) and predicted (solid line) heart rate with time prior to administration of doxazosin in guinea-pigs.

Predicted mean blood pressure-time profiles for the vehicle treated animals used in the doxazosin study are shown in figure 5.17. The linear pattern with time can be observed for most individuals, although for V10 and V12, the pattern appears slightly different for the first 20 minutes compared to the remainder of time when vehicle was administered. Whether this is a true vehicle effect or not cannot be determined with this
small number of individuals. Profiles both increase and decrease with time and to
different extents as seen across the individual animals used in all three studies.

5.4 Pharmacodynamic modelling of the cardiovascular response to doxazosin

The cardiovascular responses to doxazosin in all three animal models were a decrease in
mean blood pressure and an associated increase in heart rate. This is in agreement with
what is known about the mechanism of action of doxazosin (see section 5.1). Integrated
PKPD models with fixed PK and baseline parameters and appropriate inhibitory or
stimulatory effect functions were used to model the data.

5.4.1 Rat study

As was the case for the milrinone rat study, the doxazosin plasma concentrations were
measured in different animals to those in which cardiovascular responses to vehicle and
doxazosin were obtained. Therefore when fixing parameters in the PKPD model,
population estimates from the PK model were used for all individuals but baseline
values were different for each individual. Since it was again observed that on average
heart rate and mean blood pressure could vary independently from drug effect the $R_{av}$
parameter was allowed to vary. Due to inter-occasion variability producing errors, each
dosing occasion was again treated as a different individual but with their respective
individual baseline parameters fixed.

5.4.1.1 Mean blood pressure

5.4.1.1.1 PD model selection

Comparison of the response-time profile for MBP in rats dosed with doxazosin with the
respective concentration-time profile did not clearly indicate whether a direct or delayed
effect would be a suitable fit. In fact the response profile was not consistent with those
typically seen for the other compounds. Instead of a gradual onset of effect, there
appeared to be an initial drop in MBP, followed by a slight increase to a plateau and
then a gradual return to baseline. It was therefore uncertain that any of the models
would provide a good fit to the data. Comparison of the goodness of fit statistics for a
direct $I_{\text{max}}$ model to an effect compartment model and an indirect response model with
inhibition of $k_{in}$, indicated that the direct model gave the best fit (table 5.15).
Comparison of the goodness of fit plots also confirmed this. To further improve the
model, inter-individual variability was applied to the structural parameters. This
Chapter 5: Doxazosin

resulted in a significant drop in log-likelihood (-41.6, 2df) and an improved visual fit to the data.

Table 5.15: Overview of model selection for the rat doxazosin MBP PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $I_{\text{max}}$; no $\eta$</td>
<td>12298.43</td>
<td>12308.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $I_{\text{max}}$; no $\eta$</td>
<td>12853.98</td>
<td>12865.98</td>
<td></td>
<td>High SE, implausible IC$_{50}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no $\eta$</td>
<td>12800.49</td>
<td>12812.49</td>
<td></td>
<td>High SE, implausible IC$_{50}$</td>
</tr>
<tr>
<td>Direct; $I_{\text{max}}$; all $\eta$</td>
<td><strong>12256.83</strong></td>
<td><strong>12270.83</strong></td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

5.4.1.1.2 Final PD model fit

The final doxazosin rat mean blood pressure PD model was selected as a direct $I_{\text{max}}$ effect model, with inter-individual variability on $I_{\text{max}}$ and IC$_{50}$, and additive residual error. Structural parameters were estimated with good precision (RSE=7-25%; table 5.16) and additive residual error was low. Variability for $I_{\text{max}}$ was low but for IC$_{50}$ was high. As for milrinone the approach taken may have over-inflated this value.

Table 5.16: Parameter estimates for the final rat doxazosin MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{max}}$</td>
<td>0.089</td>
<td>7</td>
</tr>
<tr>
<td>IC$_{50}$ (nM)</td>
<td>0.347</td>
<td>25</td>
</tr>
<tr>
<td>Inter-individual variability in $I_{\text{max}}$ (%)</td>
<td>23</td>
<td>53</td>
</tr>
<tr>
<td>Inter-individual variability in IC$_{50}$ (%)</td>
<td>86</td>
<td>48</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>4.04</td>
<td>2</td>
</tr>
</tbody>
</table>

Diagnostic plots show an acceptable fit to the data (figures 5.18 and 5.19). The scatter plots of predicted against observed mean blood pressure display an even distribution of points around the line of unity and the deviation from the line is not too great in the case of the individual predictions. Weighted residuals are evenly distributed around zero with a good proportion of the points falling within ±2 standard deviations. Higher residuals are mainly seen for the time points between 500 & 1200 minutes and at the higher MBP values. As mentioned in previous chapters this corresponds with the rats’ active phase where higher intra-individual variability occurs. However there also appears to be a group of residuals falling below -2SD at approximately the same early time point. Inspection of the individual predicted mean blood pressure-time profiles (figure 5.20) indicates that this may be related to the unusual early response to
doxazosin. Although the majority of the low dose profiles appear to be predicted well, there are a couple of the medium and high dose profiles where it can be seen that the initial drop in MBP is missed, particularly for rat 6. The individual plots also show that due to the slight increase observed after the initial drop, the model does underestimate MBP for most of the high doses during this plateau phase.

Figure 5.18: Observed versus predicted mean blood pressure for the final rat doxazosin MBP PD model using population (A) or individual (B) parameters.

Figure 5.19: Population weighted residuals (PWRES) versus time (A) or mean blood pressure (B) for the final rat doxazosin MBP PD model.
Figure 5.20: Individual plots of observed (circles) and predicted (line) mean blood pressure with time in rats following administration of 1(LD), 10(MD) or 30(HD) mg/kg doxazosin.
5.4.1.2 Heart rate

5.4.1.2.1 PD model selection

Comparison of the HR response and concentration profiles for rats dosed with doxazosin indicated that peak response occurred at approximately the same time as peak concentration and thus a direct effect model would be the most suitable. Initial modelling of the data with a direct $E_{\text{max}}$ model and additive error produced a reasonable fit to the data except for some animals where the initial increase in HR due to the administration effect was consistently under-predicted. It is perfectly feasible that the level of effect due to administration could vary between occasions, therefore estimation the parameter P (extent of administration effect) and its variability were included to try and improve the fit.

Table 5.17: Overview of model selection for the rat doxazosin HR PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $E_{\text{max}}$; no $\eta$</td>
<td>20430.64</td>
<td>20440.64</td>
<td>-</td>
<td>Poor admin effect pred</td>
</tr>
<tr>
<td>Direct; $E_{\text{max}}$; $P$ est; $P$ $\eta$</td>
<td>20351.09</td>
<td>20365.09</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; $P$ est; $P$ $\eta$</td>
<td>20353.75</td>
<td>20369.75</td>
<td>-</td>
<td>High $k_{e0}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; $P$ est; $P$ $\eta$</td>
<td>20346.86</td>
<td>20362.86</td>
<td>-</td>
<td>High $k_{\text{out}}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; $P$ est; all $\eta$</td>
<td>20237.56</td>
<td>20259.56</td>
<td>Y</td>
<td>High SE &amp; shrinkage $\omega$ $E_{\text{max}}$ $k_{\text{out}}$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; $P$ est; $\eta$ EC$_{50}$</td>
<td>20240.76</td>
<td>20258.76</td>
<td>Y</td>
<td>High $k_{\text{out}}$</td>
</tr>
<tr>
<td>Direct; $E_{\text{max}}$; all $\eta$</td>
<td>20247.53</td>
<td>20265.53</td>
<td>Y</td>
<td>High SE &amp; shrinkage $\omega$ $E_{\text{max}}$</td>
</tr>
<tr>
<td><strong>Direct; $E_{\text{max}}$; $\eta$ EC$_{50}$</strong></td>
<td><strong>20246.38</strong></td>
<td><strong>20262.38</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

The direct effect model with estimation of P produced a significant drop in log-likelihood (-79.55, 2df; table 5.17) and an improved fit. However, when the direct effect model was compared to the delayed effect models, the fit statistics actually indicated that the indirect model was superior. The value for $k_{\text{out}}$ was very high though (59.22h$^{-1}$), which would effectively reduce the model to a direct effect. Addition of variability to the structural parameter of the indirect model produced a significant fall in log-likelihood (-109.3, 3df) and reduced the population estimate of $k_{\text{out}}$, although it was still high at 9.96h$^{-1}$. However, the omegas for $E_{\text{max}}$ and $k_{\text{out}}$ were estimated with poor precision (RSE=92-147%) and high shrinkage (85-95%). Removal of these etas resulted in a similar -2LL value and no issues with precision. However the $k_{\text{out}}$ had returned to a very high value (58.5h$^{-1}$), thus it was decided that actually a direct effect model would
be sufficient to describe the data. Addition of inter-individual variability to the structural parameters of the direct $E_{\text{max}}$ model resulted in a significant drop in log-likelihood (-103.56, 2df), however the omega for $E_{\text{max}}$ was poorly estimated (RSE=182%) and had high shrinkage (94%). Removal of this eta resulted in a similar -2LL value and no further issues.

5.4.1.2.2 Final PD model fit

The final doxazosin rat heart rate PD model was selected as a direct $E_{\text{max}}$ effect model, with inter-individual variability on $EC_{50}$, and additive residual error. Structural parameters were estimated with good to moderate precision (RSE=7-32%; table 5.18). Variability for $EC_{50}$ was high but the residual error was low, meaning the majority of the variability had been adequately explained.

Table 5.18: Parameter estimates for the final rat doxazosin HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.285</td>
<td>7</td>
</tr>
<tr>
<td>$EC_{50}$ (nM)</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td>Inter-individual variability in $EC_{50}$ (%)</td>
<td>116</td>
<td>37</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>26.1</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 5.21: Observed versus predicted heart rate for the final rat doxazosin HR PD model using population (A) or individual (B) parameters.
Figure 5.22: Individual plots of observed (circles) and predicted (line) heart rate with time in rats following administration of 1(LD), 10(MD) or 30(HD) mg/kg doxazosin.

Diagnostic plots show an acceptable fit to the data (figures 5.21 and 5.23). The scatter plots of predicted against observed heart rate display an even distribution of points.
around the line of unity. Weighted residuals are evenly distributed around zero with a good proportion of the points falling within ±2 standard deviations. Once again the majority of the points falling outside of the ±2SD range are for the rats’ active phase where random intra-individual variability is greater. Individual predicted mean heart rate-time profiles give a good overall description of the observed data and the drug effect (figure 5.22).

![Figure 5.23: Population weighted residuals (PWRES) versus time (A) or predicted heart rate (B) for the final rat doxazosin HR PD model.](image)

**5.4.2 Dog study**

Since the same dogs were used to collect all concentration and response data, individual estimates of baseline and PK parameters were fixed in the integrated PKPD model. The 72 hour washout between doses was sufficient so the different doses could be modelled as different occasions. However, due to the issues with inter-occasion modelling in Monolix, each dose was treated as a different individual. Although individual PK and baseline parameters were kept fixed, $R_{av}$ values were allowed to vary to account for any differences in average blood pressure/heart rate.

**5.4.2.1 Mean blood pressure**

**5.4.2.1.1 PD model selection**

Comparison of the doxazosin plasma concentration and mean blood pressure response time course did not give any indication of a delayed response. In fact for 2 of the 4 dogs the peak of response actually appeared to occur before the peak concentration. It was therefore assumed that a direct effect model would give the best fit to the data. However, when the goodness of fit statistics for the direct $I_{max}$ model were compared to the corresponding delayed effect models, the indirect model was indicated as producing
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the best fit. Comparison of the fits however did not show any difference for the more complex model and the \( k_{\text{out}} \) value was actually fairly high (4.37h\(^{-1}\)), which would effectively reduce the model to a direct effect. Therefore it was decided to continue model refinement with the direct effect model. An administration effect was again observed for the animals but a function was not included as the associated parameters \( P \) and \( k_{\text{Adm}} \) could not be precisely estimated.

Table 5.19: Overview of model selection for the dog doxazosin MBP PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; ( I_{\text{max}} ); no ( \eta )</td>
<td>8698.94</td>
<td>8708.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect cpt; ( I_{\text{max}} ); no ( \eta )</td>
<td>8726.13</td>
<td>8738.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect; ( I_{\text{max}} ); no ( \eta )</td>
<td>8696.08</td>
<td>8708.08</td>
<td></td>
<td>Lowest AIC but high ( k_{\text{out}} )</td>
</tr>
<tr>
<td>Direct; ( I_{\text{max}} ); all ( \eta )</td>
<td>8571.75</td>
<td>8585.75</td>
<td>Y</td>
<td>( I_{\text{max}} \sim 1 ); High SE IC(_{50}); High SE &amp; shrinkage ( \omega )</td>
</tr>
<tr>
<td>Direct; ( I_{\text{max}} ); IC(_{50}) ( \eta )</td>
<td>8571.41</td>
<td>8583.41</td>
<td>Y</td>
<td>( I_{\text{max}} = 1 )</td>
</tr>
<tr>
<td>Direct; ( I_{\text{slope}} ); no ( \eta )</td>
<td>8801.94</td>
<td>8809.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct; ( I_{\text{slope}} ); ( \eta )</td>
<td><strong>8562.22</strong></td>
<td><strong>8572.22</strong></td>
<td>Y</td>
<td><strong>FINAL MODEL</strong> Lower AIC</td>
</tr>
</tbody>
</table>

Addition of inter-individual variability to the structural parameters of the \( I_{\text{max}} \) model resulted in a significant drop in log-likelihood (-127.19, 2df). However both structural parameters were poorly estimated (RSE=78-91%), \( I_{\text{max}} \) was almost 1 and the omega for \( I_{\text{max}} \) was extremely imprecise (RSE=2x10\(^5\)% and had complete shrinkage (100%). Removal of the eta for \( I_{\text{max}} \) did improve the model with respect to IC\(_{50}\) precision but the \( I_{\text{max}} \) estimate was 1 and there were errors in calculating its precision. Since the \( I_{\text{max}} \) estimate was physiologically implausible and did not reflect the maximum effect observed for the dose range studied, it was decided a linear model may be more appropriate. A direct inhibitory linear effect model with no inter-individual variability did not result in improved fit statistics. However, addition of variability to \( I_{\text{slope}} \) resulted in a significant drop in log-likelihood (-239.72, 1df) and an AIC value that was lower than any of the \( I_{\text{max}} \) models’ values.

5.4.2.1.2 Final PD model fit

The final doxazosin dog mean blood pressure PD model was selected as a direct inhibitory linear effect model, with inter-individual variability on \( I_{\text{slope}} \) and additive residual error. The \( I_{\text{slope}} \) parameter was estimated with good precision (RSE=25%; table
5.20). Variability for $I_{\text{slope}}$ was high but additive residual error was low, indicating most of the variability had been accounted for within the $I_{\text{slope}}$ parameter.

Table 5.20: Parameter estimates for the final dog doxazosin MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{slope}}$ (nM$^{-1}$)</td>
<td>0.0379</td>
<td>25</td>
</tr>
<tr>
<td>Inter-individual variability in $I_{\text{slope}}$ (%)</td>
<td>85</td>
<td>42</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>4.53</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 5.24: Observed versus predicted mean blood pressure for the final dog doxazosin MBP PD model using population (A) or individual (B) parameters.

Diagnostic plots show an adequate fit to the data (figures 5.24 and 5.25). The scatter plot of individual predicted against observed mean blood pressure displays an even distribution of points around the line of unity. However for the population predicted values there appears to be a trend for under-prediction of the lower values. There are also a few under-predicted points at the higher values, which are the points associated with the administration effect that could not be predicted. Weighted residuals are evenly distributed around zero with a good proportion within ±2 standard deviations. The majority of those falling outside of this range are related to the administration effect as can be seen by the early time point.

Individual predicted mean blood pressure-time profiles are shown in figure 5.26. Overall they give a good description of the observed data and the extent of drug effect. However in some of the plots for dogs 1 (e.g. HD) and 3 (e.g. MD) it can be noticed that the peak observed effect is occurring before the peak predicted effect and is due to the higher $ka$ values and hence slower absorption predicted for these 2 individual animals.
Figure 5.25: Population weighted residuals (PWRES) versus time (A) or predicted mean blood pressure (B) for the final dog doxazosin MBP PD model.

Figure 5.26: Individual plots of observed (circles) and predicted (line) blood pressure with time in dogs administered 0.1(LD), 0.3(MD) or 1(HD) mg/kg doxazosin.
5.4.2.2 Heart rate

5.4.2.2.1 PD model selection

As for mean blood pressure, comparison of the doxazosin plasma concentration and heart rate response time courses did not give any indication of a delayed response. This once again suggested that a direct effect model would give the best fit to the data. However, comparison of the goodness of fit statistics (table 5.21) for the direct $E_{\text{max}}$ model and delayed effect models actually indicated the indirect model was superior. The $k_{\text{out}}$ estimate was extremely large though ($2.5 \times 10^4 \text{h}^{-1}$), which effectively reduces the model to a direct effect. Comparison of the fits did not show any advantage for the more complex model and thus once again it was decided to continue model refinement with the direct effect model.

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $E_{\text{max}}$; no $\eta$</td>
<td>11439.28</td>
<td>11449.28</td>
<td>-</td>
<td>Implausible $E_{\text{max}}$; High SE all</td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; no $\eta$</td>
<td>25605.42</td>
<td>25617.42</td>
<td>-</td>
<td>Implausible $E_{\text{max}}, k_{\text{ref}}$; High SE all; NaN errors</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; no $\eta$</td>
<td>11425.21</td>
<td>11437.21</td>
<td>-</td>
<td>Lower AIC; Implausible $k_{\text{out}}$</td>
</tr>
<tr>
<td>Direct; $E_{\text{max}}$; all $\eta$</td>
<td>11428.65</td>
<td>11442.65</td>
<td>Y</td>
<td>Implausible $E_{\text{max}}$; High SE all High SE &amp; shrinkage $\omega$ all</td>
</tr>
<tr>
<td>Direct; $E_{\text{slope}}$; no $\eta$</td>
<td>11439.26</td>
<td>11447.26</td>
<td>-</td>
<td>Lower AIC</td>
</tr>
<tr>
<td><strong>Direct; $E_{\text{slope}}$; $\eta$</strong></td>
<td><strong>11426.81</strong></td>
<td><strong>11436.81</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

The direct effect $E_{\text{max}}$ model presented with its own issues, with extremely poor precision of parameters estimates (RSE=1830-1850%) and an implausible $E_{\text{max}}$ estimate (46.5). Addition of inter-individual variability to both structural parameters resulted in a significant drop in log-likelihood (-10.63, 2df) and improved parameter precision but not to an acceptable level (RSE=193-207%). The $E_{\text{max}}$ estimate was also reduced but still implausible (4.95). In addition the omegas for both $E_{\text{max}}$ and $E_{C50}$ were extremely poorly estimated (RSE=985-1390%) and displayed high shrinkage (76-81%). A linear effect model was therefore considered as an alternative. A direct stimulatory linear model gave a good visual fit to the data and resulted in improved goodness of fit statistics. Addition of inter-individual variability to the $E_{\text{slope}}$ parameter resulted in a significant drop in log-likelihood (-12.45, 1df) and no other issues.

5.4.2.2.2 Final PD model fit
Chapter 5: Doxazosin

The final doxazosin dog heart rate PD model was selected as direct stimulatory linear effect model, with inter-individual variability on $E_{\text{slope}}$, and additive residual error. The $E_{\text{slope}}$ parameter was estimated with good precision (RSE=15%; table 5.22), inter-individual variability was moderately low, as was additive residual error.

Table 5.22: Parameter estimates for the final dog doxazosin HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{slope}}$ (nM$^{-1}$)</td>
<td>0.0523</td>
<td>15</td>
</tr>
<tr>
<td>Inter-individual variability in $E_{\text{slope}}$ (%)</td>
<td>38</td>
<td>66</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>12.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Diagnostic plots (figures 5.27 and 5.28) show an acceptable fit to the data. The scatter plots of predicted against observed heart rate display an even distribution of points around the line of unity, although there is more deviation from the line than observed in other cases. Weighted residuals are evenly distributed around zero with a reasonable proportion of the points falling within ±2 standard deviations. Individual predicted heart rate-time profiles are shown in figure 5.29. As for mean blood pressure, overall they give a good description of the observed data and the extent of drug effect. However in the plots for dog 1 it can be noted that the peak observed effect is occurring before the peak predicted effect. It can also be noted that for all dogs the intra-individual variability between consecutive points is high.

Figure 5.27: Observed versus predicted heart rate for the final dog doxazosin HR PD model using population (A) or individual (B) parameters.
Figure 5.28: Population weighted residuals (PWRES) versus time (A) or predicted heart rate (B) for the final dog doxazosin HR PD model.

Figure 5.29: Individual plots of observed (circles) and predicted (line) heart rate with time in dogs administered doses of 0.1(LD), 0.3(MD) or 1(HD) mg/kg doxazosin.
5.4.3 Guinea-pig study

Since doxazosin plasma concentrations and cardiovascular responses were measured in the same individual animals, individual PK estimates were subsequently fixed in the integrated PKPD model. In addition baseline response parameters were derived from pre-dose data for each animal (see section 5.3.3) and as a result were also fixed to individual values.

5.4.3.1 Mean blood pressure

5.4.3.1.1 PD model selection

The design for the doxazosin guinea-pig study was the same as for L-NAME and milrinone, thus it was once more difficult to determine which model might be appropriate just from a comparison of concentration and response time-courses. The fit of a direct $I_{\text{max}}$ model was therefore compared to those of an effect compartment model and an indirect model with inhibition of $k_{\text{in}}$. As had been the case for the other compounds the indirect model appeared to be the best model according to the fit statistics (table 5.23), although the precision for IC$_{50}$ was poor (RSE=55%).

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $I_{\text{max}}$; no $\eta$</td>
<td>2166.87</td>
<td>2172.87</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Effect cpt; $I_{\text{max}}$; no $\eta$</td>
<td>2130.70</td>
<td>2138.70</td>
<td>-</td>
<td>V. high SE IC$<em>{50}$, $k</em>{e0}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no $\eta$</td>
<td>2018.55</td>
<td>2026.55</td>
<td>-</td>
<td>Lowest AIC; High SE IC$_{50}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; all $\eta$</td>
<td>1702.36</td>
<td>1716.36</td>
<td>Y</td>
<td>High SE IC$<em>{50}$; High SE &amp; shrinkage $\omega$ k$</em>{\text{out}}$</td>
</tr>
<tr>
<td>Indirect; $I_{\text{max}}$; no $\eta$ k$_{\text{out}}$</td>
<td>1701.88</td>
<td>1713.88</td>
<td>Y</td>
<td>High SE IC$_{50}$</td>
</tr>
<tr>
<td><strong>Direct; $I_{\text{max}}$; all $\eta$</strong></td>
<td><strong>1789.59</strong></td>
<td><strong>1799.59</strong></td>
<td><strong>Y</strong></td>
<td><strong>FINAL MODEL</strong></td>
</tr>
</tbody>
</table>

Addition of inter-individual variability to all structural parameters resulted in a significant drop in log-likelihood (-316.19, 3df) but precision for IC$_{50}$ worsened (RSE=61%) and the omega for k$_{\text{out}}$ was also poorly estimated (RSE=599%) and had high shrinkage (91%). Removal of the k$_{\text{out}}$ eta resulted in an improved fit according to the fit statistics but the precision of the IC$_{50}$ was still poor (RSE=65%). Since poor precision can be an indication of over-parameterisation, it was considered that like for the rat and dog models, a direct effect model might actually be the most appropriate model. Addition of inter-individual variability to the structural parameters of the direct
I_{max} model produced a significant drop in log-likelihood (-377.28, 2df) and no issues with parameter precision.

5.4.3.1.2 Final PD model fit

The final doxazosin guinea-pig mean blood pressure PD model was selected as a direct I_{max} effect model, with inter-individual variability on I_{max} and IC_{50}, and additive residual error. Structural parameters were estimated with good to moderate precision (RSE=8-35%; table 5.24) and additive residual error was low. Variability for I_{max} was low but for IC_{50} was high. Part of this may due to the differing baseline slopes.

Table 5.24: Parameter estimates for the final guinea-pig doxazosin MBP PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_{max}</td>
<td>0.386</td>
<td>8</td>
</tr>
<tr>
<td>IC_{50} (nM)</td>
<td>0.542</td>
<td>35</td>
</tr>
<tr>
<td>Inter-individual variability in I_{max} (%)</td>
<td>34</td>
<td>60</td>
</tr>
<tr>
<td>Inter-individual variability in IC_{50} (%)</td>
<td>95</td>
<td>53</td>
</tr>
<tr>
<td>Additive residual error (mmHg)</td>
<td>1.83</td>
<td>3</td>
</tr>
</tbody>
</table>

Diagnostic plots (figures 5.30 and 5.32) show good fit to the data for both population and individual models. The scatter plots of predicted against observed mean blood pressure display an even distribution of points around the line of unity and little deviation from the line for the individual model.

![A](image1.png) ![B](image2.png)

Figure 5.30: Observed versus predicted mean blood pressure for the final guinea-pig doxazosin MBP PD model using population (A) or individual (B) parameters.
Weighted residuals are evenly distributed around zero with no observable pattern and a good proportion of points within ±2 standard deviations.

![Population weighted residuals (PWRES) versus time (A) or mean blood pressure (B) for the final guinea-pig doxazosin MBP PD model.](image)

**Figure 5.31**: Population weighted residuals (PWRES) versus time (A) or mean blood pressure (B) for the final guinea-pig doxazosin MBP PD model.

Individual predicted mean blood pressure-time profiles give a good overall description of the observed data and the drug effect (figure 5.31).

![Individual plots of observed (circles) and predicted (line) mean blood pressure with time in guinea-pigs administered multiple doses of doxazosin.](image)

**Figure 5.32**: Individual plots of observed (circles) and predicted (line) mean blood pressure with time in guinea-pigs administered multiple doses of doxazosin.

5.4.3.2 Heart rate

5.4.3.2.1 PD model selection

As was the case for mean blood pressure, it was difficult to determine the possibility of a delay due to the lack of concentration measurements. A direct $E_{\text{max}}$ model was
therefore compared with both delay models, to see which gave the best fit. Comparison of the goodness of fit statistics (table 5.25) indicated that the direct model gave the best fit, which corresponded with the choice for rat and dog. Addition of inter-individual variability to the structural parameters improved the model with a significant drop in log-likelihood (-745.93, 2df) and better visual fit to the data.

Table 5.25: Overview of model selection for the guinea-pig doxazosin HR PD data

<table>
<thead>
<tr>
<th>Model</th>
<th>-2LL</th>
<th>AIC</th>
<th>Sig</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct; $E_{\text{max}}$; no $\eta$</td>
<td>2708.77</td>
<td>2714.77</td>
<td>-</td>
<td>Lowest AIC</td>
</tr>
<tr>
<td>Effect cpt; $E_{\text{max}}$; no $\eta$</td>
<td>2711.45</td>
<td>2719.45</td>
<td>-</td>
<td>High SE $k_0$</td>
</tr>
<tr>
<td>Indirect; $E_{\text{max}}$; no $\eta$</td>
<td>2708.76</td>
<td>2716.76</td>
<td>-</td>
<td>Implausible $k_{out}$</td>
</tr>
<tr>
<td>Direct; $E_{\text{max}}$; all $\eta$</td>
<td>1962.84</td>
<td>1972.84</td>
<td>Y</td>
<td>FINAL MODEL</td>
</tr>
</tbody>
</table>

5.4.3.2.2 Final PD model fit

The final doxazosin rat heart rate PD model was selected as direct $E_{\text{max}}$ effect model, with inter-individual variability on $E_{\text{max}}$ and $EC_{50}$, and additive residual error. Structural parameters were estimated with good to moderate precision (RSE=7-32%; table 5.26) and additive residual error was low. Variability for $E_{\text{max}}$ was moderate but for $EC_{50}$ was high.

Table 5.26: Parameter estimates for the final guinea-pig doxazosin HR PD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.169</td>
<td>17</td>
</tr>
<tr>
<td>$EC_{50}$ (nM)</td>
<td>0.398</td>
<td>36</td>
</tr>
<tr>
<td>Inter-individual variability in $E_{\text{max}}$ (%)</td>
<td>45</td>
<td>54</td>
</tr>
<tr>
<td>Inter-individual variability in $EC_{50}$ (%)</td>
<td>92</td>
<td>55</td>
</tr>
<tr>
<td>Additive residual error (bpm)</td>
<td>3.35</td>
<td>4</td>
</tr>
</tbody>
</table>

Diagnostic plots (figures 5.33 and 5.34) show an acceptable fit to the data. The scatter plot of individual predicated against observed heart rate displays an even distribution of points around the line of unity and little deviation. However for the population predicted plot there is some slight bias towards under-prediction at lower values and over-prediction at higher values, likely due to the baseline differences. Weighted residuals are evenly distributed around zero with no observable pattern and the majority within ±2 standard deviations.
Individual predicted heart rate-time profiles give a good overall description of the observed data and the drug effect (figure 5.35).

Figure 5.33: Individual plots of observed (circles) and predicted (line) heart rate with time in guinea-pigs administered multiple doses of doxazosin.

Figure 5.34: Observed versus predicted heart rate for the final guinea-pig doxazosin HR PD model using population (A) or individual (B) parameters.
5.5 Prediction of human cardiovascular response to doxazosin

5.5.1 Human PK & PD data

5.5.1.1 Literature search

A literature search for doxazosin studies in human subjects revealed several potentially suitable articles. Once again a number of these had been performed in patients, in this case in individuals with hypertension e.g. Conrad et al., (1988); Donnelly et al., (1989). There were however a series of studies from the same group that measured the cardiac response to doxazosin in healthy volunteers (Elliott et al., 1982; Elliott et al., 1986; Vincent et al., 1983).

The first study, Elliott et al., (1982), was a placebo controlled single dose design in 6 young healthy male volunteers. Intravenous boluses of either placebo (saline vehicle) or 12µg/kg doxazosin were administered to the subjects one week apart in a randomised manner. Blood pressure (systolic and diastolic) and heart rate were measured by an automated blood pressure monitor and on an ECG recording respectively. Measurements were taken in both the supine position and after 1-2 minutes standing. Only the standing results were reported as doxazosin had no effect on supine measurements. In addition to cardiovascular response, blood samples were taken to measure doxazosin concentrations. Timings for blood pressure and heart rate measurements and for blood sampling were not reported in the methodology section, however they could be approximately estimated from the response and concentration figures. The approximate timings for cardiovascular responses were pre-dose (0) and at 10, 20, 40 minutes, and 1, 1.5, 2, 4, 6, 8, 24 hours post-dose. Timings were similar for...
doxazosin concentrations with blood samples taken at approximately 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 and 24 hours post-dose.

The second study, Vincent et al., (1983), looked at the effect of a 2mg oral dose of doxazosin, in addition to an intravenous dose. The article did not give any details about being placebo controlled, however the data was re-used in the group’s third study (Elliott et al., 1986) and reported placebo responses in that paper. Study design was similar to the Elliott 1982 study; blood pressure was measured by an automated blood pressure monitor and heart rate on an ECG recording. Measurements were taken in both the supine position and after 2 and 5 minutes standing. Again the methods did not specify the timings of these but from the graph they were approximated as pre-dose (0) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours post-dose. Blood samples for measurement of doxazosin concentrations were also taken at these timings and at 10, 12 and 24 hours post-dose.

As mentioned above the third study, Elliott et al., (1986), made use of the results from the first and second studies. Placebo MBP & HR response data were obtained from this study, since they were not reported in Vincent et al., (1983).

5.5.1.2 Modelling of PK and baseline data
The majority of data was reported graphically in all 3 studies, thus graphs were digitized to obtain concentration/MBP/HR points for modelling.

The only data that did not require modelling were the intravenous PK data (Elliott et al., 1982), where fitted model parameters were reported. However the model used was an empirical sum of two exponentials, so clearance and volume parameters had to be derived from the reported macro-constants. In addition the concentrations had been measured in whole blood so required conversion to plasma values. Luckily the Vincent study reported parameters from whole blood and plasma, enabling the calculation of the doxazosin blood-to-plasma ratio (B/P). The average calculated B/P value of 0.635 was used to convert the macro-constants to plasma values and then CL, \( V_1 \), Q and \( V_2 \) were derived using standard equations. The resulting values were 0.063L/h/kg for CL, 0.631L/kg for \( V_1 \), 0.644L/h/kg for Q and 0.321L/kg for \( V_2 \).

The average concentration-time profile in the Vincent study was in whole blood and thus required conversion to plasma concentration using the B/P ratio. In addition data were converted to nano-molar units for consistency with the pre-clinical species.
A 1-compartment, 1st order absorption model with proportional error provided an adequate fit to the data. The estimated parameter values were $1.22 \text{h}^{-1}$ for $k_a$, $7.59 \text{L/h}$ for $\text{CL/F}$ and $72.9 \text{L}$ for $\text{V/F}$. The volunteers’ body weights were not reported but assuming a standard 70kg, this results in values of $0.108 \text{L/h/kg}$ for $\text{CL/F}$ and $1.04 \text{L/kg}$. The estimates were highly precise (RSE=1-3%) and proportional error was extremely low at 2.85%. The fit of the data is shown in figure 5.36.

![Figure 5.36](image.png)

**Figure 5.36**: Plot of observed (circles) and predicted (line) plasma doxazosin concentration with time in healthy volunteers following oral administration of 2mg doxazosin. Data from Vincent *et al.*, (1983).

The cardiovascular measurements following placebo were taken over a 24 hour time frame for the Elliott *et al.*, (1982) study. This would allow a full circadian model to be fit if the time points were at regular enough intervals throughout that period. However there was a large time gap between 8 and 24 hours where no measurements were taken. Since the 24 hour time point was included purely to show that MBP/HR had returned to baseline after doxazosin dosing, it was decided to ignore this final time-point for baseline fitting. This left measurements for 8 hours, which was the same as the Vincent study. There was no indication of the time of day either of the studies were started but it was assumed to be the morning to allow 8 hours worth of measurements to be taken. Since it is known that there is a circadian rhythm during the daytime, a single 12 hour cosine model was fit to each of the data sets. The model successfully described the data in each case. Parameter estimates are shown in table 5.27 and are reasonably consistent between studies and the two measures. In both studies the degree of change is lower for MBP and peak response occurs later, although for the latter the difference is much greater for the Elliott *et al.*, (1982) study.
Table 5.27: Parameter estimates for the human MBP and HR baseline model

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBP (mmHg or bpm)</td>
<td>HR (mmHg or bpm)</td>
</tr>
<tr>
<td>$R_{av}$</td>
<td>90.2</td>
<td>91.5</td>
</tr>
<tr>
<td>$R_{amp}$</td>
<td>3.9</td>
<td>6.62</td>
</tr>
<tr>
<td>$T_z$ (h)</td>
<td>10.8</td>
<td>7.14</td>
</tr>
</tbody>
</table>

For the Elliott et al., (1982) study parameters were estimated with good precision for both MBP (RSE=1-17%) and HR (RSE=1-21%). Additive residual errors were low in both cases (1.08mmHg and 3.08bpm). Fits of the Elliott et al., (1982) MBP and HR placebo response data are shown in figure 5.37.

For the Elliott et al., (1986) study parameters were again estimated with good precision for HR (RSE=1-15%) and for $R_{av}$ and $T_z$ for MBP (RSE=1-3%). The $R_{amp}$ parameter was imprecise (RSE=79%) but this was likely due to jumps in diastolic blood pressure seen at 1 and 2 hours. Without these time points the amplitude would most likely increase, however the fit was considered sufficient for purpose since the data was taken from the same individuals dosed with doxazosin. Additive residual errors were again low in both cases (1.08mmHg and 3.08bpm). Fits of the MBP and HR placebo response data are shown in figure 5.38.
5.5.2 Predictions

Integrated PKPD models for prediction of human cardiovascular response to doxazosin were scripted based on the models derived for each of the 3 pre-clinical species. PK parameters for the oral dosing were fixed to those derived from the mean concentration time profile in the Vincent study. For the intravenous study the parameters were fixed to those derived from the reported macro-constants in the Elliott et al., (1982) study. Predicted human doxazosin plasma concentrations were converted to unbound concentrations using the fraction unbound in plasma value of 0.017 reported in Kaye et al., (1986). Baseline parameters were fixed to those derived from the modelling of placebo data. However, the $R_{av}$ parameter was slightly adjusted for each prediction to account for differences in the MBP/HR observed values at time 0.

5.5.2.1 Mean blood pressure

The prediction of doxazosin effect on human mean blood pressure was described either via a direct $I_{max}$ effect model or a direct inhibitory linear effect model. The former was used with PD parameters derived from either the rat or the guinea-pig study and the latter with values derived from the dog study.

Table 5.28: Summary of final doxazosin MBP PD model parameters for all 3 species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Dog</th>
<th>Guinea-pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{slope}$ (nM$^{-1}$)</td>
<td>-</td>
<td>0.0379</td>
<td>-</td>
</tr>
<tr>
<td>$I_{max}$</td>
<td>0.089</td>
<td>-</td>
<td>0.386</td>
</tr>
<tr>
<td>IC$_{50}$ (nM)</td>
<td>0.347</td>
<td>-</td>
<td>0.542</td>
</tr>
</tbody>
</table>
These values are summarised in table 5.28. As can be seen from the table there is some consistency in the IC\textsubscript{50} values for rat and guinea-pig, which are in the same order of magnitude.

The predictions for both the oral and intravenous dose are shown in figure 5.39. As can be observed the figure the rat and dog models both under-predict the effect of doxazosin on mean blood pressure in humans However, the guinea-pig model does seem to capture the magnitude of effect quite well. Unfortunately the time course of the effect is not accurately predicted as there appears to be a delay in humans that was not observed for the 3 pre-clinical models. Overall the results indicate that although the direct model is incorrect, the extent of effect is well reflected by the guinea-pig values for efficacy and potency.

![Figure 5.39: Prediction of observed human mean blood pressure (circles) following administration of 2mg p.o. (A) or 12µg/kg i.v. (B) doxazosin, using the PD model and parameter estimates for rat (solid line), dog (dashed line) or guinea-pig (dotted-dashed line).](image)

5.5.2.2 Heart rate

The prediction of doxazosin effect on human heart rate was described either via a direct \(E_{\text{max}}\) effect model or a direct stimulatory linear effect model. The former was used with PD parameters derived from either the rat or the guinea-pig study and the latter with values derived from the dog study. These values are summarised in table 5.29. As can be seen from the table there is no consistency in the values across the 3 species, however when compared with MBP values the dog & guinea-pig values are of the same order of magnitude.
Table 5.29: Summary of final doxazosin HR PD model parameters for all 3 species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Dog</th>
<th>Guinea-pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{slope}}$ (nM$^{-1}$)</td>
<td>-</td>
<td>0.0523</td>
<td>-</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.285</td>
<td>-</td>
<td>0.169</td>
</tr>
<tr>
<td>EC$_{50}$ (nM)</td>
<td>24</td>
<td>-</td>
<td>0.398</td>
</tr>
</tbody>
</table>

The predictions for both the oral and intravenous dose are shown in figure 5.40. As was the case for mean blood pressure, it is the guinea-pig model that produces the best prediction of the effect of doxazosin on heart rate in humans. However, the extent of effect is more closely predicted for the oral dose compared to the intravenous dose, where a considerable degree of under-prediction is observed. Like all other cases except MBP for doxazosin the rat model shows barely any change from baseline. The dog model also shows only modest changes from baseline. For all 3 species the time-course of effect is incorrectly predicted. Once again there appears to be a delay in effect in humans that was not observed in the animal studies. Overall the results indicate that the direct model is again incorrect but the extent of effect is reasonably reflected by the guinea-pig values, for oral administration of doxazosin at least.

Figure 5.40: Prediction of observed human heart rate (circles) following administration of 2mg p.o. (A) or 12µg/kg i.v. (B) doxazosin, using the PD model and parameter estimates for rat (solid line), dog (dashed line) or guinea-pig (dotted-dashed line).
Chapter 6

Discussion and Conclusions
6 Discussion and Conclusions

Pharmacokinetic-pharmacodynamic modelling and simulation methods are well established techniques, used throughout the therapeutic development of new chemical entities, from pre-clinical to late clinical phases. Usage of these techniques within the safety sciences has to date been extremely limited. Thus, the overall aim of this project is to show the potential benefit of applying these methods to existing safety pharmacology data in an effort to ensure any adverse effects are understood and can be predicted in man. The first stage of fulfilling this aim was to investigate the application of PKPD modelling techniques to available safety pharmacology data and the second to prediction of human drug response.

6.1 PK

Correct description of the plasma concentration profile is vital for obtaining the correct pharmacodynamic parameters within a PKPD model. Compartmental models were used to determine the change in concentration with time and overall seemed to give a good description of the data.

6.1.1 L-NAME

L-NAME had been chosen as the first reference compound to study as the effects on the cardiovascular system are highly reproducible. However, since it is actually the active metabolite L-NOARG which exerts these cardiovascular effects, it was L-NOARG plasma concentrations that were measured. This potentially added complexity to the PK modelling as one compound had been administered (L-NAME) and another measured (L-NOARG).

Fitting of a standard compartmental PK model to L-NOARG concentration data following i.v. dosing of L-NAME assumes an instantaneous conversion of L-NAME to L-NOARG. For an oral dose, the absorption term in a standard PK model actually reflects a combination of both L-NAME absorption and its conversion to L-NOARG. To investigate the need to independently account for this metabolic conversion, a 1st order absorption PK model was modified to include a central compartment for L-NAME as well as L-NOARG. The modification introduced an additional parameter, \( k_{\text{met}} \), to describe the conversion of L-NAME to L-NOARG.
The results of the PK fitting for the species where L-NAME had been dosed orally (rat and dog) did not support the inclusion of the extra parameter. It has been shown that the conversion from L-NAME to L-NOARG is an extremely rapid process in vivo in rats (Brouillet et al., 1995) and ex vivo in canine plasma (Krejcy et al., 1993). As long as the conversion is complete and irreversible, as is indicated, the need to account for it within the model could be considered unnecessary. The model developed to account for the conversion may also have identifiability issues due to the lack of L-NAME measurements. Although no formal identifiability analysis has been performed, if there were a need to determine $k_{met}$, L-NAME plasma concentrations would most likely be required. Alternatively the $k_{met}$ parameter could be determined from previous studies where both compounds’ concentrations are measured and then implemented as a fixed value.

Peak L-NOARG plasma concentrations for both rat and dog occurred within 1 hour in most cases. However, blood samples were obtained for very few early time points, with the first measurement taken at 1 hour for dogs. This indicated a rapid absorption of L-NAME in addition to the rapid conversion to L-NOARG. Insufficient measurements within this time-frame is the most likely reason for the failure to precisely estimate a 1st-order absorption rate constant for either species. Consequently, a constant rate of absorption (zero-order) had to be assumed to obtain acceptable parameter precision. Little information is available regarding the absorption of L-NAME and it appears the only reason it is dosed in preference to L-NOARG is due to its increased solubility (Griffith et al., 1996). However, L-NOARG has been directly dosed both orally and intravenously in a number of rat studies and bioavailability shown to be high at ~90% (Piotrovskij et al., 1993; Tabrizi-Fard et al., 1996; Tabrizi-Fard et al., 1994). Absorption was generally rapid for the oral administration of L-NOARG, with a mean $T_{max}$ of 1.17 hours; although the variability was high at 78% (Piotrovskij et al., 1993). This corresponded well with the inter-individual variability seen in the duration of absorption ($T_{k0}$) for L-NAME, which was 82%. Due to its low lipophilicity an active transport system was hypothesised for L-NOARG absorption across the gut wall and it could be possible the same occurs for L-NAME. It appears the use of L-NOARG instead of L-NAME would have been possible and could have by-passed any issues with the conversion, however there do not appear to be any studies of L-NOARG in humans reported in the literature. In either case, it appears timings of blood sampling was not optimal to accurately model the absorption/(conversion) phase.
Following the absorption and conversion of L-NAME to L-NOARG, the distribution and elimination of L-NOARG in rats was adequately described by a 1-compartment model. However the model did show some tendency for over-prediction at some higher concentrations. For the individual model, these were identified as the 4 hour time points for the rats receiving the highest dose and could be predicted well with application of a 2-compartment model. However the 2-compartmental model was not supported by the fit statistics and did not improve the population model, which was used in the PKPD model. Examination of dose corrected concentrations from the individual rats (see appendix 1.4) indicated that the concentrations from the 100mg/kg dose were consistently lower than for the 10 and 30mg/kg doses. This would explain the consistent over-prediction of the higher concentrations. However, since the mechanism leading to lower relative concentration is unknown, it is unaccounted for within that model and thus is reflected in the high residual variability instead.

Published studies of L-NOARG PK in rats all consistently show that plasma concentrations follow a bi-exponential decline after i.v. dosing. However following oral dosing the nature of the decline was not clear due to substantial secondary peaks in virtually all of the profiles (Piotrovskij et al., 1993). It is unclear as to whether this phenomenon occurred for rats in the Pfizer L-NAME study due to the lack of data points for most of the animals. Information was not available as to whether the missing data points were removed due to such peaks occurring. Since peaks were also seen in some i.v. studies (Piotrovskij et al., 1994a; Piotrovskij et al., 1993) entero-hepatic recycling was considered as a possible cause. However it has been discounted due to negative experiments in bile duct cannulated rats (Piotrovskij et al., 1994b).

PK parameter values from the published studies of L-NOARG consistently show a low clearance (~0.07L/h/kg) and a high steady state volume (~2L/kg). Low clearance and high volume values were also obtained from the modelling of the rat PK data, although the value for clearance was 4 times higher (0.281L/h/kg) and volume was nearly twice as high (3.89L/kg) as those published for L-NOARG. Despite the potential difference due to the administration of L-NAME, values from the current study were only obtained over 24 hours whereas for all published studies measurements were obtained for at least 3 days due to L-NOARG’s long half life. One of the published studies reported a potential non-linearity in clearance for doses of 100mg/kg. This was not observed in the current study, since dose-corrected concentrations were actually found to be lower at this dose level. Little is actually known about the elimination of L-NOARG apart from
that it appears to be mediated via a metabolic process due to the low presence of unchanged compound in urine (Piotrovskij et al., 1994a).

Distribution and elimination of L-NOARG in dogs differed from the rat and required a 2-compartment model to describe the bi-phasic nature of the profiles. This is likely due to the different study design where both doses were administered to the same dogs one week apart, enabling the long half life of the L-NOARG to be captured. PK parameter estimates also differed between the species, with clearance and steady state volume displaying more agreement with the published rat values (CL/F=0.0455L/h/kg and $V_{ss}/F=2.19$L/kg, where $V_{ss}/F=V_1/F+V_2/F$). There is one published study of L-NOARG in conscious dogs in the literature (Huber et al., 1992) and although plasma concentrations after oral administration were measured, no PK analysis was performed. Extraction of the average plasma concentrations from the reported graph enabled the PK parameters to be calculated using a 1-compartment, 1st order absorption model. The resulting parameters were 0.00894L/h/kg for CL/F and 0.756L/kg for V/F, which were 5- and 3-fold lower than values calculated from the current study, respectively. However, the values derived from the published study should be heeded with caution, since few time points were measured and only over a 24 hour period, they were average values and the first two time points (1 and 3 hours) had extremely large variability (CV~50%).

The available data for the guinea-pig was very sparse, with only one concentration measured for each dose. With this little data the only model that could be supported was a simple one compartment model. The infusion protocol administered was selected to achieve specific steady-state concentrations. However, the model did not appear to predict the concentrations to be at steady-state at the time-points selected. With the use of a mixed effect approach further information could be gained if the samples were taken at different time points for different animals and could be a design consideration for future studies.

The model required inter-individual variability in $V$ to give the best fit, which is unusual and this value was moderately high at 60%. In addition, when the clearance value is adjusted to the same units as the other species, 2.23L/h/kg, it becomes clear that it is hugely different. Clearance is consistently low in both rat and dog; however the value for the guinea-pig is high and close to the liver blood flow (Yates et al., 1979). In
addition volume was much lower than in the dog and rat at 0.744L/kg. This value still indicates distribution into the tissues but not to the same extent as the other species.

One issue that was not taken into account for the guinea-pig was the conversion of L-NAME to L-NOARG. L-NAME concentrations were available but were very low compared with the L-NOARG concentrations (~5 %), indicating that the conversion was rapid. Initial modelling included the L-NAME concentrations but was unable to accurately determine either $k_{\text{met}}$ or the L-NAME volume of distribution. Values for L-NOARG parameters were unaffected by the inclusion of L-NAME so it was decided to simplify the model and exclude it.

### 6.1.2 Milrinone

Modelling of plasma concentration-time data for milrinone was more straightforward than for L-NAME and only required the use of standard PK models.

Rat and dog were again dosed orally but this time absorption could be described by a 1st order absorption model in both cases. Absorption was rapid for both species; with $k_a$ values of 9.54h$^{-1}$ and 6.14h$^{-1}$, maximum concentrations were predicted at approximately 10 minutes for rat and 30 minutes for dog. Inter-individual variability in $k_a$ was found to significantly improve the model for rats and at 37% could be considered due to natural variability between the animals. The only potential issue to highlight is that the first blood sample in rats was taken at 15 minutes, which was later than all the individual animals’ predicted maximum concentration except one. Any future studies with this compound should take this into account when selecting sampling times.

The profiles for all rats were biphasic and thus as expected a 2-compartment model was the best fit to the data. However, an additive residual error model was required to give precise estimates for some of the parameters, which is not typical. The additive error value of 338nM was reasonably low when compared to the plasma concentrations for the 2 higher doses but high as a proportion of the concentrations for the lowest dose. It appears that the profiles for the low dose had a less well defined shape, which may explain the need for a higher residual error. Since it is residual error it does not give any insight into why the profiles are less distinct.

The apparent clearance (CL/F) determined for the rat was moderate at 1.12L/h/kg. This agreed well with the literature value of 0.975L/h/kg (Brocks et al., 2005). Since milrinone is mainly excreted unchanged via the kidneys and only eliminated to a minor
extent by metabolism (Baker et al., 1984), it is unsurprising that bioavailability is high at ~90% and that these values for clearance following i.v. and oral dosing are similar. Since the kidney is the major route of elimination, it would be expected that clearance is related to kidney function. Since the derived value is higher than the glomerular filtration rate (GFR) multiplied by the fraction unbound in plasma (fu), the elimination is not purely passive. Active tubular secretion must also be involved resulting in a value of approximately half the renal blood flow value for rats of 2.21L/h/kg (Davies et al., 1993).

Volume of distribution values (1.62 and 3.28L/kg) indicated extensive distribution within tissues. Brocks et al., (2005) reported bi-phasic profiles for milrinone in rats after i.v. dosing, with volume values of 0.397 and 0.282L/kg. Although much lower than the Pfizer study these values are still indicative of distribution into the tissues.

Milrinone plasma concentration profiles for dogs were adequately described by a 1-compartment model. However, once again the time points for blood sampling were not optimal, since all time points after 4 hours were below the limit of quantification (BLQ) and for 1 animal the 4h time point was also BLQ. Since the lower limit of quantification for this study was unknown very few concentrations were available to determine the PK parameters. A published study of i.v. milrinone PK data (Edelson et al., 1983) indicated a biphasic profile, best fit by a 2-compartment model. However, since absorption can mask the fast distribution phase in an oral PK profile, differences in the shapes of i.v. and oral profiles are often observed.

The clearance value of 0.267L/h/kg was approximately half the reported i.v. clearance in dogs of 0.531L/h/kg (Edelson et al., 1983). This published value is approximately half the renal blood flow for dogs (Davies et al., 1993) and thus would concur with the values observed for rats. The lower estimated value is still greater than GFR*fu and thus indicates active tubular excretion. It is possible that the clearance was underestimated and if the LLOQ had been known or time points more carefully selected a more accurate estimate could have been achieved.

The estimated apparent volume of distribution of 0.829L/kg again indicated distribution into the tissues but not to as great an extent as for rat. The value was lower than the $V_{ss}$ from the Edelson study (1.41L/kg), although this appeared to be due to a particularly high peripheral volume for one of the animals as the mean of the other 2 animals was 0.92L/kg.
A 1-compartment model was fit to the guinea-pig since the study design was the same as for L-NAME. Again the profile did not appear to result in steady-state levels for the time points measured. There are no published studies of milrinone in guinea-pigs, however the estimated clearance of 1.18L/h/kg was in good agreement with the rat value and had a low inter-individual variability of 17%. The volume of distribution estimate of 0.217L/kg, once again indicates some distribution into the tissue and agrees well with the published rat value.

6.1.3 Doxazosin

Modelling of doxazosin PK data again only required standard models to achieve a good fit. Study designs were the same as for milrinone, thus rat and dog were dosed orally and a 1\textsuperscript{st} order absorption model was suitable in both cases. Absorption was much slower than for the other two compounds; with population $k_a$ values of 1.88h\textsuperscript{-1} and 1.19h\textsuperscript{-1}, maximum concentrations were predicted at approximately 70 minutes for rat and 2.25 hours for dog. However, the time of maximum concentration varied between dogs and thus inter-individual variability in $k_a$ was required in the model and estimated as 55%. In fact none of the four dogs had peak concentrations around the 2.25h mark; the distribution appeared bimodal with peak concentrations occurring at approximately 1.5-1.75h for two animals and at approximately 3-3.25h for the other two. A previous study of doxazosin PK in a number of pre-clinical species and humans indicated that absorption in dogs was incomplete with approximately 40% excreted unchanged in the faeces (Kaye et al., 1986). The vehicle used for the safety pharmacology study was 20% PEG 200 in purified water, presumably to increase the absorption; however it is not known whether it was complete in this case. Peak concentrations for the same dose in another study in dogs were approximately 3-fold higher (Witte et al., 2002) and the same dose administered in a pilot study to dog 4 was again 3-fold higher. This does appear to indicate variable and incomplete absorption for doxazosin in these animals; however, PEG has also been shown to affect plasma drug measurements (Shou et al., 2003; Weaver et al., 2006). Without further details of the bioanalytical methods, underprediction of concentration due to PEG cannot be discounted.

Profiles for both rat and dog appeared mono-phasic and a 1-compartment model was sufficient for a good fit. However it must once again be noted that the blood sampling times may not have been optimal since samples were not taken between 6 and 24 hours. In a number of cases, for both species, concentrations were BLQ at 24 hours. The
LLOQ was available for dog and thus was accounted for within the model, however optimal sampling times may be something to consider for the design of future safety pharmacology studies.

The apparent oral clearance estimated for rats was high at 5.08L/h/kg, while the reported value for i.v. clearance was 1.8L/h/kg (Kaye et al., 1986). This would indicate a bioavailability of 35%, slightly lower than the 50% value reported (Kaye et al., 1986). The CL/F value estimated in dogs was 2.51L/h/kg. When compared to the CL value of 0.78L/h/kg reported in Kaye et al., (1986) a value of 31% for bioavailability is indicated. This is inconsistent with the reported F value of 60% (Kaye et al., 1986). Since 40% of the dose was excreted unchanged in the faeces in the Kaye study, this would indicate that F is equal to the fraction absorbed (fa) and no pre-systemic metabolism occurred. The CL/F value of 0.73L/h/kg reported for the Witte study indicates complete bioavailability and hence also complete absorption. These differing results for dog indicate that there may be issues with absorption but that there is little/no first pass metabolism. The low F value for the Pfizer study may therefore be due to very poor absorption from the formulation and would explain the much lower concentrations compared to Witte et al., (2002).

Kate et al., (1986) reported that metabolism was the main route of elimination for doxazosin in all species, however a difference in metabolic profiles between the species was observed. It is known that CYP3A4 is the main enzyme responsible for metabolism of doxazosin in man and although clearance is reasonably low, some first pass extraction in the gut and liver would be expected. The main metabolite in rat was the same as for human, thus it could be conceived that the equivalent CYP enzyme is involved. With a higher clearance than human, it is likely that first pass metabolism contributed to the lower bioavailability. The metabolic profile for the dog was completely different though. The metabolites quantities were small compared to the large proportion excreted unchanged in the faeces and one of the main metabolites was a glucuronide. It is therefore feasible that little first pass metabolism occurs in the dog, which supports the theory that low bioavailability is largely due to poor absorption.

Apparent volume of distribution values were extremely high for both rat (18.4L/kg) and dog (23.6L/kg), indicating extensive distribution into the tissues. These apparent values are much higher than the true values from i.v. dosing, which were 3L/kg for rat and 5L/kg for dog (Kaye et al., 1986). However, the volume of distribution values derived
from the other oral study in dogs (Witte et al., 2002) were 6-8L/kg for the 3 doses levels, similar to the true value.

Since the study design for guinea-pig was the same as for L-NAME and milrinone a 1-compartment model was fit to the doxaozsinc plasma concentration data. This time the profiles did appear to result in more steady-state like levels but not for all individuals. There are no published studies of doxazosin in guinea-pigs but in comparison to rats the estimated clearance of 2.74L/h/kg indicates a more rapid elimination. The volume of distribution value (1.93L/kg) indicates similar extensive distribution into the tissues.

6.2 Baseline

When characterising a drug effect, accounting for the baseline response is vitally important to avoid biased or imprecise results. Often data are normalised to baseline, either as a ratio, through subtraction of baseline or as a percentage change relative to baseline. However, the baseline measurements themselves can hold information which is likely to be lost in this process and so is not the preferred approach. Various approaches to handling baseline in a non-linear mixed effects analysis, including normalisation, treating baseline as a covariate and modelling the baseline to give population values and inter-individual variability have been analysed (Dansirikul et al., 2008). The conclusion, when both bias and imprecision were considered, was that the modelling approach was best and thus was the approach taken in this project.

6.2.1 Circadian rhythm models

Many baseline responses will be of a continuous nature however, there are also many physiological variables that display time-dependent biological rhythms, such as circadian rhythms. In these cases it is particularly important to characterise the baseline response, although there appears to be a lack of models available to do so.

6.2.1.1 Sallstrom model

The Sallstrom model (Sallstrom et al., 2005) was designed specifically to capture the circadian rhythms of heart rate, blood pressure and temperature for rats used in PD studies. The model could be considered to have a good foundation for description of cardiovascular rhythms as is based on the van der Pol oscillator, which has been previously used to describe the regulation of a heart beat (van der Pol et al., 1928). It
also accounts for the effect of the artificial lighting environment used in typical animal study designs.

The model could also be considered relatively complex, with 2 differential equations and 3 parameters to describe the underlying oscillations and lighting effect and another 2 parameters required to scale the oscillations to the actual physiological variable values. Vehicle data from each of the three compounds’ studies were initially modelled separately but determining precise parameter estimates from such few animals proved difficult. For example when fitting the model to baseline mean blood pressure measurements from the milrinone study it was impossible to derive precise estimates for gamma and delta. The issue was solved by modelling all 3 sets of vehicle data together to provide as accurate population estimates as possible and determine the variability that was necessary.

The resulting population parameters showed reasonable agreement with the parameter estimates determined from the Sallstrom data set. For mean blood pressure, population values for $R_{\text{amp}}$, delta and D (0.109, 0.00233min$^{-1}$ and 0.0418) were very close to the Sallstrom values (0.1, 0.0018min$^{-1}$ and 0.042), although the population value for gamma was much lower (0.0521min$^{-1}$ vs. 0.2min$^{-1}$). For heart rate there were some further differences, population values for $R_{\text{amp}}$ and D were both lower than reported by Sallstrom (0.266 and 0.0215 vs. 0.37 and 0.036) and gamma and delta both higher (0.126min$^{-1}$ and 0.00317min$^{-1}$ vs. 0.092min$^{-1}$ and 0.0022min$^{-1}$). In both cases $R_{av}$ also differed but this is to be expected with different sets of animals and the different strains used.

For both haemodynamic measures, inter-individual variability for the reference level ($R_{av}$) significantly improved the model. This is to be expected with biological variations between individual animals and was low in both cases (8% for MBP and 7% for HR). In addition though, inclusion of inter-individual variability for gamma and delta also significantly improved the model for both measures. Inter-individual variability for HR gamma and delta were reasonably low at 30% and 20% respectively, however for MBP they were much higher, particularly gamma at 114%, delta was 51%. Gamma and delta are the 2 parameters that determine the time course and shape of the oscillations which are the basis of the model. Since the oscillations define the rhythm aspect of the model (which theoretically is the same across individuals) such high variability is unusual.
Across the 24 hour period for which the baseline data were measured, no apparent irregularities were noted in the fits. However, when the eta values were re-estimated for the heart rate data as part of the rat L-NAME PD model, some irregularities were observed. For 1 individual in particular (ID 14), there was an extra oscillation and for another (ID 17) the oscillation appeared early. Further investigation into the individual values for these 2 individuals revealed particularly high delta estimates (0.056 and 0.046min\(^{-1}\) respectively). In addition, one approach to modelling the rat haemodynamic response to milrinone and doxazosin attempted but not reported here was the continuous approach used for L-NAME dog data. When the individual Sallstrom model parameters were used to predict baseline for longer than 24 hours, more unusual patterns and extra oscillations appeared in the profiles.

Simulations performed with the 2 differential equations forming the basis of the Sallstrom model, indicated that unusual patterns and more/less frequent oscillations were produced when values for gamma and delta differed greatly from the estimates reported in the Sallstrom paper (see appendix 1.5). Since the Sallstrom data set was measured over a period of 4 days, the pattern of change over 24 hours would have been more clearly characterised. In addition, the values used for modelling were the average for a set of animals rather than the individual data, removing the need for individual values. If the Sallstrom model were to be used to describe rat blood pressure or heart rate baselines in future studies, fixing the gamma and delta values to the estimates from the Sallstrom paper and allowing individual differences to be described by the parameters that scale the oscillations to the physiological measure (R\(_{av}\) and R\(_{amp}\)) would be the recommended approach.

A review of the literature shows that the model has not been widely adopted. The only other study to utilise it was from the same group (Visser et al., 2006). Here it was used to describe the baseline temperature in rats as part of a PKPD modelling of the hypothermic response to clomethiazole.

An assessment of the Sallstrom model (van Steeg et al., 2008) evaluated its use in describing baseline heart rate data during PKPD analysis of safety pharmacology data. The approach was slightly different in respect to the fitting, as a simultaneous population fit of the PKPD data was attempted as opposed to the sequential approach taken here. The reasoning for this was that precise estimates of the PK parameters could not be obtained using the sparse PK data alone. Problems occurred with the fitting of
the PKPD data when the Sallstrom model was used and overall the conclusion was that this model was overparameterised and interfered with the identification of the PK and PD parameters. Thus, the use of the model was seen as impractical for use with safety pharmacology data and a simpler model preferred.

6.2.1.2 Cosine models

A review of the literature regarding circadian rhythms indicates that cosine models are the most widely applied for analysis of such data. In terms of cardiovascular parameters, cosine models have been used to describe the 24 hour profiles of blood pressure and heart rate in humans (Hermida et al., 2002) and in other species such as rats (Lemmer et al., 1993). Within PKPD analysis multiple cosine models have been used to describe the human baseline for blood pressure (Hempel et al., 1998; Lindauer et al., 2010) and QT interval (Piotrovsky, 2005). A single cosine model was also used to describe baseline heart rate data during a PKPD analysis of safety pharmacology data for both dogs and humans (Langdon et al., 2010).

The baseline heart rate data from the studies used in this project showed good agreement with that presented in Langdon et al., (2010). However this is to be expected since the Langdon study was also a PKPD analysis of Pfizer safety pharmacology data obtained from the same animal colony. The researchers used a single cosine model with a 24 hour period to describe the circadian rhythm as opposed to the dual cosine model with 24 and 12 hours periods selected as the best fit in this project. However as explained in section 3.3.2.2.2 the main need for the dual cosine model came from the dogs used in the doxazosin study. For the majority of the animals an increase in heart rate was observed during the period of time after the lights came on in the morning (the beginning and end of the time periods as observed in the profiles), which could also be seen in the Langdon data. In most cases the 24 hour fluctuation in heart rate was much larger than this morning increase but for the dogs used in the doxazosin study the 24 hour change was very small and the morning increase became more prominent.

Although the models are different, population values for the 24 hour period of the dual cosine model can be compared to the respective values in the Langdon study. This comparison shows that reference baseline heart rate ($R_{av}$) is higher (78.9bpm vs. 68.2bpm), amplitude is lower (4.97bpm vs.12.4bpm) and the time of peak heart rate was slightly later (12.2h vs.10.4h) in the joint analysis. Since the study design was the same these peak times related to approximately 9pm in the joint analysis and 7pm in the
Langdon study. Inter-individual variability for $R_{av}$ and $T_z$ were similar for the joint analysis and the Langdon study (10% vs. 8% and 9% vs. 12% respectively). However no variability was applied to $R_{amp}$ in the Langdon study whereas it was very high (108%) in the joint analysis. This indicates much greater consistency between the baselines of the 4 dogs used in the Langdon study than across the 11 dogs used in the joint analysis.

No other papers reporting the fit of a cosine model to dog heart rate data were located but there were papers that reported 24 hour heart rate data. In general these did show agreement in terms of the values and the pattern where values fluctuated from 68-88bpm with peak heart rate at 7pm (Ashkar, 1979).

A single cosine model was used to describe the circadian pattern of mean blood pressure in dogs. In addition to the simpler model the pattern of the MBP circadian rhythm also differed to the heart rate data with peak values occurring around midday ($T_z = 3.65h$) rather than early evening. Unfortunately no reports of cosine models fitted to blood pressure data from dogs were located in the literature but there are some reports of the changes with time. The pattern of blood pressure with time between 6pm and 12pm agreed with the cosine model with the peak appearing around midday (Anderson et al., 1990). Values did appear a little lower though with a mean reference value of 83mmHg compared to the population estimate of 106mmHg for $R_{av}$ and the change over that time frame was approximately 2.1mmHg compared to 3.42mmHg for $R_{amp}$. Blood pressure data was not reported in Ashkar (1979) but they did measure total peripheral resistance (TPR), a major determinant of MBP. The 24 hour TPR pattern appeared to agree well with the MBP pattern from the joint analysis with peak values at approximately midday. Other studies reporting 24 hour blood pressure measurement were located but it was more difficult to determine the circadian pattern due to large spikes caused by feeding or the presence of researchers in the room, which were not filtered out (Miyazaki et al., 2002; Soloviev et al., 2006). However, the absolute values did appear more consistent with the Pfizer studies at ~100mmHg.

The degree of change in the MBP over the 24 hour period varied considerably. With an IIV value of 55% for $R_{amp}$, values were as little as 1.58mmHg to as much as 7.94mmHg. Unlike for heart rate, the lower or higher values were not associated with any of the individual studies. There was one peculiarity though, a completely reversed pattern for one of the dogs from the milrinone study. The high inter-individual variability value for
Tz of 72% was due to this individual and not representative of the population. The reason for the unusual profile was unknown but was consistent across doses, therefore appears to be something physiological.

Overall the circadian pattern of heart rate and mean blood pressure in the dogs were well described by the cosine models and thus they appear appropriate for use with safety pharmacology data.

6.2.2 Other effects on baseline response

6.2.2.1 Anaesthetic effect

The time frame used for the guinea-pig study (90 minutes) was not sufficient to display a full circadian rhythm; however there were changes over the timeframe, particularly for heart rate. Although the individual guinea-pigs were studied at various times throughout the day this did not appear to be a factor in the pattern observed. In fact the reason for the changes appears to be the anaesthetic used. The effects of various different anaesthetic regimens on the cardiovascular response have been studied in guinea-pigs (Mooney et al., 2012). The effect of pentobarbital was the same as seen in this project with a decrease of ~15bpm in heart rate and ~1-2mmHg in mean blood pressure over 45 minutes. The effect of other anaesthetic regimens produced either no change over time (isoflurane or pentobarbital with fentanyl pre-treatment) or a larger decrease (fentanyl, fluanisone and midazolam combination). The changes due to anaesthesia were modelled using linear functions for both mean blood pressure and heart rate and appeared to describe the data satisfactorily.

6.2.2.2 Known external effects

A number of factors including feeding, handling and dosing can affect the observed haemodynamic responses. When this happens at a critical time, it is essential to account for it within the model to ensure it does not impact on the prediction of pharmacological effect. In the conscious animal studies, transient increases in both heart rate and blood pressure were observed upon administration of vehicle. This was modelled using an empirical function describing an exponential increase and decrease in the variable as described in Sallstrom et al., (2005). In the rat, precise parameter estimates could be obtained and the function was built into baseline model. The same was the case for heart rate in dogs but not for mean blood pressure. The administration effect lasted longer in rats than in dogs, where typically an increase was only observed for the 0.25 hour time
point. Since the data had been averaged into 15 minute time bins, information about the administration effect was effectively lost and the increase could not be distinguished from residual error. A more precise estimation of the effect may be possible with the use of 1 minute as opposed to 15 minute time points for the first hour after dosing.

As mentioned in section 2.1.3.2, increases in blood pressure and heart rate were observed during the feeding time for dogs. However, since this was 6-7 hours after dosing, it was decided to simply remove this data as was not at a critical time.

6.2.2.3 Unknown external effects

For the conscious animals studies transient increases were observed at times other than recorded events such as dosing and feeding. These were likely due to sudden external stimuli such as noise or other environmental factors. Where these increases were large and obviously inconsistent with the baseline, the points were removed (see section 2.1.3.2). This was mainly performed for rats, although a few points were also removed for dogs. In addition to these transient spikes, the heart rate and blood pressure in these studies could generally be considered reasonably ‘noisy’. Due to homeostasis feedback mechanisms, haemodynamic variability occurs naturally when the animals are at rest and values oscillate around a set point. This variability did appear to be high in the animals, particularly for dogs and in some cases may have been influenced by other factors.

The degree of variability can be observed in the residual errors. For rat MBP and HR baseline models the residual error was approximately 5% of the average value. However the actual values, 5.17mmHg and 24.2bpm are right at the border of what would be considered as a potential level for concern in safety pharmacology (>5mmHg, >25bpm). For the dog, again residual error was only ~5% of average baseline for MBP but for HR it was higher at ~15%. When the actual values of 5.61 mmHg and 11.5bpm are compared to the overall 24 hours change due to the circadian rhythm (2 x R_{amp}), it can be seen that the residual error levels are equivalent to the 24 hours changes (6.84mmHg and 9.94bpm). They are also slightly into the potential level of concern (>5mmHg, >10bpm). This could be considered a cause for concern as the extent of drug effect could be partially masked by the apparent level of natural variability.
6.3 PD

6.3.1 Models assessed

A limited number of pharmacodynamic models were assessed for their fit to the haemodynamic responses to the three compounds. Although the compounds in this project were reference compounds with well established mechanisms of effect, typical safety pharmacology studies would be performed for compounds in development, where any cardiovascular effects may be unexpected and have unknown mechanisms. In such cases the models can only ever be empirically based and would typically be either a direct effect model if the time-courses of concentration and effect were aligned or an effect-compartment model if a delay was observed. The effect compartment model is designed to account for delays due to distribution to the effect site and thus depending on the pharmacokinetics of the compound, may be misspecified and lead to incorrectly determined values. Temporal delays may also be caused by other mechanisms which relate to the pharmacodynamic (e.g. receptor binding) or system processes (e.g. transduction), for which alternative models exist (see section 1.1.2.2). Due to the mechanism of action and the site at which they act (see sections 3.1, 4.1 and 5.1), the rate limiting step for the compounds used in this project is likely to be at the transduction stage (although slow binding processes have not been ruled out). Typical signal transduction models contain a series of transit compartments to describe the cascade of events that occur between receptor/enzyme/mediator activation or inhibition and the physiologic response. Where details regarding the transit times between each step are unknown (as in these and many cases) the model is simplified to a single equation with one time function \((\tau)\). If a baseline is then incorporated into this equation, it becomes mathematically identical to the indirect response models with inhibition or stimulation of the production of the response (Mager et al., 2001). Indirect response models are based on the turnover of a biomarker, which the drug alters to give rise to its effect. The biomarker is often an endogenous mediator but can be a physiologic response, such as blood pressure or heart rate. The indirect response model therefore gives a fairly general model which can be used to describe the effect on the haemodynamics due to an unknown but system related process. Consequently, it was chosen as a third PD model option in this project and provided an alternative model to account for non-PK related temporal effect delays.
6.3.2 Model fits

In general the fits achieved with the final PKPD models were adequate, although there were a few issues. These related to three main areas: the design of the studies, the baseline model and limitations of the Monolix software.

6.3.2.1 Study design

With respect to the study design, there were 2 main issues. The first was the timings of blood sampling compared to the timings of the response measurements. This was mainly an issue for the guinea-pig study design where only 1 blood sample was taken for each dosing level. The benefit of using a PKPD model is that the concentration and response measurements do not have to be sampled at the same time points. However for the guinea-pig studies the sparseness of the concentration measurements limited the PK model possibilities to only one. Thus it could not be said with certainty that the model accurately predicted the concentration over the whole course of the experiment or whether there was any temporal delay in the response. For 2 of the 3 compounds only a direct model could be justified for the guinea-pig studies. For milrinone an indirect model did show a statistically better fit, however the values for $k_{\text{out}}$ where very large when compared to the other 2 species ($32.8\,\text{h}^{-1}$ for MBP and $50.9\,\text{h}^{-1}$ for HR). It could very easily be argued that the model effectively became direct, as was the case for doxazosin where high $k_{\text{out}}$ values were seen, however the indirect model was still selected for milrinone in guinea-pigs as the models for rat and dog were both indirect too.

The second issue relating to study design was that the dose ranges selected for some studies did not appear to cover the whole effect range. This meant for some studies (milrinone in rat and dog and doxazosin in dog) the maximum effect model had to be substituted with the linear effect model. When the maximum effect models were fit to the data for these studies, the $I_{\text{max}}$ values of 1 indicated complete inhibition (i.e. a blood pressure of 0) and $E_{\text{max}}$ values ranged from 2 – 5 which resulted in physiologically implausible heart rate values. The $C_{50}$ values associated with the maximum values were also above the maximum concentration observed for these dose levels.

The physiological limit of blood pressure and heart rate was a factor not considered within the modelling process. Blood pressure and heart rate, as many physiological functions, are regulated by a number of feedback mechanisms to ensure the circulation
is adequately maintained within a specific range. The simplest option to ensure predicted PD values are feasible would be to incorporate the physiological limits of blood pressure and heart rate for each species into the PD models used. The work of Yao *et al.*, (2006) showed that physiological limits could be incorporated into indirect response models and enabled more accurate estimation of the true PD parameter values. The other option is to develop a model with feedback mechanisms incorporated. Francheteau *et al.*, (1993) developed a physiologically based cardiovascular model to describe the effects of dihydropyridine drugs on mean blood pressure and cardiac output. The model incorporated a number of cardiovascular variables, including total peripheral resistance (TPR), mean blood pressure, heart rate, stroke volume (SV) and cardiac output (CO). It also accounted for the baroreceptor reflex, which is involved in the regulation of blood pressure via feedback mechanisms increasing/decreasing blood pressure directly and via increases/decreases in heart rate. It was hoped that this model could be used to describe the effects of the compounds on MBP and HR simultaneously; however there are a number of factors which would need to be addressed to do this. Firstly the baseline was assumed to be constant, thus incorporation of the circadian rhythm would be required. Secondly changes in cardiac contractility were noted for all of the compounds but particularly milrinone. These would cause changes in the stroke volume, which was fixed in the model. Lastly, for L-NAME and milrinone, the effects were delayed, how this would be incorporated into the model would need to be investigated.

### 6.3.2.2 Fixed baseline

The second cause for PD fitting issues was related to the baseline models and the use of fixed baseline parameters. Using fixed population values for the Sallstrom baseline led to poor fitting of rat heart rate profiles in the presence of L-NAME. However, simultaneously estimating individual baseline and effect parameter values for the rats dosed with L-NAME caused alternative issues with the resulting baseline predictions (see sections 3.4.1.2.2 and 6.2.1.1). Baseline values for rats in the milrinone study were fixed to individual values determined after administration of vehicle. However, the fits for some individuals’ heart rate profiles were still not good and possibly due to inter-occasion variability in the baseline. Due to the complexity of the Sallstrom model and issues observed with L-NAME, simultaneous fitting of baseline and effect was not undertaken with milrinone but if a simpler baseline model were used it may be a more appropriate approach.
A fixed baseline also caused issues for the fit of dog heart rate profiles in the presence of L-NAME (see section 3.4.2.2.1). Here the baseline was simply re-estimated during the PKPD fit and no problems were observed. This therefore agrees with the suggestion that baseline and effect should be estimated simultaneously.

6.3.2.3 Monolix limitations

Issues with the limitations of Monolix or potentially with the non-linear mixed effects approach itself were noted when modelling the CV response to L-NAME in the dog. Identical fit statistics for each model meant it was impossible to determine the best model fit using the statistics and other model fit measured had to be used alone. Whether this was a problem with the method of log-likelihood calculation (via the Monolix importance sampling method) or whether this was due to issues with the very small number of data-rich individuals is unknown. However it meant that this approach of modelling the continuous timeline was not used for the milrinone and doxazosin rat and dog studies. Instead it was decided to model each dose as a different occasion, which for these drugs was an option due to complete washout of these drugs between doses. However this caused further issues, producing error messages during the fitting process when variability (inter-individual or inter-occasion) was introduced. The only option remaining was to treat each dosing occasion as a different individual. This approach worked and appeared to give good fits but it must be noted that inter-individual variability was high in a lot of cases. The reason for this is that the value actually represents a combination of inter-individual and inter-occasion variabilities. If the occasion approach had worked, inter-occasion variability would have only been applied to baseline parameters, however with this method there was no way to remove it for the PD parameters. For rat and dog milrinone models, there was particularly large variability for $k_{\text{out}}$ (73-140%). It was suspected that the $k_{\text{out}}$ parameter was trying to capture changes from baseline unrelated to drug effect i.e. random variability due to the normal fluctuations. The fixed baseline was another factor in this but the variability should be treated as over-inflated. For doxazosin rat and dog models where the effect was direct, large variabilities were estimated for the potency or slope values (85-116%). Although the fits appear good these large variabilities are not ideal and the use of an occasion method (with either different software or a newer version of Monolix) would give more reliable estimates.
6.3.2.4 Unusual profile

In one specific case, mean blood pressure response to doxazosin in rat, the shape of the profile was unusual. Instead of a gradual onset of effect, either following or lagging behind the concentration-time profile, there appeared to be an initial drop in MBP followed by a slight increase to a plateau and then a gradual return to baseline. This was not consistent with the profile observed in dogs or for the other compounds and thus it was not expected that any of the models would provide a really good description of the data. The reason for this unusually shaped profile was also unknown but the sudden drop could initiate a reflex feedback mechanism, which prevented the decrease in pressure falling too far. How this could be modelled was not investigated further but may require a non-concentration dependent function with negative feedback to describe the initial drop, coupled with a normal concentration dependent model to describe the remaining effect. The fit of the direct effect model was adequate but the resulting values may not truly represent the effect seen in rats.

6.3.3 Consistency of models and values

The models used for each compound was highly consistent across species and PD measures with the same models used for all 3 species for milrinone and doxazosin. The only exception was L-NAME where the guinea-pig haemodynamics were described using a direct model, whereas the rat and dog effects were best fit by an indirect model. As described in section 6.3.2.1 the guinea-pig model study design did not really allow for any temporal delay to be observed, thus really only a direct effect could be assumed. However it must also be noted that the rate of input of other compounds has been shown to influence the haemodynamic response (Kleinbloesem et al., 1987) and thus the different rate of appearance of drug in plasma following oral dosing versus intravenous dosing may affect the observed response.

The PD values derived for each compound were not at all consistent across species. In some cases, potency or slope values were in the same order of magnitude for 2 of the 3 species but these differed between compounds and between measures. In many cases the potency or slope values were also an order of magnitude different between the 2 cardiovascular measures. The only exceptions to this were L-NAME in the rat, where potency values for MBP and HR were virtually the same (44.8 and 41µM respectively) and milrinone and doxazosin in the guinea-pig, where potency values for MBP and HR were in the same order of magnitude (0.494 and 0.126µM for milrinone and 0.542 and
0.398nM for doxazosin). Where an indirect model was used, the $k_{out}$ values also varied across species and did not appear to just be weight-dependent as the values were still not equivalent after scaling to human.

Comparison of the results with literature studies showed variable agreement. For L-NAME, a number of studies described the effect on mean blood pressure in the rat (Bernareggi et al., 1999; Gardiner et al., 1990; Wang et al., 1995; Wang et al., 1993). Intravenous bolus doses of 10-30mg/kg showed an increase in MBP of 36-43%. This is reasonable consistent with the predicted $E_{\text{max}}$ value of 35.7%, although the i.v. doses may lead to higher concentrations than observed after oral administration and thus the maximum effect may be slightly underpredicted. However a PKPD study of L-NOARG in anaesthetised rats (Tabrizi-Fard et al., 1998) reported a figure of 32.5% for MBP $E_{\text{max}}$, which is very consistent with the value from this project. The 20mg/kg i.v. infusion also resulted in concentrations almost twice as high as the Pfizer study. Although it must also be noted that higher i.v. bolus doses of L-NOARG (30mg/kg) in conscious rats led to increase of approximately 40%, again higher than either PKPD model predicts. Whether this is due to different administration or animal variability is unknown.

Fewer studies reported the effect of L-NAME/L-NOARG on heart rate in rats. Gardiner et al., (1990) reported a decrease of 28% following a 10mg/kg i.v. bolus dose of L-NAME, greater than the modelled $I_{\text{max}}$ value of 14.5%. However, higher bolus doses of L-NOARG (30mg/kg) resulted in more consistent decreases of 14.5-20% (Wang et al., 1995; Wang et al., 1991). Without concentration measurements it is difficult to know if this is due to concentration differences or animal variability. Heart rate was not modelled as part of the reported L-NOARG PKPD study (Tabrizi-Fard et al., 1998), since the decrease was not deemed significant. However the graphs appear to indicate a drop of ~5%, less than observed in other cases but this could be due to the effect of slower administration or the anaesthetic affecting the baroreceptor response.

The maximum effect values for L-NAME effect on MBP and HR in the dog showed reasonable agreement with a literature study of L-NAME in conscious dogs (Picker et al., 2001). Administration of i.v. bolus doses of 10-50mg/kg L-NAME resulted in an increase in MBP of 22% and a decrease in HR of 29-34%, compared to an $E_{\text{max}}$ of 19.5% and an $I_{\text{max}}$ of 42.2%. However when compared to a study of the oral
administration of 30mg/kg L-NOARG (Huber et al., 1992), less consistency was seen (MBP increase 32%, HR decrease 16%) even though concentrations were similar.

One study of L-NAME in guinea-pigs was located (Bernareggi et al., 1999) and the increase in MBP observed at a much higher dose (20mg/kg), was reasonably consistent with the E\text{max} value (58% vs 72% respectively). Unfortunately the influence on heart rate was not studied.

There did not appear to be any milrinone PKPD studies for any of the species in the literature however for each species a study on the haemodynamic effect could be located. Since a linear model was used for rat and dog, the highest predicted effect were used for comparison. For rat, a decrease of ~30% was calculated for MBP. Using the population slope value for HR gave a predicted increase of 80%. This was not observed in the actual data and was due to the approach taken as the higher doses had smaller slope values. A more representative value of the observed effect was 30%. A study of milrinone in anaesthetised rats, measured concentration and effects for i.v. bolus doses of 0.3-10mg/kg (Verrijk et al., 1990). The 3mg/kg dose gave the closest concentrations to those observed in the Pfizer study. For this dose, the decrease in MBP was similar (~30%) but the increase in HR was much lower (~17%) than observed in the Pfizer study. However it must be noted that the route of administration and the anaesthesia may both have effects on these values.

For the Pfizer dog study, milrinone produced a decrease of ~15% in MBP and an increase of ~35% in HR for 0.3mg/kg. Alousi et al., (1984) reported the effect of 1mg/kg orally administered milrinone in conscious dogs as a ~10% decrease in blood pressure and ~40% increase in heart rate, which are both reasonably consistent with the Pfizer study. The slope values estimated for milrinone in dog would likely overpredict this effect, which highlights the importance of an appropriate dose range. The effects of milrinone were also reported in anaesthetised dogs, which displayed a much higher effect on blood pressure and lower effect on heart rate, which again indicates the potential effect of anaesthesia on the cardiovascular system.

For the guinea-pig, a published safety pharmacology study (Marks et al., 2012) used a very similar study design with respect to anaesthesia and dosing regimen to the Pfizer study. Results showed lower effects for both MBP (~30% decrease) and HR (10% increase) when compared with the maximum effect values predicted in this project (46% for MBP and 35.7% for HR). The only difference in the study design was the
administration of 15 minute infusions, rather than the 5 minute loading infusion and 10 minute maintenance infusion. The latter could cause larger effects due to higher initial concentrations. Unbound plasma concentrations were reported in the Marks study; however the values appeared to be more consistent with total concentrations from the Pfizer study and did not help explain the observed differences.

Unfortunately the literature appeared to be lacking in studies of doxazosin effects in pre-clinical species and studies could be located for either rat or guinea-pig. Two studies were located for dog (Alabaster et al., 1986; Mizuno et al., 2000); however these were both in hypertensive animals and only measured blood pressure. The decrease observed in these studies (~20%) did agree well with the effect observed in this project (~15%) though. The only mention of heart rate in these studies was to comment on the lack of an increase. This differs greatly from the effect seen in the Pfizer study, where an increase of 35-60% was seen for the top dose. However, issues with the timings of concentrations vs. response did mean the model did not predict such an extent (only ~20%), which is maybe why it failed to predict the human response (see sections 5.5.2.2 and 6.4).

6.4 Cross-species scaling

One of the major challenges in achieving successful prediction of human cardiovascular effects within the safety field is the lack of knowledge regarding the mechanism of action. With the possible exception of QT prolongation, significant changes in cardiovascular parameters such as blood pressure, heart rate, contractility etc. can be related to one or more of the numerous pathways involved in the cardiovascular system regulation. Recent examples of the application of PKPD modelling techniques to pre-clinical cardiovascular safety data have been published for compounds that caused PR and QRS interval prolongation (Fleury et al., 2011) and increased heart rate (Langdon et al., 2010) via unknown mechanisms. For the former the main concern was to understand the concentration effect relationship due to an observed temporal delay and no comparison with human data was made; however the latter compared the model results between dog and human and a ~2-fold higher effect slope was observed for dog. A similar study of QT interval prolongation (Ollertam et al., 2006) showed that the reference compound dofetilide was ~2-fold less potent in dogs than humans (i.e. EC$_{50}$ for dogs 2 x higher than in man). Since these are isolated cases, the main objectives of this project were to see if there are consistent differences in the concentration-effect relationship across species for compounds with different mechanisms of action and
whether prediction of human effect could be achieved without taking a mechanistic approach.

Values derived from modelling in this project showed that in most cases the animal species under-predicted the extent of the human response. Generally this was by much more than 2-fold though and not consistent across the compounds. The most consistent prediction came from the dog. For both L-NAME and milrinone the extent of heart rate response was predicted and the order of magnitude of effect was the same. Fitting of average human data revealed that the IC$_{50}$ for L-NAME was approximately 3-fold lower than in dog and the milrinone slope was 1.5 fold higher, thus showing a greater effect on HR in man. This differed from the Langdon study where HR effect was greater in the dog. However it must be acknowledged that this was not a full accurate analysis of the human values due to limited data (and why the results were not reported). Also the milrinone heart rate fit was not ideal due to the baseline applied, which was not necessarily representative of those individuals (no placebo data). For mean blood pressure, L-NAME EC$_{50}$ and milrinone slope values for humans were similar to those estimated for heart rate. This meant neither were in the same order of magnitude as dog and explains why the extent of MBP effect in humans was under-predicted.

Unfortunately the successful prediction of heart rate effect by dog was not replicated for doxazosin. The mean blood pressure values also differed by a much greater extent, leading to a very poor prediction. There were however concerns over the PK data for doxazosin in the dog. The concentrations appeared at odds with other observed data and may have been related to poor absorption. Safety pharmacology guidance actually mentions that route of administration should be the same, except where huge differences in bioavailability are observed. The PD heart rate model for dog actually under-predicted the extent of effect seen in some dogs due to differences in the time courses, which were not accounted for. Since peak heart rate actually occurred before peak concentration, the direct model was not sufficient to capture the early increases. This may have been due to issues with the concentration but also highlights another important factor not accounted for in the modelling. Heart rate changes are not necessarily due to a direct action of the drug but instead are a result of feedback systems within the cardiovascular system. To obtain a more accurate estimate of effect, a model would be necessary to account for such feedback systems linking the various cardiovascular variables e.g. Francheteau et al., (1993).
Chapter 6: Discussion and Conclusions

A comparison of the rat and human values showed a huge difference in sensitivity of effect. In all but one case the rat predictions did not really change from baseline. However there was no consistency in the degree of difference between human and rat values, ranging from 9-74 fold. The only exception to this was for doxazosin mean blood pressure where some effect was seen. However, as mentioned in section 6.3.2.4 the profile was unusual and the model did not give the best fit so the prediction may just be coincidental.

The guinea-pig predictions were particularly inconsistent. For L-NAME no real change from baseline was predicted since the potency values for MBP and HR were 20-fold higher than for human. For milrinone mean blood pressure was under-predicted but heart rate was over-predicted. For doxazosin the guinea-pig values gave the best prediction capturing the extent of effect for both MBP and HR, for the oral study at least. The reasons why the guinea-pig would predictive for one drug and not for another are unknown. The fact that the animals are anaesthetised may play a role. Although it has been shown that the baroreceptor response is intact under such conditions, it is decreased compared to conscious animals (Marks et al., 2012; Mooney et al., 2012). Other changes to the cardiovascular system may also occur or there may be aspects of the study design e.g. dose, which were not optimal.

In all cases, even when the extent of effect was predicted, the time course of the effect was not. Effects that appeared indirect in animals were direct in human (L-NAME) and vice-versa (doxazosin). Although as previously mentioned cardiovascular changes can differ depending on the rate of administration and that study design can affect the model options, this also indicates that there may be other differences in drug effects between species. However, without use the use of a more mechanistic modelling approach and separation of the pharmacological and physiological effects, it is impossible to give any further insight.

6.5 Conclusions

The use of compartmental models provided an adequate description of the PK data. For L-NAME the conversion to L-NOARG was not required within the model, however due to a lack of data in absorption/conversion phase only a zero-order input could be justified. Some dose non-linearity was seen for rats where the highest dose produced lower concentrations than predicted by the model. Since a literature study actually
observed higher concentration for such a dose, it is uncertain if this is a real property of drug or simply due to animal variability and too few data points.

There were some issues with PK study design. For the guinea-pig too few data points limited the model options to one. In a number of other cases there were a lack of data points at the right times either making it difficult define absorption phase or an accurate estimate of clearance. Although where LLOQ data was available it was used to try and overcome the latter. It appears that standard timings where used in all cases, however different sampling time may be required for compounds with different PK. Involvement of the PK group with study designs could help determine appropriate sampling times.

In general the PK model values agreed well with the literature. The only real concern was doxazosin in the dog where variable and incomplete absorption may have been an issue. Large difference between concentrations between this study and the literature were seen and a much lower bioavailability. The literature indicates that doxazosin bioavailability in the dog is predominantly due to the fraction absorbed which is not necessarily equal to 1.

The Sallstrom model was used to describe the circadian rhythm seen for rat baseline MBP and HR and overall the model described the baseline well. However its complexity required all baseline data to be fit at once to obtain precise estimates for the parameters that controlled the 24 hours oscillations (γ and δ). Overall population values showed good agreement with values obtained in Sallstrom study although inter-individual variability in γ and δ resulted in some large individual differences, which caused some issues. If γ and δ values varied greatly from the published values some strange oscillation patterns could occur. If the baseline model were to be used again, fixing γ and δ values may be required, although the model is probably too complex for standard use.

Cosine models are generally used to describe circadian rhythm patterns and provided a good description of the dog data. Good agreement with literature values and the pattern was observed for both MBP and HR. However HR required a dual cosine model due to increases associated with morning lighting combined with low 24 hours change in individual dogs from the doxazosin study. Overall cosine functions appear good models for MBP and HR circadian rhythm patterns.
Changes in guinea-pig baseline appeared to be related to anaesthesia and were described well by a linear model. Administration effect in conscious animals could be predicted for all except MBP in dogs. Since the time frame of effect was short it may be that more time points within the first hour are required to capture it within the model. Baselines for conscious animals were also affected by other external effects causing transient increases and in general the data were very variable. Filtering out such effects should be considered as some degree of drug effect could be masked by variability due to other causes.

A limited number of PD models were assessed due to the lack of mechanistic understanding for normal safety pharmacology scenarios. Where a delay in effect was seen indirect models gave better fits than effect-compartment models.

Some issues with study design affected the PKPD modelling. Again the lack of concentration time points for guinea-pig was one, as it meant any delay in effect could not be accurately determined. If some cases dose ranges were not sufficient to predict the maximum effect, although physiological limits would also affect this and were not incorporated. Feedback mechanisms play an important role in the maintenance of the cardiovascular system responses and an alternative approach would be to use a more physiological model. Other problems were caused by using a fixed baseline, which would be resolved by a simultaneous fit of baseline and PD, if the baseline is simple enough to allow it. There were also problems with the use of Monolix; statistical or run issues with continuous study design, or an attempt to model inter-occasion variability meant each occasion had to be treated as a separate individual. This led to overestimation of variability and possibly affected the accuracy of the population estimates. Despite these problems, overall there was a reasonable fit of the models to the data. The only exception was doxazosin’s effect on rat MBP where there was an unusual profile. A model with feedback mechanism include may be required to capture this correctly.

Models were consistent across animals with the exception of guinea-pig in one case, which was possibly due to differences in effect with route of administration. There was no consistency in the values either across species or between CV measures, except for the order of magnitude in some cases. There was reasonable consistency of results with the effects seen in literature.
In general the animal PD models under-predicted the effect seen in human although there was not a consistent pattern of the level of under-prediction across compounds. The only possible exception was for HR in the dog where extent of effect was predicted for both L-NAME and milrinone. However, difference in animal potency values for MBP meant it was not predictive of human MBP effect, which had similar potency as HR. Unfortunately dog was not predictive for doxazosin HR. However this may be due to the potential issues with PK and/or the direct model not predicting early effects HR effects, for which feedback mechanisms may be required. The guinea-pig model was particularly inconsistent across compounds, but did show some good predictions for both doxazosin MBP & HR. Time course of effect was predicted poorly in all cases and once again a more mechanistic model may be required to capture this.

Overall PKPD modelling can be used to describe safety pharmacology data in different species. If study design aspects with respect to sampling times and dose ranges could be addressed and modelling issues such as fixed baselines and issue with the software tackled, more accuracy could be obtained. Dog showed some potential for prediction of CV measures. Use of more complex CV models with accurate feedback mechanisms included may help this. The use of such models in safety pharmacology would be possible if is just biological aspects that are more complex and may help to give more insight into mechanism of action. Guinea-pig may also offer a possible predictive animal model but reasons for inconsistency of its predictiveness would need to be explored across a greater range of compounds.
Chapter 7

References
7 References


Chapter 7: References


MHW-Japan (1975). Notes on application for approval to manufacture (import) new drugs.


Chapter 8

Appendices
Appendices

Appendix 1.1: Studies not analysed

- Isoflurane anaesthetised dog: anaesthetic caused changes to the cardiovascular system. Blood samples were damaged in transit, leading to insufficient samples.
- Urethane/\(\alpha\)-chloralose anaesthetised dog: L-NAME not study compound, data set incomplete.
- Sling restrained dog: L-NAME not study compound, data set incomplete. Issues with doxazosin PK data.
- Verapamil dosed rats (Sprague Dawley/Wistar Han): PK data for all doses unavailable when apparent dose non-linearity.
- Verapamil dose guinea-pigs: PK data unavailable

Appendix 1.2: Average data plots

Appendix 1.2.1: Rat L-NOARG plasma concentrations with time following doses of 10 (red), 30 (green) or 100mg/kg (blue) of L-NAME.
Appendix 1.2.2: Dog L-NOARG plasma concentrations with time following doses of 10 (red) or 40mg/kg (blue) of L-NAME.

Appendix 1.2.3: Guinea-pig L-NOARG plasma concentrations with time following multiple ascending doses of L-NAME. Each colour represents a different animal.
Appendix 1.2.4: Rat milrinone plasma concentrations with time following doses of 0.35 (red), 3.5 (green) or 10.5mg/kg (blue).

Appendix 1.2.5: Dog milrinone plasma concentrations with time following a dose of 0.1mg/kg.
Appendix 1.2.6: Guinea-pig milrinone plasma concentrations with time following multiple ascending doses. Each colour represents a different animal.

Appendix 1.2.7: Rat doxazosin plasma concentrations with time following doses of 1 (red), 10 (green) or 30mg/kg (blue).
Appendix 1.2.8: Dog doxazosin plasma concentrations with time following a dose of 0.3mg/kg.

![Graph showing plasma concentrations over time for dogs after a dose of doxazosin.]

Appendix 1.2.9: Guinea-pig doxazosin plasma concentrations with time following multiple ascending doses. Each colour represents a different animal.

![Graph showing plasma concentrations over time for guinea-pigs after multiple ascending doses of doxazosin.]

Appendix 1.2.10: Rat mean blood pressure with time following doses of 10 (red), 30 (green) or 100mg/kg (blue) of L-NAME or vehicle (black).

Appendix 1.2.11: Rat heart rate with time following doses of 10 (red), 30 (green) or 100mg/kg (blue) of L-NAME or vehicle (black).
Appendix 1.2.12: Dog mean blood pressure with time following doses of 10 (red), 20 (green) or 40mg/kg (blue) of L-NAME or vehicle (black).

Appendix 1.2.13: Dog heart rate with time following doses of 10 (red), 20 (green) or 40mg/kg (blue) of L-NAME or vehicle (black).
Appendix 1.2.14: Guinea-pig mean blood pressure with time following multiple ascending doses of L-NAME.

Appendix 1.2.15: Guinea-pig heart rate with time following multiple ascending doses of L-NAME.
Appendix 1.2.16: Rat mean blood pressure with time following doses of 0.35 (red), 3.5 (green) or 10.5mg/kg (blue) of milrinone or vehicle (black).

Appendix 1.2.17: Rat heart rate with time following doses of 0.35 (red), 3.5 (green) or 10.5mg/kg (blue) of milrinone or vehicle (black).
Appendix 1.2.18: Dog mean blood pressure with time following doses of 0.03 (red), 0.1 (green) or 0.3mg/kg (blue) of milrinone or vehicle (black).

Appendix 1.2.19: Dog heart rate with time following doses of 0.03 (red), 0.1 (green) or 0.3mg/kg (blue) of milrinone or vehicle (black).
Appendix 1.2.20: Guinea-pig mean blood pressure with time following multiple ascending doses of milrinone (green) or vehicle (black).

Appendix 1.2.21: Guinea-pig heart rate with time following multiple ascending doses of milrinone (green) or vehicle (black).
Appendix 1.2.22: Rat mean blood pressure with time following doses of 1 (red), 10 (green) or 30mg/kg (blue) of doxazosin or vehicle (black).

Appendix 1.2.23: Rat heart rate with time following doses of 1 (red), 10 (green) or 30mg/kg (blue) of doxazosin or vehicle (black).
Appendix 1.2.24: Dog mean blood pressure with time following doses of 0.1 (red), 0.3 (green) or 1mg/kg (blue) of doxazosin or vehicle (black).

Appendix 1.2.25: Dog heart rate with time following doses of 0.1 (red), 0.3 (green) or 1mg/kg (blue) of doxazosin or vehicle (black).
Appendix 1.2.26: Guinea-pig mean blood pressure with time following multiple ascending doses of doxazosin (red) or vehicle (black).

Appendix 1.2.27: Guinea-pig heart rate with time following multiple ascending doses of doxazosin (red) or vehicle (black).
Appendix 1.3: Excluded data examples

Appendix 1.3.1: Transient increases in the mean blood pressure time profile of rat 8, dosed with 10.5mg/kg milrinone.

Appendix 1.3.2: Transient increases in the heart rate time profile of rat 8, dosed with 10.5mg/kg milrinone.
Appendix 1.3.3: Transient increases in the mean blood pressure time profile of dog 1 dosed with 0.3mg/kg milrinone.

Appendix 1.3.4: Transient increases in the heart rate time profile of dog 1 dosed with 0.3mg/kg milrinone.
Appendix 1.3.5: Abnormally high heart rate for dog 4 in the L-NAME study

Appendix 1.4: L-NAME rat dose-corrected PK
Appendix 1.5: Sallstrom model oscillations

Appendix 1.5.1: Oscillations for Sallstrom estimates $\gamma = 0.2\text{min}^{-1}$ $\delta = 0.0018\text{min}^{-1}$

Appendix 1.5.2: Oscillations for $\gamma = 0.2\text{min}^{-1}$ $\delta = 0.0036\text{min}^{-1}$
Appendix 1.5.2: Oscillations for $\gamma = 0.4 \text{min}^{-1}$ $\delta = 0.0009 \text{min}^{-1}$