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DEVELOPMENT AND VALIDATION OF A SEMI-PHYSIOLOGICAL PHARMACOKINETIC (PBPK) MODEL TO PREDICT SYSTEMIC AND PULMONARY EXPOSURES AFTER INTRAVENOUS, ORAL ADMINISTRATION AND PULMONARY INHALATION OF SELECTED DRUGS, Budesonide, Tobramycin and Ciprofloxacin, In Humans

Bishoy Hanna

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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<th>Abbreviation</th>
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<tbody>
<tr>
<td>$A_c$</td>
<td>Amount of drug in central compartment</td>
</tr>
<tr>
<td>$A_{cl}$</td>
<td>Amount of drug in central lung compartment</td>
</tr>
<tr>
<td>$A_g$</td>
<td>Amount of drug in GIT</td>
</tr>
<tr>
<td>$A_p$</td>
<td>Amount of drug in peripheral compartment</td>
</tr>
<tr>
<td>$A_{pl}$</td>
<td>Amount of drug in peripheral lung compartment</td>
</tr>
<tr>
<td>ADME</td>
<td>Absorption, distribution, metabolism and excretion</td>
</tr>
<tr>
<td>AGs</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BUD</td>
<td>Budesonide</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>CFTR</td>
<td>Cystic Fibrosis Transmembrane Regulator</td>
</tr>
<tr>
<td>CIP</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>CLU</td>
<td>Central lung unbound compartment</td>
</tr>
<tr>
<td>CLS</td>
<td>Central lung sequestration compartment</td>
</tr>
<tr>
<td>$CL_{tot}$</td>
<td>Total Clearance</td>
</tr>
<tr>
<td>$CL_{ren}$</td>
<td>Renal Clearance</td>
</tr>
<tr>
<td>$CL_{nonren}$</td>
<td>Non-renal Clearance</td>
</tr>
<tr>
<td>$c_p(t)$</td>
<td>Plasma Concentration-time Profile</td>
</tr>
<tr>
<td>$c_s(t)$</td>
<td>Sputum Concentration-time Profile</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DEM</td>
<td>Dose emitted from inhaler</td>
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Din  Fraction of the dose that remains in the inhaler
DtGI  Dose to GI tract
DtL  Dose to Lung
DPI  Dry Powder Inhaler
F_{CD}  Fraction of the dose deposited in the central Lung
F_{ex}  Fraction of the dose exhaled
F_{oral}  Fraction of the dose surviving absorption from GIT to central compartment
F_{PD}  Fraction of the dose deposited in the peripheral lung
GIT  Gastrointestinal Tract
HV  Healthy Volunteers
INH  Inhalation
IV  Intravenous
k_{10}  First-order elimination rate constant from central compartment
k_{12}  First-order rate distribution constant from central compartment to peripheral body compartment
k_{21}  First-order re-distribution rate constant from peripheral body compartment to central compartment
k_{c\text{ratio}}  Capacity ratio for central lung unbound \( (k_{ca}/k_{cd}) \)
k_{cls\text{ratio}}  Capacity ratio for central lung sequestered \( (k_{cls2}/k_{cls1}) \)
k_{c\text{sum}}  Central lung unbound equilibration rate constant \( (= k_{ca} + k_{cd}) \)
k_{cls1\text{sum}}  Central lung sequestration equilibration rate constant \( (= k_{cls1} + k_{cls2}) \)
\( k_{ca} \)  Uptake of drug from the unbound central lung compartment to the sequestration central lung compartment

\( k_{cd} \)  Efflux from the central lung unbound to central lung sequestration

\( k_{cls1} \)  Distribution from the central body compartment to the central lung sequestration

\( k_{cls2} \)  Absorption from central lung sequestration to central body compartment

\( k_{cm} \)  First-order mucociliary clearance rate constant from central lung to GIT

\( k_{GA} \)  First-order rate constant from GIT to central compartment

\( k_{pa}^{\text{ratio}} \)  Capacity ratio for peripheral lung unbound \( (k_{pa}/k_{pd}) \)

\( k_{pls}^{\text{ratio}} \)  Capacity ratio for peripheral lung sequestration \( (k_{pls2}/k_{pls1}) \)

\( k_{p}^{\text{sum}} \)  Peripheral lung unbound equilibration rate constant \( (= k_{pa} + k_{pd}) \)

\( k_{pls1}^{\text{sum}} \)  Peripheral lung sequestration equilibration rate constant \( (= k_{pls1} + k_{pls2}) \)

\( k_{pa} \)  Uptake of drug from the peripheral lung unbound to the peripheral lung sequestration

\( k_{pd} \)  Rate constant for efflux from the peripheral lung unbound to peripheral lung sequestration

\( k_{pls1} \)  Rate constant for distribution from the central body compartment to the PLS

\( k_{pls2} \)  Rate constants for absorption from peripheral lung sequestration to central body compartment
$k_{pm}$ First-order mucociliary clearance rate constant from peripheral lung unbound to central lung unbound

LLOQ Lower limit of quantitation in an analytical method

M&S Modeling and Simulation

MOA Mechanism of Action

MCS Monte-Carlo Simulation

NCA Non-compartmental Analysis

PBPK Physiological Pharmacokinetic

PK Pharmacokinetic

PLU Peripheral lung unbound compartment

PLS Peripheral lung sequestration compartment

PO Oral dosing

PUL Pulmonary

RD Repeat dose

SD Single dose

$t_{1/2}$ Half-life

TOB Tobramycin

$V_0$ Volume of distribution of the central compartment

$V_dss$ Steady-state volume of distribution

VI Visual inspection of noncompartmental analysis log-linear regression

VPC Visual Predictive Check

WHP Box and whisker plot
Abstract

DEVELOPMENT AND VALIDATION OF A SEMI-PHYSIOLOGICAL PHARMACOKINETIC (PBPK) MODEL TO PREDICT SYSTEMIC AND PULMONARY EXPOSURES AFTER INTRAVENOUS, ORAL ADMINISTRATION AND PULMONARY INHALATION OF SELECTED DRUGS, BUDESONIDE, TOBRAMYCIN AND CIPROFLOXACIN, IN HUMANS

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Using a semi-PBPK modeling/quantitative meta-analysis approach, this project investigated what factors affect pulmonary and systemic exposures of Budesonide (BUD), Tobramycin (TOB), and Ciprofloxacin (CIP) after inhalation:

Three structurally different pulmonary disposition models were developed for each drug, including pulmonary absorption (all three), excretion (TOB and CIP) and sequestration (TOB) in a peripheral and central lung compartment. Systemic disposition parameters were estimated using available human mean plasma ($c_p(t)$) and sputum ($c_s(t)$) concentration profiles after IV administration, and GI absorption parameters were estimated from these profiles after oral administration. Pulmonary disposition parameters were estimated from $c_p(t)$ and $c_s(t)$ profiles after inhalation using various devices along with their published pulmonary deposition characteristics. Appropriate covariate models accounted for effects of Cystic Fibrosis on the systemic disposition/GI absorption for TOB and CIP. Monte Carlo Simulations (MCS) were used...
to optimize parameters and validate the final models and parameter spaces against published data.

Despite limited available data, especially $cs(t)$ for BUD and CIP (after IV administration), the point estimates for the final model parameters were mechanistically plausible for all three drugs and consistent with their known differences in physicochemical and ADME properties. Model predictions adequately described the observed $cp(t)$ and $cs(t)$ profiles as well as exposure metrics across studies.

As the most lipophilic drug, BUD showed the fastest pulmonary absorption rates and highest $F_{pul}$ (83%). TOB, a very hydrophilic drug, exhibited (intracellular) pulmonary sequestration, resulting in slow pulmonary absorption and excretion and low $F_{pul}$ (10%). CIP - as zwitterion - showed relatively slow pulmonary absorption and excretion, leading to low $F_{pul}$ (8%); pulmonary excretion accounted for 27% of CIP overall elimination.

Results of a formal parameter sensitivity analysis demonstrated that, for all three drugs, after inhalation, (1) their systemic exposures ($cp(t)$) depend primarily on $CL_{tot}$ along with $F_{pul}/sequestration$ combined with $F_{oral}$; (2) increasing pulmonary exposures ($cs(t)$) can be accomplished by slowing down pulmonary absorption rates ($k_{ca}$) and/or slowing down mucociliary clearance from the lungs into the GI tract ($k_{cm}$) – affirming the overall hypothesis guiding the project.
CHAPTER 1

INTRODUCTION

1.1. Inhalation as a Dosing Route

 Historical evidence has shown that inhalation (INH) of drugs for medicinal purposes has been around since 1554 BC in Egypt.¹ INH as an intended route of drug administration is typically utilized for either, a) its ability to deliver the drug directly to its site of action, i.e. the lungs, or b) delivering the drug into the blood stream (systemic circulation) while avoiding any hepatic first-pass elimination, which reduces bioavailability after oral administration.² Delivering drug directly to the lungs may allow use of a lower dose, and minimizes the systemic exposure of the drug relative to intravenous (IV) or oral dosing (PO) routes.³

     After INH, a fraction of the dose will: 1) remain in the INH device, 2) be deposited in the mouth where it may be exhaled, 3) be swallowed and delivered in the GI tract where it may be available for GI absorption (similar to PO administration), and 4) deposited in the lungs for further disposition, namely pulmonary uptake, sequestration, and
mucociliary clearance. Mucociliary clearance can transport drug from the lung into the GI for possible GI absorption.4

The fraction of the dose that undergoes each of the four fates listed will depend on factors such as the efficiency of the device, the INH maneuver, patience compliance with the INH technique, patient health/disease state, and other factors.5 Some studies that evaluate drug deposition after INH further characterize the fraction of the dose that is deposited in the lungs by specifying whether it is deposited into the peripheral lung or in the central lung.6 Given that some disease states require targeting of the central lung, while other disease states require the targeting of the peripheral lung, a number of authors have discussed the necessity of obtaining peripheral versus central lung deposition device data.7

Cellular structure and airway geometry varies based on the region within the lung.8 As such, drug disposition once deposited in the lung will also vary. Drugs deposited in the lung can undergo processes including: dissolution, absorption, enzymatic metabolism, macrophage degradation, intracellular sequestration, and/or mucociliary clearance of the drug into the esophagus/GI tract.9 Accordingly, a model developed for describe INH of drugs, should attempt to capture these different processes.

1.2. Semi-PBPK Modeling of Inhaled Drugs

While the PK within the systemic circulation can be well described based on available experimental data, less is known about the pulmonary kinetics of drugs after IV administration or oral INH. To date, no modeling work has been done in an attempt to describe or predict the pulmonary drug concentration after IV administration even
though this is a frequently utilized dosing route. For example, given that the primary function of antibiotics is to prevent/treat pulmonary infections, their site of action is the lungs.

Notably, some authors have attempted to compare isolated sputum drug concentrations to minimum inhibitory concentrations (MIC) values for bacteria responsible for pulmonary infections, however, no work so far has attempted to develop a mathematical model the concentration time profile in the lungs or sputum.\textsuperscript{10,11} Others have looked at of bacteria concentrations in sputum and have drawn conclusions regarding the likely effectiveness of various antibacterial therapies.\textsuperscript{12–14} This method however, may not be an appropriate indicator of pulmonary drug exposures. A possible method to directly determine drug levels within the lung is to administer the compound and obtain a lung biopsy. While this method may be considered the most accurate and reliable technique for determining PUL drug concentrations, it is an invasive and dangerous procedure.

Physiologically -based Pharmacokinetic (PBPK) modeling and simulation (M&S) is an alternative method to estimate and predict drug concentrations, not only lung concentration(s), but also within systemic body compartments, including the bloodstream. However, PK Modeling, and more so PBPK modeling, is heavily dependent on the data available for the construction of the final model and parameter estimation, namely the physiological, biochemical, and physicochemical processes that occur in biological systems under certain physiological and pathological conditions.\textsuperscript{15} This information, however, may not be readily available, or the reliability of the available data may be in question or in conflict with published literature. As such, PBPK models reflect
current scientific knowledge, attempting to include all available experimental data, so as to provide the best-informed predictions. It is, however, important to note that the validity and quality of the simulations and conclusions reached are highly dependent on the utilized model, and how well informed the model is, i.e., the information incorporated in the construction of the model.

1.3. Sputum Composition, Physiological Components, and Purpose

Lining the surface of the lungs is a substance known as the airway surface liquid (ASL), which is composed of the periciliary fluid in immediate contact with the pulmonary (PUL) epithelium and the mucus layer resting on top of the periciliary fluid. The periciliary fluid is a low-viscosity aqueous layer that surrounds the cilia of the epithelia and allows them to beat in order to propel the mucus layer towards the trachea, and ultimately to the esophagus. The principal polymeric components of the mucus layer are mucins (specifically MUC5AC or MUC5B), which are high-molecular-weight, heavily-glycosylated proteins that are produced by epithelial tissues, and serve to create the gel-like viscosity of the mucus. While the periciliary fluid’s main function is to allow the cilia to beat, the mucus also serves an immunological purpose as a form of chemical barrier against external pathogens. It finally serves to maintain airway hydration and plays a role in cell signaling. When the mucus layer is propelled by the cilia and expectorated, the resulting matrix is called sputum.

Most studies of airway secretions in people with lung disease have been done using expectorated sputum, given the relative ease of obtaining specimens, compared to the difficulty in obtaining normal mucus from healthy airways.
Researchers have attempted to estimate the volume of the airway surface liquid via two primary methods; the first, estimated based on a bronchoalveolar lavage method, and the second, morphometry-extrapolation of the area and thickness of the fluid layer covering the respiratory epithelia: The bronchial lavage method is thought to lead to an underestimation of the ASL volume, as the recovery by lavage is typically incomplete, and hence leads to a lower calculated volume.\textsuperscript{19} The surface area of the lung has been previously compared to the area of a tennis court,\textsuperscript{20} however, more recently, researchers have suggested that it would only be half that area,\textsuperscript{21} and some have proposed that the area should be calculated based on body weight.\textsuperscript{22}

1.4. Fundamentals of Covariate Modeling

In addition to PBPK modeling, covariate models often need to be used to describe observable/predictable sources of PK variability arising from known patient characteristics. A covariate is any patient-specific variable such as body weight, age, gender, or renal function that is specific to an individual and may explain some of the between-subject variability observed in drug exposure or drug response (PK/PD).

The implementation of covariate models can be accomplished for either continuous or categorical covariates. The most frequently utilized mathematical implementation of these covariate models for continuous covariates across literature are: linear, proportional, piecewise, power, and exponential. Typically, power models (also known as allometric models) have been utilized for both volume of distribution ($V_{dss}$) and total clearance ($CL_{tot}$), given biological plausibility and clinical relevance.\textsuperscript{23} It is noteworthy, however, that the scaling factors for both those models are not the same,
namely, $V_{ds}$ typically has a scaling factor of 1.0 and $CL_{tot}$ typically has a scaling factor of 0.75.

As compared to continuous models, categorical models are binary, i.e. male/female, or healthy/diseases. Covariate models are able to aid in bridging the gap across various studies in the literature, as they are able to explain some of the inter-patient variability that may arise due to disease state, body weight, and other patient specific factors.
CHAPTER 2

HYPOTHESIS, SPECIFIC AIMS AND OVERALL STRATEGY

2.1. Hypothesis

After inhalation of drugs that target the lungs, increasing pulmonary exposures can be accomplished by slowing down uptake from the lungs into systemic circulation and/or reducing mucociliary clearance from the lungs into the GI tract. Additionally, systemic levels can be decreased primarily by increasing the total clearance of the drug from the body rather than reducing pulmonary absorption.

2.2. Specific Aims

The overall objective of this research is to utilize semi-PBPK modeling for selected drugs to determine which physiological processes/PBPK model parameters have the most influence on systemic and pulmonary exposures after INH administration. The following specific aims will be addressed to accomplish this objective:

1. Collect available literature studies reporting $c_p(t)$ and $c_s(t)$ profiles for BUD, TOB, CIP after IV, PO, and INH administration to humans.
2. Develop and validate a structural semi-PBPK model for each drug including relevant disease state and appropriate covariate models and optimize model parameters.
   a. The final model(s) have a common sub-model describing pulmonary disposition in order to allow comparison across drugs.
   b. For each drug, the final model will be validated by matching model predictions with available $c_p(t)$ and $c_s(t)$ profiles, as well as reported systemic and pulmonary exposure metrics.

3. Using the final PBPK model and optimized model parameter space, determine which model parameters have the most influence on systemic and pulmonary exposures using a sensitivity analysis.
   a. Perform a sensitivity analysis to identify which model parameters elicit the largest fold-change in both systemic and pulmonary exposure.

4. Compare across drugs to relate physiochemical properties to pulmonary absorption, sequestration, and excretion

2.3. Selection of Final Three Drugs For Investigation

To ensure that the sensitivity of the systemic and pulmonary exposure to model parameters was not an experimental artifact, as well as to increase the generalizability of the results from this work, the semi-PBPK modeling was performed on three drugs: budesonide (BUD), tobramycin (TOB), and ciprofloxacin (CIP).

These drugs were selected based on various criteria:
First, these drugs were selected due to the availability of published $c_p(t)$ profiles (and $c_s(t)$ for TOB and CIP) after both IV and INH administration. This was necessary since the model development and validation step required these data sets.

In order to allow for generalization of the conclusions from the results of this work, the selected compounds also varied in their underlying physical chemical properties: For example, the lipophilicity of the three compounds included BUD as moderately lipophilic compound ($\log(P) = 1.7$), while CIP is also a lipophilic compound ($\log(P) = -0.13$), and TOB which is a hydrophilic compound ($\log(P) = -7.3$).

Furthermore, their ionizability within physiological pH range varied. BUD will be uncharged in the physiological pH range with a pKa = 12.9, TOB is polycationic with pKa's = 6.7, 8.3, and 9.9, and CIP is likely to be zwitterionic (pKa = 6.1, and 8.7). However BUD, TOB, and CIP all have similar molecular weight (431, 468, and 331 g/mole, respectively).

Presumably due to their difference in lipophilicity and charge, BUD has a plasma protein binding of 80% (predominantly to albumin), TOB has negligible plasma protein binding, and CIP has a plasma protein binding of 20-40%.

As a result of, these drugs vary in their clearance pathways: BUD predominantly undergoes hepatic metabolism, TOB is virtually exclusively cleared via renal pathways, and CIP is known to be subject to both renal and non-renal clearance pathways. Their oral bioavailability varies as well, with BUD’s $F_{oral}$ of $\approx 10\%$ due to extensive hepatic first-pass metabolism, TOB with negligible $F_{oral}$ as it is too polar to cross the GI epithelial, and CIP has a reported $F_{oral}$ 70% presumably due to some first pass metabolism and possible GI efflux.
As can be seen, while there are sufficient *in-vivo* PK data available for these drugs after the dosing routes of interest, the three selected drugs are sufficiently different from each other such that the results from this work should be generalizable.

### 2.4. PK and Semi-PBPK Models Utilized for Analysis

Three structurally different semi-PBPK models were utilized within this work to simultaneously describe systemic and pulmonary.

#### 2.4.1. Model #1

The first model, Model #1, includes the drug absorption organ only. Figure 1 depicts the movement of drug as it is administered by IV, PO, or INH routes:

After INH, the emitted dose, which is the fraction of the dose that escapes the inhalation device, is obtained from deposition information, based on the metered dose and the fraction of the dose that remains in the inhaler (D_in). The model assumes that a fraction of the nominal dose (as known as device dose, or total dose) is instantaneously deposited in the oropharynx (DtGI) where it is swallowed and available for subsequent absorption from the gastrointestinal (GI) tract.

Alternatively, portions of the nominal dose are deposited into the lung (DtL). The DtL is fractionated into the peripheral lung compartment (F_pdl), central lung compartment (F_cd). Lastly, a fraction of the dose is exhaled (F_ex). Drug that is deposited in the peripheral lung compartment is available for (a) absorption into the central compartment by the rate constant k_pa or (b) for mucociliary clearance into the central lung compartment via k_pm. Similarly, drug that is either transferred (from the peripheral lung compartment) or originally deposited (from the device) into the central lung
compartment is available for (a) absorption into the central compartment by the rate constant $k_{ca}$ or for (b) mucociliary clearance into the GIT compartment via $k_{cm}$.

Lastly, drug that is either cleared (from the central lung compartment) or deposited (from the device) into the GIT compartment is available for gastrointestinal absorption into the central compartment by the rate constant $k_{ga}$, given oral bioavailability ($F_{oral}$).

Drug that reaches the central compartment distributes to and redistributes from the peripheral body compartment via first-order rate constants $k_{12}$ and $k_{21}$, respectively, while also undergoing first-order elimination from the central compartment via $CL_{tot}$.

Deposition parameters, pulmonary disposition, oral absorption, and systemic disposition parameters are color coded in the three figures below as: black, blue, yellow, and green parameters.

**Figure 1  Semi-PBPK Model #1**

Scheme describing pulmonary deposition, absorption, and systemic disposition following IV, PO, or INH administration

\[
\frac{d\text{inhaler}}{dt} = -k_{in} * D_m * (1 - D_{in})
\]

\[
\frac{d\text{PLU}}{dt} = k_{in} * D_m * (1 - D_{in}) * F_{pd} - \text{PLU} * (k_{pa} + k_{pm})
\]
\[
\frac{d\text{CLU}}{dt} = k_{\text{in}} \cdot D_{\text{m}} \cdot (1 - D_{\text{in}}) \cdot F_{\text{cd}} - \text{CLU} \cdot (k_{\text{ca}} + k_{\text{cm}}) + \text{PLU} \cdot k_{\text{pm}}
\]

\[
\frac{d\text{GI}}{dt} = k_{\text{in}} \cdot D_{\text{m}} \cdot (1 - D_{\text{in}}) \cdot F_{\text{gd}} + \text{CLU} \cdot k_{\text{cm}} - \text{GI} \cdot k_{\text{ga}}
\]

\[
\frac{d\text{CC}}{dt} = \text{CLU} \cdot k_{\text{ca}} + \text{PLU} \cdot k_{\text{pa}} + \text{PC} \cdot k_{21} + \text{GI} \cdot k_{\text{ga}} \cdot F_{\text{oral}} - \text{CC} \cdot (k_{21} + \frac{\text{CL}_{\text{tot}}}{V_{0}})
\]

\[
\frac{d\text{PC}}{dt} = \text{CC} \cdot k_{12} - \text{PC} \cdot k_{21}
\]

**Equations 1** Differential equation expression for Model #1

**2.4.2. Model #2**

In Model #1, after IV administration of a drug, drug cannot distribute into the lungs from the systemic circulation (central compartment). This precludes pulmonary exposures after IV administration and is contrary to reported data for some drugs that show *in-vivo* pulmonary exposure after IV administration (i.e., drug concentration in sputum). Furthermore, after INH, it may be biologically plausible that drug that is absorbed into the systemic circulation is able to redistribute back to the lung, however, Model #1 does not allow for this.

To address this limitation, Model #2, incorporates the lungs as absorption and excretion organs. Figure 2. shows Model #2, accounting for distribution from the central compartment into the lungs after any dosing route. This is accomplished through the addition of two new rate constants; \(k_{\text{pd}}\) and \(k_{\text{cd}}\), which describe distribution from the central compartment to the peripheral and central lung compartments, respectively.
Figure 2 Semi-PBPK Model #2

Scheme describing pulmonary deposition, absorption, and systemic disposition following IV, PO, or INH administration.

\[
\frac{d\text{inhaler}}{dt} = -k_{\text{in}} \cdot D_m \cdot (1 - D_{\text{in}})
\]

\[
\frac{d\text{PLU}}{dt} = k_{\text{in}} \cdot D_m \cdot (1 - D_{\text{in}}) \cdot F_{pd} + CC \cdot k_{pd} - \text{PLU} \cdot (k_{\text{pa}} + k_{pm})
\]

\[
\frac{d\text{CLU}}{dt} = k_{\text{in}} \cdot D_m \cdot (1 - D_{\text{in}}) \cdot F_{cd} + \text{CLS} \cdot k_{cd} - \text{CLU} \cdot (k_{ca} + k_{cm}) + \text{PLU} \cdot k_{pm}
\]

\[
\frac{d\text{GI}}{dt} = k_{\text{in}} \cdot D_m \cdot (1 - D_{\text{in}}) \cdot F_{gd} + \text{CLU} \cdot k_{cm} - \text{GI} \cdot k_{ga}
\]

\[
\frac{d\text{CC}}{dt} = \text{CLS} \cdot k_{ca} + \text{PLU} \cdot k_{pa} + PC \cdot k_{21} + \text{GI} \cdot k_{ga} \cdot F_{oral} - \text{CC} \cdot (k_{cd} + k_{pd} + k_{21} + \frac{\text{CL}_{\text{tot}}}{V_0})
\]

\[
\frac{d\text{PC}}{dt} = \text{CC} \cdot k_{12} - PC \cdot k_{21}
\]

Equations 2 Differential equation expression for Model #2

2.4.3. Model #3

Some studies have shown that certain compounds, for example TOB, can be sequestered within pulmonary epithelium.\textsuperscript{24} To account for this sequestration, Model #2
was expanded to include two pulmonary sequestration compartments along with corresponding the rate constants to mimic pulmonary sequestration of drugs.

Here the drug is an absorption, sequestration and excretion organ. This new model is shown in Figure 3, and will be referred to as Model #3:

$k_{pls1}$ and $k_{cls1}$ allow for drug distribution from the central compartment into the peripheral and central lung sequestration compartments (PLS and CLS) respectively. Similarly, $k_{pls2}$ and $k_{cls2}$ represent absorption from the PLS and CLS into the central compartment, respectively. The differential equations describing the intercompartmental movement of the drug in Model 3 are reported in Equations 3.

\[
\frac{d\text{inhaled}}{dt} = -k_{in} \times D_m \times (1 - D_{in})
\]

**Figure 3 Semi-PBPK Model #3**

Scheme describing pulmonary deposition, absorption, and systemic disposition following IV, PO, or INH administration. Plasma concentrations (systemic exposure) are calculated by dividing the amount of drug that is in the central compartment by the central compartment volume ($V_0$). For both TOB and CIP, sputum concentrations are calculated by dividing the amount of drug in the central lung unbound compartment with the central lung volume (CLV). CLV is set to 80% of total lung volume, which was obtained from literature.²⁵
\[
\frac{dPLU}{dt} = k_{in} \cdot D_m \cdot (1 - D_{in}) \cdot F_{pd} + PLS \cdot k_{pd} - PLU \cdot (k_{pa} + k_{pm})
\]
\[
\frac{dCLU}{dt} = k_{in} \cdot D_m \cdot (1 - D_{in}) \cdot F_{cd} + CLS \cdot k_{cd} - CLU \cdot (k_{ca} + k_{cm}) + PLU \cdot k_{pm}
\]
\[
\frac{dGI}{dt} = k_{in} \cdot D_m \cdot (1 - D_{in}) \cdot F_{gd} + CLU \cdot k_{cm} - GI \cdot k_{ga}
\]
\[
\frac{dPLS}{dt} = PLU \cdot k_{pa} - PLS \cdot (k_{pd} + k_{pls2}) + CC \cdot k_{pls1}
\]
\[
\frac{dCLS}{dt} = CLU \cdot k_{ca} - CLS \cdot (k_{cd} + k_{cls2}) + CC \cdot k_{cls1}
\]
\[
\frac{dCC}{dt} = CLS \cdot k_{cls2} + PLS \cdot k_{pls2} + PC \cdot k_{21} + GI \cdot k_{ga} \cdot F_{oral}
\]
\[-CC \cdot (k_{cls1} + k_{pls1} + k_{21} + \frac{CL_{tot}}{V_0})
\]
\[
\frac{dPC}{dt} = CC \cdot k_{12} - PC \cdot k_{21}
\]

**Equations 3** Differential equation expression for Model #3

### 2.5. Model Optimization and Parameter Estimation

#### 2.5.1. IV NCA

Estimation of NCA was performed on \( c_p(t) \) profiles following IV, PO, and INH for the three drugs. The slope of the terminal phase (\( \lambda \)) of the \( c_p(t) \) profile was estimated by a log-linear regression in excel using the Analysis ToolPak add-in for Excel 2016. Area under the curve and area under the moment curve was estimated using the trapezoidal rule as described by Gibaldi.\(^26\) \( AUC_{t=\infty} \) was estimated by dividing the last measured concentration by \( \lambda \), \( AUC_{t=\infty} \) was then calculated the sum of \( AUC_{0-t} \) and \( AUC_{t=\infty} \). Total
clearance \((CL_{tot})\) was estimated as the total administered dose divided by \(AUC_{\infty}\). Systemic mean residence time \((MRT^{IV})\) was estimated by \(\frac{AUMC_{\infty}}{AUC_{\infty}}\) (for infusion studies the calculation was modified to \(\frac{AUMC_{\infty}}{AUC_{\infty}}-T_{inf}/2\)), and then was used to estimate volume of distribution \(V_{dss}\) when multiplied by \(CL_{tot}\).

A meta-analysis of the available data was performed to explore dose proportionality by investigating how \(AUC\) relates with the various tested doses after IV administration and after INH.

### 2.5.2. PO NCA

For PO studies, \(F_{oral}\) was estimated using NCA by dividing the product of \(AUC^{PO}\) and \(CL_{tot}^{IV}\) by the orally administered dose \(Dose^{PO}\). Mean absorption time after oral administration \((MAT^{po})\) was calculated by subtracting the systemic mean residency time after IV administration \((MRT^{IV})\) from the mean residency time after PO administration \((MRT^{po})\). Subsequently, \(k_{ga}\) was estimated initially by taking the ratio of 1 over \(MAT^{po}\).

The total clearance and systemic mean residence time utilized in estimating \(F_{oral}\) and \(MAT\), respectively, were the overall means of the HV and CF groups estimated using IV data sets.

### 2.5.3. INH NCA

For INH studies, bioavailability after INH \((F_{inh})\) was calculated as shown in Equation 4 below using NCA values for \(AUC\) after both INH and IV administration and their respective doses. The device dose represents the nominal dose placed in the INH device.
F_{inh} = \frac{\text{Device dose}}{\text{Dose}_{IV}} \frac{\text{AUC}_{0-\infty}^{\text{INH}}}{\text{AUC}_{0-\infty}^{\text{IV}}}

\textbf{Equation 4} NCA-calculated bioavailability after INH

After INH, drug can reach systemic circulation after absorption from either the lungs or from the GI tract. As such, F_{INH} depends on (1) fraction of the dose that is orally bioavailable (F_{oral}), and (2) the fraction of the dose that is bioavailable through the lungs (pulmonary bioavailability = F_{pul}). The contribution of each of the two pathways is weighted by the amount of the dose that is originally deposited in that pathway:

Specifically, the contribution of F_{pul} to F_{INH} will depend on the fraction of the dose deposited in the lung (DtL), and the contribution of F_{oral} to F_{INH} will depend on the fraction of the dose deposited in the GI tract (DtGI). This is expressed in Equation 5.

\[ F_{\text{inh}} = F_{\text{pul}} \times \frac{DtL}{DtL + DtGI} + F_{\text{oral}} \times \frac{DtGI}{DtL + DtGI} \]

\textbf{Equation 5} F_{INH} as the sum of F_{pul} and F_{oral} multiplied by their relative contributions to F_{inh}.

Rearranging equation 5 to solve for F_{pul}, we derive Equation 6. Equation 6 allows estimation of F_{pul} based on F_{INH} and F_{oral}.

\[ F_{\text{pul}} = \frac{F_{\text{inh}} \times (DtL + DtGI) - F_{\text{oral}} \times DtGI}{DtL} \]

\textbf{Equation 6} Estimating pulmonary bioavailability (F_{pul}) after inhalation
In addition to this equation, there is an additional method that will be used to estimate $F_{pul}$ when adequate systemic exposure data with sufficient plasma data is available after with and without administration of a charcoal block solution after drug INH. This method will be described in Chapter 5.

### 2.5.4. Covariate Analysis

For PO studies, exploratory plots of $F_{oral}$, $MAT^{PO}$, and $k_{ga}$ vs dose, age, body weight, and disease state were inspected to determine if any these covariates may affect these PK parameters.

### 2.5.5. NCA Acceptance Criteria

The validity of NCA performed was determined based on several criteria. Firstly, the log-linear regression performed to obtain lambda for the reported terminal points on the $c_p(t)$ profiles needed to pass a visual inspection, specifically that all points were evenly distributed above and below the regression line. Furthermore, the $r^2$ of the regression had to be greater than 0.9. Regression results were deemed acceptable if the sampling schedule was long enough such that two terminal half-lives were covered. The extrapolated AUC could not contribute more than 30% of the $AUC_{\infty}$. Additional confidence in the results of the NCA was gained if the estimated PK parameters were similar to the literature reported parameter exposure metrics. Finally, $c_{max}$ and $t_{max}$ had to be captured for both PO and INH studies i.e. points on both sides of $c_{max}$.

### 2.5.6. Model Fitting and Parameter Estimation

Initial estimates for systemic disposition and oral absorption parameters were obtained from NCA. Pulmonary disposition parameter initial estimates were obtained
from similar modeling works in literature, with exception of mucociliary clearance initial estimates, which were obtained from *in-vivo* studies.\textsuperscript{27,28}

The acceptability of the model fits that were performed was also based on numerous acceptance criteria. Firstly, a visual inspection of the model predicted results relative to the reported $c_p(t)$ profile was crucial to determine if the estimate fit was acceptable. Additionally, the overall goodness of fit for the model was evaluated by checking if the $r^2$ was greater than 0.90 and if the model selection criteria (MSC) was greater than 3. Lastly, confidence in the estimated values of the various model parameters was based on the CV of each of the parameters being less than 30% and the correlation between parameters being less than 0.5 and greater than -0.5 model-estimated parameters. The results of the model fitting were also compared with PK parameters obtained from NCA, to confirm agreement.

**2.5.7. Monte Carlo Simulations**

MCS were performed by first specifying the number of simulations (n) the model should be run. Parameter variability distributions were specified, with a mean and a coefficient of variation.

The results of MCS were also assessed against several acceptance criteria. A visual predictive check (VPC, 5% - 95% percentiles) relative to reported $c_p(t)$ and $c_s(t)$ profiles was also performed. Mean (and SD when available) had to be within this VPC. When possible, studies were grouped together based on dose, route, population, or INH device. Reported mean exposure metrics for both plasma and sputum exposure were compared with model-predicted exposures. Individual difference model-predicted and reported exposures had to be less than 50% for plasma exposure and less than 150%
for sputum exposure. The average of these differences across each of the individual data had to be not different from zero. Additionally, an informal comparison between model predicted exposure metric variability (SD) and reported variability (SD when available) was performed. Comparison of the predicted and reported variabilities was used for parameter variability estimation (CV’s associated with parameter estimate uncertainty).
A common chronic disease that affects nearly 26 million individuals across the United States, asthma is a complex disease that involves airway inflammation, sporadic airflow obstruction, and bronchial hyperresponsiveness. According to the Center of Disease Control, asthma in the U.S is associated with over fifty-six billion dollars in annual expenses. Patients may experience symptoms such as wheezing, labored breathing, chest tightness, coughing, and shortness of breath. Although it has not been clearly pinpointed as to why some patients develop asthma and others do not, both environmental and genetic factors have been identified as key contributing factors. In some patients, exposure to airborne substances, such as pollen, dust mites, mold spores, and pet dander have been known to trigger asthma, while in others, physical activity (exercise-induced asthma), strong emotions and stress have also induced asthma. Cells that respond to these triggers include (but are not limited to) mast cells,
T-lymphocytes, macrophages, neutrophils, and epithelial cells. Exposure to these triggers leads the patients to have 1) an inflamed/thickened airway wall, 2) tightened muscles leading to a constricted airway, and 3) increased mucus production. These three symptoms lead to a narrowed airway and hence limited air flow. It is this limited air flow that causes the symptoms mentioned above.

### 3.1.2. Treatment of Asthma

Treatment options for asthma include methylxanthines, as well as inhaled β-adrenergic agonists, muscarinic cholinergic antagonists, and glucocorticoids. Of these treatments, one of the mainstay options used by asthma patients to manage symptoms and slow down disease progression are the orally inhaled glucocorticoids, including BUD. BUD has been shown to exhibit anti-inflammatory response in including T lymphocytes, eosinophils, mast cells, and dendritic cells both in-vitro and in-vivo. It is potent glucocorticoid, exhibiting high local pulmonary anti-inflammatory activity after inhalation. When clinically tested at a dose of 800 µg twice daily, it was shown that BUD reduced the acute and delayed pulmonary reactions of asthma as measured by a reduction in the forced expiratory volume in one minute (FEV₁). While it has been shown to be efficacious, the use of INH BUD is limited by its systemic absorption, leading to inhibition of the hypothalamic-pituitary-adrenal axis, increasing the risk for adverse reactions, e.g., stunting of bone growth in pediatric patients.

### 3.1.3. Relevant Physiochemical Properties of Budesonide

BUD is a small lipophilic molecule with a log(P) value of 1.7 and a molar mass of 431 g/mole. The epimer structures of BUD are shown in Figure 4. Additionally, BUD
has a pKa of 12.9, which is not physiologically relevant. The aqueous solubility of BUD was experimentally determined to be between $19 \cdot 10^{-3}$ and $26 \cdot 10^{-3}$ g/L.\(^{36}\) At a concentration of 30 µM in the apical compartment of a Calu-3 cell monolayer model, the linear concentration-time profile allowed to determine a $P_{app}$ value of $8.59 \cdot 10^{-6}$ cm/sec, which indicated high permeability.\(^{37}\) Similar studies were also performed in Caco-2 cell monolayers. Permeability was estimated in both directions (A-B and B-A) with resulting $P_{app}$ values equal to $11 \cdot 10^{-6}$ cm/sec, and $8.7 \cdot 10^{-6}$ cm/sec respectively.\(^{38}\) Based on the experimental $P_{app}$ values both in Caco-2 and Calu-3 cell models, it can be concluded that BUD is a high permeability compound, crossing epithelia via transcellular diffusion.

![Epimer structures of BUD](image)

**Figure 4 Epimer structures of BUD**

**3.1.4. Summary of Known PK/ADME Properties of Budesonide**

With a reported plasma protein binding 88% predominantly to albumin, BUD still exhibits extensive extravascular distribution throughout the body with a $V_d_{ss}$ after a 10 minute 500-µg IV infusion dose in HV of $2.2 \pm 0.46$ L/kg. Total clearance values ($CL_{tot}$) are also estimated after the same dosing regiments in those subjects to be $0.86 \pm 0.13$
L/min. While there are 16 detectable BUD metabolites, only two metabolites account for the majority of the elimination. 6β-hydroxybudesonide and 16α-hydroxyprednisone are two major metabolites and were predominantly formed by the CYP3A4 enzyme. There was no evidence of BUD undergoing renal elimination, which was well supported in literature and is likely due to its lipophilic nature. Additionally, it has been shown that BUD can undergo reversible fatty acid conjugation within airway tissue.

When administered with via IV infusion as well as PO administration in HV, the calculated experimental $F_{oral}$ was 10.7%. In light of the high clearance value and the known metabolic pathways, it was noted that BUD undergoes extensive first-pass metabolism and as such is considered a high hepatic extraction ratio drug. After inhalation from a Turbuhaler® (again with a known IV reference data set), the authors reported that the systemic availability of BUD was 38%.

3.2. Objectives

The major objectives of the chapter were to:

1. Develop a semi-mechanistic PBPK model for INH BUD;
2. Estimate and optimize model parameters (point and variability estimates);
3. Validate the final model and parameters on plasma exposures using Monte-Carlo simulations (MCS);
4. Determine the sensitivity of plasma exposures to model parameters.

3.3. Methods

3.3.1. Summary of Identified IV, PO and INH studies

All six identified of BUD after IV administration studies were included in the analysis performed. Four of the studies utilized HPLC-MS as the analytical method,
while the two other studies utilized HPLC-Radiometry methods. All studies were performed in HV and provided a total of six mean level $c_p(t)$ profiles.

Four studies were identified in which BUD was administered orally. Of the four studies, two studies were not intended to characterize the oral absorption rate constant and hence had an insufficient sampling schedule. Additionally, one study utilized an inadequate analytical method for measuring plasma concentrations and hence as not included in the analysis. One study was included in analysis and provide a single mean $c_p(t)$ profile. The dose was formulated in tablet form in the included study.

Thirteen studies were identified where BUD was INH. Of the thirteen studies, eight studies did not report any $c_p(t)$ profiles, and two studies did not report what device were utilized. Accordingly, only three studies where the dose was administered through a Turbuhaler were utilized in the analysis that was performed.

### 3.3.2. Digitization and Noncompartmental PK Analysis

NCA was performed on the digitized $c_p(t)$ profiles for each of the individual studies across the dosing routes (IV, PO, and INH). To assess PK dose-linearity, AUC vs dose and log(AUC) vs log(dose) plots were inspected. For the PO study, $F_{oral}$ was estimated using NCA by dividing the product of $AUC_{PO}$ and $CL_{tot}^{IV}$ by the orally administered dose ($D_{po}$).

Mean absorption time after oral administration ($MAT_{po}$) was calculated by subtracting the systemic mean residency time after IV administration ($MRT_{IV}$) from the mean residency time after PO administration ($MRT_{po}$). Subsequently, $k_{ga}$ was estimated by taking the ratio of 1 over $MAT_{po}$. These calculations were performed with $CL_{tot}$ and
MRT\textsuperscript{IV} of each of the individual IV studies, a $F_{oral}$ and MAT\textsuperscript{po} range was determined. NCA was not performed for INH studies.

### 3.3.3. Semi-PBPK Models Tested

Model #1 was used for both fit to the reported data for BUD as well as the MCS performed. Elimination from the central compartment was modeled using an elimination micro-rate constant $k_{10}$. The differential equation for the central compartment (CC) was derived (Equation 7) and the explicit solution (Equation 8) was solved for using the Laplace transformation method.

\[
\frac{dCC}{dt} = PL * k_{pa} + CL * k_{ca} + GI * k_{ga} * F_{oral} + PC * k_{21} - CC * (k_{12} + k_{10})
\]

**Equation 7** Differential equation for central compartment

\[
CC(t) = C_p a e^{(-\alpha t)} + C_p b e^{(-\beta t)} + C_p c e^{(-k_c t)} + C_p p e^{(-k_p t)} + C_p g a e^{(-k_g a t)}
\]

**Equation 8** Explicit solution derived by Laplace transformation calculating central compartment concentration at time $t$ after INH

### 3.3.4. Software

The NCA will then be performed in MS Excel, modeling work was performed with Scientist® v3.0, and MCS will be performed with Matlab® R2015a.

For the included studies, captured $c_p(t)$ and $c_s(t)$ profiles were digitized using the GetData© Graph Digitizer Version 2.24 software. NCA was performed on the plasma concentration data. Model parameter fitting was performed in Scientist v. 3.0.0.215
Build: 1334 using a stiff integrator. MCS and data visualization was performed in Matlab® R2015a. Since the explicit solution was derived via the Laplace transform method, differential equations and numerical solvers were not programmatically utilized.

3.4. Results

3.4.1. Summary of Final IV, PO, and INH Studies Identified

A search of the literature on PubMed and Google Scholar resulted in the identification of 6 publications where BUD was administered as an IV infusion. These publications and their respective key study design elements are listed in Table 1.34,44–48 These studies enrolled between 3 and 24 subjects and tested doses ranging from 0.1 mg to 0.5 mg that were infused over the course of 5 to 10 minutes.

An additional four studies were identified in which BUD was administered orally. These four studies including the relevant study design parameters are tabulated in Table 2.34,48–50 The administered doses ranged from 2.0 to 4.8 mg. Only one of the five PO administration studies was acceptable. One study was excluded from analysis due to an inadequate sampling schedule that would not allow for the estimation of oral absorption parameters. A second study was excluded due to an insensitive analytical method that was used for the detection of plasma concentrations. Lastly, a study was excluded from analysis due to the use of an ad-hoc formulation (an aqueous solution was administered instead of a tablet) that showed altered $c_p(t)$ and absorption PK parameters.

A total of 3 studies were identified where BUD was administered by INH. These studies and their key study design elements are listed in Table 3.44,51,52 All three studies were suitable for analysis as they satisfied the inclusion criteria, and provided $c_p(t)$
profiles, and plasma exposure metrics. Only one device was tested in these three studies, and the same dose was tested in all three studies (1 mg). The Turbuhaler DPI was the inhalation device utilized in this study, for which the deposition data was also reported in literature.\textsuperscript{53}

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>BUD 101</th>
<th>BUD 102</th>
<th>BUD 103</th>
<th>BUD 104</th>
<th>BUD 105</th>
<th>BUD 106</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion Time (min)</td>
<td>9</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>First/Latest Sampling Time Points (hrs)</td>
<td>0.17 → 8</td>
<td>0.08 → 8</td>
<td>0.24 → 10</td>
<td>0.10 → 8.22</td>
<td>0.14 → 8.22</td>
<td>0.17 → 10</td>
</tr>
<tr>
<td>Number of Data Points</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total Subjects (N)</td>
<td>24</td>
<td>4</td>
<td>16</td>
<td>3</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Gender (Healthy)</td>
<td>M = 12 (✓)</td>
<td>M = 4 (✓)</td>
<td>M = 6 (✓)</td>
<td>M = 3 (✓)</td>
<td>M = 7 (✓)</td>
<td>M = 8 (✓)</td>
</tr>
<tr>
<td>Age</td>
<td>39 (22-53)</td>
<td>22-46</td>
<td>31 (20-44)</td>
<td>--</td>
<td>42 (26-56)</td>
<td>37 (27-45)</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>68 (42-92)</td>
<td>63-95</td>
<td>67 (53-94)</td>
<td>--</td>
<td>68 (49-88)</td>
<td>68 (50-95)</td>
</tr>
<tr>
<td>Assay</td>
<td>HPLC - MS</td>
<td>HPLC - Radiometry</td>
<td>HPLC - MS</td>
<td>HPLC - Radiometry</td>
<td>HPLC - MS</td>
<td>HPLC - MS</td>
</tr>
</tbody>
</table>

Table 1 Six identified studies with key study design elements wherein BUD was administered by IV infusion to HV

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>BUD 201</th>
<th>BUD 202</th>
<th>BUD 203</th>
<th>BUD 204</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Data Points</td>
<td>11</td>
<td>5</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Sampling Time Scheme</td>
<td>0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24</td>
<td>2, 3, 4, 6, 8</td>
<td>0.5, 1, 1.5, 2, 3, 4, 6, 8, 10</td>
<td>0.33, 0.5, 0.67, 1, 1.33, 1.67, 2, 3, 4, 5, 6, 8, 12</td>
</tr>
<tr>
<td>Total Subjects (N)</td>
<td>6</td>
<td>3</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Gender (Healthy)</td>
<td>M = 6 (✓)</td>
<td>M = 3 (✓)</td>
<td>M = 8 (✓)</td>
<td>M = 6 (✓)</td>
</tr>
<tr>
<td>Age</td>
<td>46.7 (43 - 56)</td>
<td>--</td>
<td>37 (27-45)</td>
<td>43.7 ± 7.1</td>
</tr>
<tr>
<td>Adhoc Formulation</td>
<td>Deuterium label + lactose</td>
<td>Tritium label + micronized</td>
<td>micronized</td>
<td>10 mL Aqueous Solution</td>
</tr>
<tr>
<td>Assay (LOQ)</td>
<td>LC - MS</td>
<td>HPLC - Radiometry</td>
<td>LC - MS</td>
<td>LC - MS/MS</td>
</tr>
</tbody>
</table>

Table 2 Four identified studies with key study design elements wherein BUD was administered PO to HV.
Table 3 Three identified studies with key study design elements wherein BUD was administered via INH to HV.

3.4.2. NCA results

NCA was performed on the six individual data sets obtained from the included studies. Visual inspection for each of the log-linear regression of the terminal slope used in estimating $\lambda$ were acceptable, $R^2$ values were all larger than 0.9853, and the AUC percent extrapolated did not exceed 15% for each of the individual data sets analyzed. The estimated PK parameters are tabulated in Table 4.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>BUD 308</th>
<th>BUD 309</th>
<th>BUD 313</th>
</tr>
</thead>
<tbody>
<tr>
<td>First and Latest Sampling Time Points (min)</td>
<td>(10, 480)</td>
<td>(15, 480)</td>
<td>(10, 480)</td>
</tr>
<tr>
<td>Number of Plasma Samples</td>
<td>10</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Total Subjects (n)</td>
<td>24</td>
<td>44</td>
<td>6</td>
</tr>
<tr>
<td>Gender (Healthy)</td>
<td>M=12 (√)</td>
<td>M=6 (√)</td>
<td>M=6 (√)</td>
</tr>
<tr>
<td>Assay</td>
<td>HPLC - MS</td>
<td>HPLC - MS/MS</td>
<td>HPLC - MS/MS</td>
</tr>
<tr>
<td>Inhalation Device</td>
<td>Turbuhaler</td>
<td>Turbuhaler</td>
<td>Turbuhaler</td>
</tr>
</tbody>
</table>

Table 4NCA estimated PK parameters for each of the individual data sets from the six included IV studies.

Inspection of the AUC vs IV dose plotted on a linear scale as well as on a log-log plot did not provide any evidence of deviation from linear PK after IV administration.
When a linear and a power model were fit to both plots respectively, the resulting $R^2$ value was 0.8293 and the exponential coefficient of 0.90. These plots are shown in Figure 5.

Figure 5 BUD IV dose escalation plots

IV infusion dose escalation plotting AUC with increasing IV dose on a linear plot with a linear regression through the estimated AUC values (left plot), and on a log-log plot with a power model fit to the estimated AUC values (right plot).

The same analysis could not be performed with the available data after PO administration as there was only a single study included in the analysis.

NCA was performed on the single PO data set available. Visual inspection for the log-linear regression of the terminal slope used in estimating $\lambda$ was acceptable, the $R^2$ value was 0.9990, and the AUC percent extrapolated was 1.2%. The estimated $F_{oral}$ ranged from 8.7% to 13.5% and the estimated $k_{ga}$ ranged from 0.0047 to 0.0088 min$^{-1}$. The observed and estimated PK parameters are tabulated in Table 5.
Table 5 NCA estimated PK parameters for the available data set from the included PO study.

### 3.4.3. Modeling of IV Data

Successful fits were obtained for each of the 6 data sets from the seven studies. No weighting factor was applied to any of the fits. MSC was higher than 3.6, \( R^2 \) values were above 0.9950 for all the selected fits, correlation matrix values were all below 0.5, coefficients of variation for each of the parameter estimates were below 30%, and visual inspection of the observed versus simulated values were all acceptable. Table 6 shows the resulting parameter values based on the fits produced by the individual data sets. The model file for the various fits are reported in the Appendix 1 as Code 1 of this chapter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (nmol/l)</td>
<td>2.64</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (min)</td>
<td>120</td>
</tr>
<tr>
<td>AUC (nmol*min/l)</td>
<td>1074</td>
</tr>
<tr>
<td>( t_{1/2} ) (min)</td>
<td>227.3</td>
</tr>
<tr>
<td>( V_{\text{RRT}_{\text{pke}}} ) (min)</td>
<td>342</td>
</tr>
<tr>
<td>% Extrapolated</td>
<td>1.2</td>
</tr>
<tr>
<td>( K^* ), VI</td>
<td>0.9990, (√)</td>
</tr>
<tr>
<td>( F_{\text{mal}} )</td>
<td>8.7 - 13.5</td>
</tr>
<tr>
<td>( k_{\text{ps}} ) (min(^{-1}))</td>
<td>0.0047 - 0.0088</td>
</tr>
</tbody>
</table>
Two compartment model parameter estimates and goodness of fit validation criteria results for each of the twelve data sets obtained from the final seven included IV studies.

MCS resulted in an acceptable parameter space that described observed $c_p(t)$ profiles and exposure metrics after IV administration. The four systemic disposition parameters were optimized and the optimized central tendency (mean) and a variation (CV) as shown in Table 7. $V_0$ followed a normal distribution with a 20% CV, while the three micro-rate constants followed a log-normal distribution with a 20% CV.

<table>
<thead>
<tr>
<th>Two Compartment Model Parameter Estimates</th>
<th>BUD 101</th>
<th>BUD 102</th>
<th>BUD 103</th>
<th>BUD 104</th>
<th>BUD 105</th>
<th>BUD 106</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{int}$ (ml/min)</td>
<td>1406</td>
<td>981</td>
<td>1340</td>
<td>1206</td>
<td>1198</td>
<td>923</td>
</tr>
<tr>
<td>$V_{a1}$ (L)</td>
<td>161</td>
<td>148</td>
<td>225</td>
<td>260</td>
<td>163</td>
<td>141</td>
</tr>
<tr>
<td>$V_1$ (L)</td>
<td>71</td>
<td>25</td>
<td>106</td>
<td>88</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>$k_{12}$ (min$^{-1}$)</td>
<td>0.056</td>
<td>0.162</td>
<td>0.020</td>
<td>0.090</td>
<td>0.180</td>
<td>0.049</td>
</tr>
<tr>
<td>$k_{21}$ (min$^{-2}$)</td>
<td>0.043</td>
<td>0.033</td>
<td>0.018</td>
<td>0.044</td>
<td>0.029</td>
<td>0.019</td>
</tr>
<tr>
<td>$k_{10}$ (min$^{-1}$)</td>
<td>0.020</td>
<td>0.039</td>
<td>0.013</td>
<td>0.014</td>
<td>0.053</td>
<td>0.024</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Two Compartment Model Validation</th>
<th>Visual Inspection</th>
<th>$R^2$</th>
<th>$R^2$</th>
<th>$R^2$</th>
<th>$R^2$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>✓</td>
<td>0.9988</td>
<td>0.9995</td>
<td>0.9998</td>
<td>0.9992</td>
<td>0.9992</td>
</tr>
<tr>
<td>MCS</td>
<td>5.31</td>
<td>6.38</td>
<td>4.25</td>
<td>3.58</td>
<td>5.96</td>
<td>5.81</td>
</tr>
<tr>
<td>$V_0$ CV</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>$k_{12}$ CV</td>
<td>16</td>
<td>7</td>
<td>24</td>
<td>21</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>$k_{21}$ CV</td>
<td>13</td>
<td>8</td>
<td>21</td>
<td>20</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>$k_{10}$ CV</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 6 IV BUD two compartment body model results

Two compartment model parameter estimates and goodness of fit validation criteria results for each of the twelve data sets obtained from the final seven included IV studies.

MCS resulted in an acceptable parameter space that described observed $c_p(t)$ profiles and exposure metrics after IV administration. The four systemic disposition parameters were optimized and the optimized central tendency (mean) and a variation (CV) as shown in Table 7. $V_0$ followed a normal distribution with a 20% CV, while the three micro-rate constants followed a log-normal distribution with a 20% CV.

<table>
<thead>
<tr>
<th>Point Estimate</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$ (L)</td>
<td>45</td>
</tr>
<tr>
<td>$k_{12}$ (min$^{-1}$)</td>
<td>0.100</td>
</tr>
<tr>
<td>$k_{21}$ (min$^{-2}$)</td>
<td>0.031</td>
</tr>
<tr>
<td>$k_{10}$ (min$^{-1}$)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Table 7 Final optimized systemic disposition model parameters point and variability estimates.
Table 8 shows the percent difference for predicted vs observed exposure metrics for each of the individual studies. The predicted AUC values did not exceed 30% of the reported values for each of the studies. Additionally, the calculated PK parameters were also within 50% of the reported values. VPC for studies BUD 101, 104, 105, and 106 are grouped in Figure 6 since these studies all utilized a dose of 0.5 mg infused over 10 minutes. VPC for BUD 102 and BUD 103 are shown in Figures 7 and 8, respectively.

<table>
<thead>
<tr>
<th>Study</th>
<th>101</th>
<th>102</th>
<th>103</th>
<th>104</th>
<th>105</th>
<th>106</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC\text{∞}</td>
<td>29%</td>
<td>16%</td>
<td>25%</td>
<td>7.9%</td>
<td>5.4%</td>
<td>12%</td>
<td>16%</td>
<td>9</td>
</tr>
<tr>
<td>t\frac{1}{2}\text{β}</td>
<td>40%</td>
<td>6%</td>
<td>11%</td>
<td>21%</td>
<td>31%</td>
<td>30%</td>
<td>23%</td>
<td>12</td>
</tr>
<tr>
<td>MRT\text{IV}</td>
<td>37%</td>
<td>1.2%</td>
<td>5.4%</td>
<td>24%</td>
<td>21%</td>
<td>14%</td>
<td>17%</td>
<td>12</td>
</tr>
<tr>
<td>CL\text{tot}</td>
<td>14%</td>
<td>32%</td>
<td>11%</td>
<td>2.6%</td>
<td>18%</td>
<td>25%</td>
<td>17%</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 8 Percent difference between reported and predicted values for AUC, t\frac{1}{2}\text{β}, MRT\text{IV}, and CL\text{tot}.

Figure 6 MCS-predicted versus observed cp(t) after IV dosing for BUD studies 101, 104, 105, and 106.
3.4.4. Modeling of PO Data

MCS resulted in an acceptable parameter space that described observed $c_p(t)$ profile and exposure metrics after PO administration. The two oral absorption parameters were optimized and the optimized central tendency (mean) and a variation (CV) as shown in Table 9. Parameter variation was 40%, which was twice the variation associated with the systemic disposition parameters.
Table 9 Final optimized oral absorption model parameters point and variability estimates.

Table 10 indicates the percent difference for each of the predicted exposure metrics; namely, AUC, $c_{\text{max}}$, $t_{\text{max}}$, and MRT$^{\text{PO}}$. The predicted AUC value was 3.7% different than the reported in the study. Additionally, the remaining exposure metrics were also within 50% of the reported values. VPC for the reported $c_p(t)$ profile is shown in Figure 9.

<table>
<thead>
<tr>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
</tr>
<tr>
<td>$c_{\text{max}}$</td>
</tr>
<tr>
<td>$t_{\text{max}}$</td>
</tr>
<tr>
<td>MRT$^{\text{PO}}$</td>
</tr>
</tbody>
</table>

Table 10 Percent difference between reported and simulated values for AUC, $c_{\text{max}}$, $t_{\text{max}}$, and MRT$^{\text{PO}}$

Figure 9 MCS-predicted versus observed $c_p(t)$ after PO dosing for BUD 201

<table>
<thead>
<tr>
<th>Point Estimate</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{ga}$ (min$^{-1}$)</td>
<td>0.0073</td>
</tr>
<tr>
<td>$F_{\text{oral}}$ (%)</td>
<td>10%</td>
</tr>
</tbody>
</table>
3.4.5. Modeling of INH Data

MCS resulted in an acceptable parameter space that described the observed \( c_p(t) \) profiles and corresponding exposure metrics after INH administration. Published scintigraphy results indicated that 27.9% of the device dose is delivered to the lungs. Parameter optimization resulted in approximately a 3:1 deposition ratio favoring the peripheral lung compartment. The fraction of the drug exhaled was less than 1%, and the fraction of the dose that remained in the inhaler was 14%. As such, the remaining 57% of the dose was deposited into the GI compartment. The variation for these deposition parameters followed a log-normal distribution and ranged from 9% to 57% as reported in literature. A summary of the central tendencies of these parameters along with their associated variabilities is reported in Table 11.

Both optimized mucociliary clearance values were below the literature estimates that were used for initial estimates. The resulting values of \( k_{cm} \) and \( k_{pm} \) were 0.016 and 0.0078 min\(^{-1}\) respectively. The optimized value for \( k_{pa} \) was twice that of \( k_{ca} \); 0.066 and 0.033 min\(^{-1}\) respectively. The variation associated with these four pulmonary disposition parameters was 30%. These parameters along with their associated variabilities are also reported in Table 11.
Table 11 Final optimized pulmonary deposition and pulmonary disposition model parameters point and variability estimates.

Deposition data obtained from pharmacoscintigraphy studies and final optimized pulmonary disposition model parameters point and variability estimates. Deposition parameters are experimental values, while pulmonary disposition was optimized using the semi-PBPK model.

Since the three INH studies analyzed all utilized the same device, the MCS were grouped and the VI plot is shown on both a linear and a logarithmic scale in Figure 10. The model slightly underpredicted the reported $c_p(t)$ profiles; however, all the observed concentrations were within the 5-95th percentiles. This slight underprediction is also observed in the under prediction of AUC as reported in Table 12. The difference between the predicted and reported AUC was below 30% for the three studies, and the difference between the predicted and the remaining exposure metrics reported in Table 12 did not exceed 50%.
Table 12 Percent difference between reported and simulated values for AUC, \(c_{\text{max}}\), \(t_{\text{max}}\), and \(\text{MRT}^{\text{INH}}\)

<table>
<thead>
<tr>
<th>Study</th>
<th>101</th>
<th>102</th>
<th>103</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(\infty)</td>
<td>-18%</td>
<td>-5%</td>
<td>-22%</td>
<td>-15.0%</td>
<td>7.26</td>
</tr>
<tr>
<td>(c_{\text{max}})</td>
<td>4%</td>
<td>-11%</td>
<td>10%</td>
<td>0.9%</td>
<td>8.80</td>
</tr>
<tr>
<td>(t_{\text{max}})</td>
<td>32%</td>
<td>8.7%</td>
<td>32.0%</td>
<td>24.2%</td>
<td>10.98</td>
</tr>
<tr>
<td>(\text{MRT}^{\text{INH}})</td>
<td>-26%</td>
<td>-15%</td>
<td>-4%</td>
<td>-15.0%</td>
<td>8.98</td>
</tr>
</tbody>
</table>

Figure 10 MCS-predicted versus observed \(c_p(t)\) after INH dosing for BUD 308, 309, and 313

3.4.6. Parameter Sensitivity Analysis Results

Sensitivity analysis results were generated for all model parameters (including the device deposition parameters) and are tabulated in Table 13.

AUC and \(c_{\text{max}}\) were most sensitive to \(V_0\) with a 25-fold change in \(V_0\) directly resulting in a 25-fold change in both AUC and \(c_{\text{max}}\). \(k_{10}\) was the second most influential model parameter on AUC, while \(F_{pd}\) was most influential on \(c_{\text{max}}\), as expected. The top three most influential parameters are tabulated in Table 14. Predicted \(c_p(t)\) profile after
the 25-fold change in $F_{pd}$ and $k_{pa}$ is shown in Figure 11, and those for $F_{cd}$ and $k_{ca}$ in Figure 12.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC</th>
<th>$C_{max}$</th>
<th>$t_{max}$</th>
<th>MRT $^{INH}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{in}$</td>
<td>2.4</td>
<td>2.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$F_{ex}$</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$F_{pd}$</td>
<td>5.2</td>
<td>10</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>$F_{cd}$</td>
<td>1.6</td>
<td>1.8</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>$k_{pm}$</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>$k_{cm}$</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>$k_{pa}$</td>
<td>1.1</td>
<td>3.1</td>
<td>9.5</td>
<td>1.2</td>
</tr>
<tr>
<td>$k_{ca}$</td>
<td>1.2</td>
<td>1.4</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>$k_{fa}$</td>
<td>1.0</td>
<td>1.3</td>
<td>1.1</td>
<td>2.1</td>
</tr>
<tr>
<td>$F_{oral}$</td>
<td>2.8</td>
<td>1.3</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>$V_0$</td>
<td>25</td>
<td>25</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>1.0</td>
<td>4.7</td>
<td>3.9</td>
<td>6.2</td>
</tr>
<tr>
<td>$k_{21}$</td>
<td>1.0</td>
<td>1.5</td>
<td>2.4</td>
<td>6.2</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>25</td>
<td>1.8</td>
<td>1.9</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 13 Sensitivity analysis results after INH of a single 2323 nmol dose.

<table>
<thead>
<tr>
<th>Sensitive Parameter</th>
<th>AUC</th>
<th>$C_{max}$</th>
<th>$t_{max}$</th>
<th>MRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>$V_0$</td>
<td>$V_0$</td>
<td>$k_{pa}$</td>
<td>$k_{10}$</td>
</tr>
<tr>
<td>Second</td>
<td>$k_{10}$</td>
<td>$F_{pd}$</td>
<td>$k_{12}$</td>
<td>$k_{12}$</td>
</tr>
<tr>
<td>Third</td>
<td>$F_{pd}$</td>
<td>$k_{21}$</td>
<td>$k_{21}$</td>
<td>$k_{21}$</td>
</tr>
</tbody>
</table>

Table 14 Top three most influential parameters for each of the evaluated exposure metrics and PK parameters
Figure 11 Resulting $c_p(t)$ profile from a 25-fold change in $F_{pd}$ (left plot) and $k_{pa}$ (right plot) after INH of 2323 nmol dose.

Figure 12 Resulting $c_p(t)$ profile from a 25-fold change in $F_{cd}$ (left plot) and $k_{ca}$ (right plot) after INH of 2323 nmole dose.

3.5. Discussion and Conclusions

3.5.1. Study Limitations

Given that BUD was developed a while ago, and the majority of the clinical investigations were performed in the early 1960’s and 1970’s, there was only limited availability of data and poor the reliability of some of the analytical methods that were experimentally used: For the IV studies, BUD given as IV infusion. IV infusion data leads to greater uncertainty in estimating distribution and redistribution rate constants
(k_{12} and k_{21}) in the two-compartment open body model. This is because as those two processes are occurring, the drug is also being infused. As such, it is difficult to accurately determine these parameters unless very frequent plasma samples are collected during both the dosing and distribution phase is. IV bolus studies with frequent early sample collection is considered optimal for determining those two parameters.

Furthermore, no sputum samples were available after any of the dosing routes. The availability of such data would have allowed for more confidence in the pulmonary disposition parameters. Additionally, there was no repeated dosing studies available for IV, PO, or INH. These studies would have been useful in studying drug accumulation in the various compartments as well as time dependence. We were also unable to obtain any data for the pediatric population. This is notable since they may often be the targeted population for this treatment. The lack of access to patient level data (both plasma and sputum) limited the ability to study the possible effects of covariate on both plasma and sputum exposure.

Lastly, the INH data available was produced from the same device across the four studies. Additional data for various devices including pressurized inhalers and nebulizers would have been useful in studying the effect of device of plasma and sputum exposure.

3.5.2. PK Dose-Proportionality

The dose dependence plots after IV infusion shown in Figures 5 support linear PK based on the high r^2 value for the linear model. Additionally, when the same data were plotted on a log-log scale (shown in the same figure), and a power model was
utilized for fitting the data, the coefficient of the exponential coefficient is close to 1.0 which also indicates linear PK. This finding is in agreement with the literature as several authors have also reported that BUD experience’s linear PK across a similar dose range. These pieces of evidence provided a strong basis for the major modeling assumption, namely that all rate constants were first-order rate constants.

3.5.3. Final Parameter Space after IV Administration

An empiric open body two compartment model of BUD with an optimized model parameter space resulted in mechanistically plausible parameter estimates and was able to adequately predict the reported \( c_p(t) \) profiles from six clinical PK studies with HV after single IV doses.

Model-optimized values indicated that are consistent with that BUD undergoes high hepatic clearance as its clearance value \((k_{10}^*V_0 = 1170 \text{ ml/min})\) approaches hepatic blood flow (1200 ml/min). This results in a short \( \beta \)-half-life \((t_{\beta}^{k_{10}} = 27 \text{ min})\). The peripheral tissue compartment is a shallow compartment \((\frac{k_{21}}{k_{12}} = 0.26)\) with intermediate capacity \((\frac{k_{21}}{k_{12}} = 1.2)\). The model indicated that BUD is widely distributed throughout the body with a \( V_{\text{dss}} \) of 190 L. This is likely due to its lipophilicity as mentioned in the introduction of this chapter. All systemic disposition parameter point estimates are consistent with published values.

3.5.4. Final Parameter Space after PO Administration
The final optimized oral absorption model and parameters for BUD suggest that BU has a low $F_{oral}$ of 10% that is due to extensive hepatic first pass metabolism. Additionally, its oral absorption was noted to be slow with an oral absorption half-life of 95 minutes. As such, BUD may experience “flip-flop” PK in, ie, the rate constant of absorption ($k_{ga}$) maybe slower than the rate constant of elimination of BUD ($k_{10}$).

Some authors have already demonstrated that this slow absorption rate is due to the absorption of BUD throughout the entire GI tract, and therefore the absorption rate may be affected by GI motility.\textsuperscript{10}

### 3.5.5. Final INH Model

The final INH model incorporated the two-compartment systemic disposition model, the oral absorption model, and two pulmonary compartments. This is Model #1 as described in chapter two. The selection of this model was based on the lack availability of sputum data, specifically, after IV administration. The model allowed for (unidirectional) absorption from the lungs (central and peripheral) to the central compartment through $k_{pa}$ and $k_{ca}$, however, the model did not allow redistribution from the central compartment back to the lungs due to the absence of any evidence. Mucociliary clearance was possible from both the peripheral and the central lung into the central lung and the GI tract compartments, respectively.

The explicit solution for each of the five compartments in the model were derived. This solution for the central compartment indicated that the four hybrid rate constants are calculated from model micro rate constants. Five intercepts incorporate dose,
deposition fractions and ratios of rate constants and had a sum of zero (i.e., some are negative).

3.5.6. Final Parameter Space after Inhalation

The final parameter set (listed in Table 15) that was optimized using the available inhalation data, was able to adequately describe the available cp(t) profiles as well as the exposure metric. The final, optimized PBPK model parameters (point estimates) indicate a small fraction exhaled from Turbuhaler (F_{ex} = 0.9%) as well as 3:1 relative peripheral-to-central pulmonary deposition (F_{pd} = 21% vs. F_{cd} = 7%) with an absorption t_{1/2} of 11 min for k_{pa} and 21 min for k_{ca}, preferential absorption from peripheral vs central compartment, respectively. Model optimization during MCS required addition of parameter variability, ranging from to 9% to 57% (CV), primarily in pulmonary deposition parameters. Furthermore, mucociliary clearance from the central lung was twice as fast as from the peripheral lung. However, due to the lack of sputum data, the ability to accurately resolve these parameters was limited.
Table 15 Final (optimized) model parameters, point and variability estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Point Estimate</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{in}$</td>
<td>14%</td>
<td>9%</td>
</tr>
<tr>
<td>$F_{ex}$</td>
<td>0.9%</td>
<td>57%</td>
</tr>
<tr>
<td>$F_{pd}$</td>
<td>21%</td>
<td>30%</td>
</tr>
<tr>
<td>$F_{cd}$</td>
<td>6.9%</td>
<td>30%</td>
</tr>
<tr>
<td>$k_{pm}$ (min$^{-1}$)</td>
<td>0.0078</td>
<td>30%</td>
</tr>
<tr>
<td>$k_{cm}$ (min$^{-1}$)</td>
<td>0.016</td>
<td>30%</td>
</tr>
<tr>
<td>$k_{pa}$ (min$^{-1}$)</td>
<td>0.066</td>
<td>30%</td>
</tr>
<tr>
<td>$k_{ra}$ (min$^{-1}$)</td>
<td>0.033</td>
<td>30%</td>
</tr>
<tr>
<td>$k_{ga}$ (min$^{-1}$)</td>
<td>0.0073</td>
<td>40%</td>
</tr>
<tr>
<td>$F_{oral}$</td>
<td>10%</td>
<td>40%</td>
</tr>
<tr>
<td>$V_0$ (L)</td>
<td>45</td>
<td>20%</td>
</tr>
<tr>
<td>$k_{12}$ (min$^{-1}$)</td>
<td>0.100</td>
<td>20%</td>
</tr>
<tr>
<td>$k_{21}$ (min$^{-1}$)</td>
<td>0.031</td>
<td>20%</td>
</tr>
<tr>
<td>$k_{10}$ (min$^{-1}$)</td>
<td>0.026</td>
<td>20%</td>
</tr>
</tbody>
</table>

3.5.7. Sensitivity Analysis

The sensitivity analysis indicated that AUC depends primarily on $V_0$, $k_{10}$, and $F_{pd}$; $c_{max}$ on $V_0$, $F_{pd}$, and $k_{12}$; $t_{max}$ on $k_{pa}$, $k_{12}$, and $k_{21}$; and MRT on $k_{10}$, $k_{12}$, and $k_{21}$. While the results of the sensitivity analysis allowed for a numerical comparison between each of the model parameters on the various exposure metrics, there appeared to be an apparent overarching theme. After inhalation, the model appears to be most sensitive to the systemic disposition parameters and least sensitive to the oral absorption.
parameters within the BUD model parameter space. None of the pulmonary disposition parameters were found to be important for plasma exposure.

3.5.8. Overall Conclusions

The final semi-PBPK model incorporated published *in-vitro* and *in-vivo* information and established a model parameter space for BUD that gave acceptable predictions and mechanistically plausible parameter estimates. Pulmonary absorption parameters ($k_{pa}$ and $k_{ca}$) were the most uncertain estimates. Most of the model parameter variability was associated with pulmonary deposition parameters. Overall, plasma exposure metrics for BUD after inhalation are more sensitive to differences in systemic disposition rather than pulmonary deposition/absorption or GI absorption.
CHAPTER 4

SEMI-PBPK MODELING OF INHALED TOBRAMYCIN

4.1. Background

4.1.1. Background on Cystic Fibrosis demographics, pathophysiology, effects on pulmonary functioning

Cystic Fibrosis (CF) diagnoses are increasing at a rate of 1000 new cases per year and over 70,000 reported worldwide which are caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene. While there are over 400 reported gene mutations that cause the disease in these patients, pulmonary (PUL) infections leading to exacerbation of the disease symptoms have been reported as the leading cause of death in CF patients and the leading cause for a shortened average life span of only 37 years. Irrespective of the patient’s disease severity classification, the most common pulmonary infection that leads to exacerbations is Pseudomonas aeruginosa, which is a gram-negative bacteria infection. This gram-negative infection occurs because the mutated CFTR gene leads to the accumulation of chloride ions within the cell, which results in the accumulation of sodium ions. Therefore, water
content increases intracellularly and decreases extracellularly. The decreased extracellular concentration of water leads to a thickening of the mucus in the intestine (leading to malnutrition), and in the lungs, resulting in the aforementioned potentially fatal infection. Patients are spending annually on average $29,000 for hospitalization related costs, and an additional $20,000 on prescription medication for treatment. Despite this spending, patient prognosis is still relatively bleak, with 30% of children and 47% of adults being hospitalized at least once for exacerbations of lung infections reported in 2007. As a result of these incidents, most pharmaceutical treatments thus far, have been aimed at symptom relief and treatment of these lung infections to prevent the aforementioned fatal pulmonary infections.

4.1.2. Treatments, IV and INH, MOA, efficacy/toxicity

Antibiotics, steroids, bronchodilators, inhaled hypertonic saline solution, oxygen therapy, mucolytic agents (Dornase Alfa), and lung transplants (for patients in which the disease is more advanced), are the current treatment options for CF pulmonary exacerbations, with administration of antibiotics being the most widely used method. According to the Cystic Fibrosis Foundation, some of the current classes of antibiotics that are clinically used include: penicillins, cephalosporins, aminoglycosides (AGs), macrolides, tetracyclines, quinolones, and aztreonam.

While their exact mechanism of action is not fully understood, AGs are known to prevent sensitive bacteria from synthesizing proteins vital for growth, by disrupting the ribosomal mRNA reading process. AGs are also responsible for creating fissures in the outer membranes of bacterial cells. Given this dual mechanism of action, AGs are
considered to be potent antibiotics that exhibit strong bactericidal activity against aerobic gram-negative and certain gram-positive bacteria.\textsuperscript{59}

Due to their strong bactericidal effects against gram-negative bacteria, AGs are often used for lung infections in CF. Patients often require several courses of IV antibiotics per year to combat these infections. Typically, these treatments require that a certain concentration be achieved such that concentration-dependent killing of bacterial cells can be accomplished. As a consequence of antibiotic use, the survival of patients with CF has improved, but the side effects of treatments have become increasingly important. A number of studies have extensively discussed the systemic toxicity of AGs (namely ototoxicity and nephrotoxicity).\textsuperscript{12,60,61} AGs can accumulate in the proximal tubule epithelial cells (either in the cytoplasm or in the endoplasmic reticulum) within the kidney where they can activate the intrinsic pathways leading to apoptosis.\textsuperscript{62} The apoptotic cascade can also be initiated within the hair cells of the inner ear by free radicals (specifically oxygen and nitrogen free radical species) which have been documented to lead to permanent hearing loss, i.e. ototoxicity.\textsuperscript{63}

It has also been reported that IV administered AGs have poor penetration into the lung, reaching mean peak sputum concentrations that are only 12\% to 20\% of the peak serum concentrations. Hence there is a desire to shift from IV administration to inhalation (INH) as this is likely to increase the pulmonary local concentrations while minimizing systemic exposures.\textsuperscript{64–66} While most of the aforementioned classes of antibiotics have IV dosage forms, few have oral (PO) dosage forms, and even fewer have orally inhaled dosage forms. Only AGs (tobramycin (TOB), gentamycin (GEN), and amikacin), Quinolones (ciprofloxacin, and levofloxacin), and aztreonam are
available for both IV and INH administration. Among the AGs, TOB has been reported to be the most active against isolated *Pseudomonas aeruginosa* with an MIC$_{50}$ of 1µg/ml and a MIC$_{90}$ of 8µg/ml. In addition to its efficacy against *Pseudomonas aeruginosa*, it was one of the earliest antibiotics (of those available for inhalation) introduced for clinical use. Accordingly, TOB has the most available data sets across the literature.

### 4.1.3. Relevant TOB-Specific Physiochemical Properties

Tobramycin is a low molecular weight compound with a molar mass of 468 grams per mole and a chemical formula of C$_{18}$H$_{37}$N$_{5}$O$_{9}$. Based on its chemical structure depicted in Figure 13 below, it has an experimentally determined partition-coefficient log(P) value of -7.3, indicating that it is a hydrophilic compound that is very soluble in water (according to the USP definition) with a solubility of 1000 mg/ml. Accordingly, it has been shown to have low permeability across Caco-2 cell models with an apparent permeability of $P_{\text{app}} = 1.78 \cdot 10^{-6}$ cm/sec, at a tested concentration of 5 mg/ml. Additionally, at a tested concentration of 100 µM, only 32% of the initial concentration was detected in the basolateral compartment, hence a $P_{\text{app}}$ could not be calculated for a Calu-3 cell model, but was assumed to be low. $P_{\text{app}}$ values for Caco-2 and Calu-3 cell models are used to gauge permeability in the GI tract and lungs, respectively. This is likely due to both its hydrophilic structure, as well as its likely polycationic charge within the physiological pH range. TOB is known to be stable at a pH range from 1-11 at temperatures ranging from 5 to 37°C.
4.1.4. Summary of ADME of TOB, IV and INH

While TOB has not been identified as a substrate for any major metabolic pathways in mice after IV administration, its oral bioavailability has been reported as negligible, primarily due to this low GI permeability, and not due to a first-pass effect.$^{14}$ Due to its hydrophilic nature, TOB is primarily eliminated by the kidneys by glomerular filtration, with 60% of the dose showing up unchanged in urine within 6 hours, and by 24 hours 85% has been recovered in urine.$^{72}$ Some authors have suggested that up to 10% of the IV administered dose could be eliminated via extrarenal mechanism such as elimination in the lung or elimination via other non-renal pathways.$^{73}$ Another plausible explanation for the remaining 15% that is not recovered in urine at the 24-hour sampling time point, is that the drug may still be distributed in tissues throughout the body.

It is known that TOB has negligible plasma protein binding, with a reported binding that is not statistically different from zero when tested under controlled conditions of physiological pH, and temperature, by means of an ultrafiltration method in human serum.$^{74}$ When a dose of 60mg/m$^2$ was administered IV in CF patients, the authors reported a volume of distribution of 0.30 ± 0.5 L/kg, and a $\text{CL}_{\text{tot}}$ value of 4.9 ±
2.1 L/h/1.73m². Similarly, in HV with a similar dose of 55 mg/m², the reported values were 0.23 ± 0.07 L/kg and 6.12 ± 1.17 L/h/1.73m², respectively.\textsuperscript{75} After 6 CF patients received a 600mg dose via an ultrasonic nebulizer, the estimated apparent volume (Vd\textsubscript{ss}/F\textsubscript{oral}) was higher than that after IV dosing at 1.74 ± 0.96 L/kg and the apparent clearance (CL\textsubscript{tot}/F\textsubscript{oral}) values were reported to be 6.98 ± 2.89 L/h.\textsuperscript{76}

PK parameter estimation has been noted to suffer from larger standard deviations, as there are numerous sources or variability that are difficult to regulate including but not limited to: device efficiency, inhalation maneuver technique, and lung function of study subjects.

**4.1.5. Summary of Literature Effect of CF on ADME, Focus on TOB**

Gastrointestinal absorption of TOB is known to be negligible due to its aforementioned hydrophilic nature, hence, there is unlikely to be detectable difference in GI absorption between HV and CF patients.\textsuperscript{57}

Pulmonary absorption on the other hand has been noted as highly variable; thus while there may exist a difference in pulmonary absorption between HV and CF patients, there has not been conclusive evidence of this presented in the literature.\textsuperscript{76}

However, compared to healthy volunteers, CF patients have been documented to have higher volumes of distribution and higher CL\textsubscript{tot}.\textsuperscript{77} Some authors have noted that the higher volumes of distribution in CF patients is secondary to their state of malnutrition and reduced levels of adipose tissue. The decrease in adipose tissue leads to a higher percentage of lean body mass, potentially explaining the larger Vd.\textsuperscript{78,79} CF patients have also been noted to suffer from hypoxemia (low concentration of oxygen in blood)
which leads to erythrocytosis (increase in red blood cells). The increase in red blood cells ultimately leads to an increase in blood volume which may be the cause for the increased volume of distribution for TOB.\textsuperscript{80}

Some investigators have theorized that the increase in CL\textsubscript{tot} in CF patients is due to their enlarged kidneys, which leads to glomerulomegaly (abnormal enlargement of glomeruli) which causes hyperfiltration. This hyperfiltration results in an increase in glomerular filtration rate and an increased urine flow, thereby increasing the renal clearance of TOB.\textsuperscript{77,81} Others, however, have speculated that in an effort to compensate for the CFTR defect, tubular cells have higher activity of other types of cellular membrane channels or increased paracellular diffusion.\textsuperscript{64} Both of these phenomena would result in higher CL\textsubscript{tot} of TOB. Finally, the contribution of clearance from the lung has also been noted as a possible explanation for the increased total clearance in CF patients, as they are known to have increased sputum production.\textsuperscript{82}

4.2. Objectives

1) Collect available \( c_p(t) \) and \( c_s(t) \) and exposure metrics for TOB after IV, and INH administration to HV and CF patients in addition to all relevant dosing information such as dose, infusion time, fraction of the dose left in the inhaler, and the dose deposited into lung.

2) Develop a structural semi-PBPK model with a nested covariate model(s) to describe sputum and plasma concentrations of TOB, after IV administration and INH in both HV and CF patients.
3) Validate the model developed for specific aim 3.3.2 with the collected data from aim.

4) Assess the sensitivity sputum and plasma exposure to each of model parameters.

5) Assess pulmonary bioavailability ($F_{pul}$) after INH administration of TOB.

4.3. Methods

4.3.1. PK Data collection

A systematic review of literature to identify studies where TOB was administered IV or was INH. Studies were included if: (1) study population size and covariates (body weight and disease state) were clearly identified, (2) either individual or mean level plasma concentration time ($c_p(t)$) profile or PK exposure metrics were reported, (3) the analytical method was deemed to be reliable (specifically, that the observed $c_p$ are larger than Lower Limit of Quantification (LLOQ)), and (4) the sampling schedule was appropriate to capture drug disposition. Optimal studies will also have reported data regarding sputum concentrations at known time points ($c_s(t)$) or sputum exposure metrics, however this was not necessary for the incorporation of a study into the analysis. When available, the central tendency and its dispersion or variability of any reported exposure metrics were also collected. Additionally, for INH studies, scintigraphy data describing the percentage of the dose that was placed in the inhalation device that ultimately reached the lungs (Dose to Lung or DtL) was also sought after from the identified studies for the tested inhalation devices or from
references studies that provided this information for the devices of interest. This data collection would directly satisfy specific aim 3.3.1

4.3.2. Digitization and Non-compartmental PK analysis

For the included studies, captured $c_p(t)$ and $c_s(t)$ profiles were digitized using the GetData© Graph Digitizer Version 2.24 software. A non-compartmental analysis (NCA) was performed on the plasma concentration data.

4.3.3. SEMI-PBPK Models Tested (Models 1 – 3)

The three semi-PBPK models schematically depicted in chapter 2 (Figure 1 - Model 1, Figures 2 - Model 2, and Figures 3 - Model 3) were tested to determine which model best described the collected data from the IV and INH studies sequentially. As depicted below, model 1 assumes (1) that equilibration of TOB within a given compartment is much faster than equilibration between compartments, (2) linear PK (all rate constants describe non-saturable non-inducible first order processes), and (3) that drug elimination only occurs through the central compartment. Model 1 was utilized to determine the systemic disposition parameters by fitting model parameters to the four IV data sets (from three studies) that did not contain sputum data. A single repeated dosing IV study was identified that contained sputum data that was utilized for obtaining model parameter estimates. Due to the presence of detectable sputum levels after IV administration, Model 1 could not be utilized for further investigation, as it did not allow for the distribution of TOB from the central compartment to the pulmonary compartments. While the introduction of the distribution pathway from the central compartment to the pulmonary compartments in Models 2 and 3 allows the models to
describe sputum levels after IV administration, it violates the third assumption listed above in that the drug is administered systemically, can now be eliminated through the GI tract after distribution to the pulmonary compartments in addition to clearance through the central compartment. These distribution pathways however, allow for the simulation of sputum concentrations after IV dosing which satisfies the model requirements listed in specific aim 3.3.2.

With the systemic disposition parameters fixed (as the average of the four individual fits generated with model 1), an attempt was made to fit pulmonary disposition parameters from model 2 and model 3 using Scientist 3.0 utilizing both the method of least-squares as well as simplex fit, to the IV data set that included both plasma and sputum values. The software however was unable to converge on values for the six or ten parameters that were to be estimated from model 2 and model 3 respectively. To minimize the number of parameters that needed to coverage while satisfying the least square optimization algorithm, mucociliary clearance \(k_{pm}\), and \(k_{cm}\) values were fixed to values estimated from a previous work, and a second attempt to estimate the remaining four or eight parameters from model 2 and 3 respectively. Although various initial estimates for the model parameters were tested, this approach did not lead to the software converging on a parameter set. Since the standard least square optimization algorithms technique did not lead to a final parameter space, the Monte Carlo Simulation approach described below was utilized to obtain the final parameter estimates.

In model 3, TOB was dosed into the central compartment (CC) of a two-compartment open body model with volume of distribution \(V_0\), distribution and
redistribution rate constants $k_{12}$ and $k_{21}$, and total clearance $CL_{tot}$. Both $V_0$ and $CL_{tot}$ were dependent on body weight power covariate models that were centered around the population body weight for either healthy or CF patients as shown in Equation 9 and 10. The lung was modeled with four compartments: (1) peripheral lung unbound (PLU), (2) peripheral lung sequestered (PLS), (3) central lung unbound (CLU), and (4) central lung sequestered (CLS). The CC distributes drug to both PLS and CLS via $k_{pls1}$ and $k_{cls1}$ and the drug redistributes back to CC via $k_{pls2}$ and $k_{cls2}$ respectively. PLS and CLS are also each connected to PLU and CLU via distribution ($k_{pa}$ and $k_{ca}$) and redistribution rate constants ($k_{pd}$, and $k_{cd}$). The PLU connects to the CLU via mucociliary clearance ($k_{pm}$), and CLU is connected to the GI compartment via mucociliary clearance/swallowing ($k_{cm}$). CLU and PLU were characterized with a total lung volume (TLV) which followed the covariate model expressed in Equation 11.

$$CL_{i_{tot}} = CL_{pop} * \left( \frac{BW_i}{BW_{pop}} \right)^{0.75} * \varepsilon_{i_{CLtot}}$$

Equation 9 Total clearance covariate model for the $i^{th}$ patient.

$$V_0^i = V_{0_{pop}} * \left( \frac{BW_i}{BW_{pop}} \right) * \varepsilon_{i_{V0}}$$

Equation 10. Volume of the central compartment covariate model for the $i^{th}$ patient.

$$TLV^i = TLV_{pop} * \left( \frac{BW_i}{BW_{pop}} \right) * \varepsilon_{i_{TLV}}$$

Equation 11. Total volume of the lung covariate model for the $i^{th}$ patient.
4.3.4. Monte Carlo Simulation MATCHING/ACCEPTABILITY criteria

Monte-Carlo simulations (MCS) were then performed with model 2 and model 3 to determine if there existed an optimal parameter space to describe all the collected IV data (both plasma and sputum). Parameter point and variability estimates were optimized using the MCS simulation by simultaneously (1) attempting to minimize the sum of the residual error for both plasma and sputum concentrations, (2) performing a visual predictive check (VPC, 5% - 95% percentiles) relative to reported $c_p(t)$ and $c_s(t)$ profiles, and (3) matching model-predicted with observed (mean) $c_p(t)$ profiles and exposure metrics. For the final IV parameter space to be accepted, the percent difference between the observed plasma and sputum exposure metrics and the simulated exposure metrics had to be less than $\pm 40\%$ for IV. Due to the increased level of variability associated with INH, the plasma exposure metrics had to be less than $\pm 45\%$ for AUC and 75% for $c_{\text{max}}$, and the sputum exposure metrics had to be less then $\pm 120\%$ for both AUC and $c_{\text{max}}$. The MCS were performed using log-normal distributions for each parameter, and initial estimates for parameter optimization were obtained from a previous study modeling BUD in HV administered via IV, PO, and oral inhalation. The number of subjects to simulate ($n$) for the MCS was determined by comparing various simulations, and selecting the lowest $n$ (to minimize computational time) while ensuring the observed variability did not appear to be minimized due to a lower value of $n$. This MCS approach served as the validation step required to address specific aim 3.3.3. MCS were performed in Rstudio Version 1.0.136 with the deSOLVE package add-in.
An attempt was made to utilize model 3 and the final optimized parameters from the IV data sets to predict $c_p(t)$ and $c_s(t)$ from INH studies (approach #1). Additionally, we attempted to identify a universal parameter space that was capable of describing both the IV and INH data sets by re-optimizing the parameters to both the IV and INH data sets simultaneously (approach #2). Given that there was no identifiable parameter space that was capable of describing all the available IV and INH data sets simultaneously, the parameter optimization was then performed solely on the available INH data sets. Optimization was performed by attempting to minimize the sum of the residual error for both plasma and sputum concentrations by performing a visual predictive check (VPC, 5%-95% percentiles) relative to reported $c_p(t)$ and $c_s(t)$ profiles, and match model-predicted with observed (mean) $c_p(t)$ profiles and exposure metrics for a single inhalation study. The optimized parameters were then utilized to predict the reported $c_p(t)$ and $c_s(t)$ as well as the exposure metrics of the three remaining studies. To be acceptable, predictions were to be within the aforementioned VPC criteria and not show any systematic bias of over-prediction or under-prediction (approach #3). Next, parameter optimization was performed using only the data sets from each of the four INH studies where the Pari nebulizer was used as the inhalation device. Parameter estimates were deemed acceptable once the predicted plasma exposure metrics were within 40% of the reported exposure metrics for the four data sets. Once the parameter space was identified, predictions of the remaining inhalation data sets (where other inhalation devices were utilized) were generated and evaluated for a systematic bias relative to the reported exposure metrics in addition to the evaluation of the VPC.
(approach #4). Lastly, the sixteen sets of data including the all available $c_p(t)$ profiles, $c_s(t)$ profiles, plasma exposure metrics, and sputum exposure metrics from the four selected inhalation studies were all simultaneously utilized for validation. In this fifth approach, results were checked to ensure that there was no consistent over or underprediction (bias) for any of the sub-group of data (HV vs CF, a specific inhalation device, a given dose, or a specific study).

4.3.5. Sensitivity analysis

As an indicator of how sensitive plasma and sputum levels are to the model parameters, a sensitivity analysis was performed with the final parameter estimates for both the IV and INH parameters for model 3. Variability across the parameters was disabled by setting all the CV’s to zero, parameter values were set to either one-fifth or five times the mean optimized value, and simulations were performed to the 24-hour timepoint. AUC and $c_{\text{max}}$ values in plasma and sputum were calculated at each of the extremes. The fold change was calculated as the ratio between the maximum and minimum values of the simulated plasma AUC multiplied by a negative one if the maximum value was obtained from when the parameter being tested was multiplied by 1/5. This was also calculated for plasma $c_{\text{max}}$, as well as sputum AUC and $c_{\text{max}}$. Both $c_p(t)$, and $c_s(t)$ profiles were generated for comparison. The top three parameters to cause the largest fold change in the four exposure metrics were determined at the end of the sensitivity analysis. This sensitivity analysis would then allow for a better understanding as to how to increase sputum concentrations or how to minimize systemic exposure as well as specifically address specific aim 3.3.4.

4.3.6. Mass balance and overall excretion simulations
The cumulative drug eliminated via each pathway (CL_{tot}, and k_{cm} (given that TOB has no oral bioavailability)) was investigated after IV administration and after INH. These mass balance excretion simulations were performed to \( t = 4 \) days with a resolution of 1 hour. Additionally, \( F_{inh} \) (fraction of the drug that shows up in systemic circulation after inhalation) will be calculated for each of the inhalation studies using the reported dose and AUC as well as the reported dose and AUC from IV study TOB 103A. This was performed to aid in estimating pulmonary bioavailability and to address specific aim 3.3.5.

4.4. Results

4.4.1. Summary and Discussion of final IV and INH studies identified

A search of the literature on PubMed and Google Scholar resulted in the identification of 8 publications where TOB was administered as an IV infusion. These publications and their respective key study design elements are listed in Table 16.\(^{10,11,73,84–88}\) Due to study design flaws, studies TOB 105 – 108 were excluded from the data analysis. Some of these studies did not have a sufficiently low enough LLOQ to capture the \( c_p(t) \) reliably, others did not have an appropriate sampling scheme that permitted any type of data analysis, and others did not report a \( c_p(t) \) or a \( c_s(t) \) profile. For the purpose of the following analysis, only studies TOB 101 – TOB 104 were utilized for analysis. These studies were characterized by infusion doses ranging from 1.5 mg/kg to 10 mg/kg administered to both healthy subjects and CF patients. The authors in TOB 103 investigated two dosing regiments; 3.3 mg/kg administered as a 30-minute infusion every 8 hours (TOB103A), and 10 mg/kg infused over 60 minutes every 24 hours
(TOB103B). TOB 104 was a unique study in that it provided both \( c_p(t) \) and \( c_s(t) \) profiles after 8 mg/kg repeat dosing.

A total of 10 studies were identified where TOB was inhaled in either HV or CF patients. These studies and their key study design elements, such as number of subjects (\( n \)), mean body weight, mean age, etc., are listed in Table 17.\(^{76,89–97}\) Additionally, the availability of \( c_p(t) \), \( c_s(t) \), exposure metrics for either plasma or sputum, and availability of deposition data is also tabulated. Of the ten identified studies, six studies were not suitable for analysis either for similar reasons as those excluded among the IV studies or due to lack of pulmonary deposition data that was necessary to perform the modeling described above. A summary of the four INH studies that were included in the analysis is reported in Table 17 along with the reported exposure metrics available for plasma and sputum data. The four studies were all single dose studies testing doses ranging from 28 mg to 300 mg that were inhaled through the Pari nebulizer, AeroDose inhaler, PulmoSphere inhaler, or the eFlow nebulizer in HV and CF patients.
Eight identified studies wherein TOB was administered IV. Tabulated key study design elements: year of publication, number of subjects, subject disease state (Healthy, CF – cystic Fibrosis, or other), dose strength and regiment, analytical method, sampling schedule, age (yrs), body weight, availability of cp(t) profile, and the availability of cs(t) profile.

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>Subject Disease State</th>
<th>Dose Strength</th>
<th>Assay method</th>
<th>PK Sampling</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Cp(t) Profile</th>
<th>Cs(t) Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>11</td>
<td>Healthy</td>
<td>1.5 mg/kg as a 30 min infusion</td>
<td>FPI</td>
<td>8, 12, 21, 30, 35, 40, 45 min, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8 hrs</td>
<td>22.9 ± 0.7 (22 - 24)</td>
<td>79.3 ± 9.1 (60 - 90)</td>
<td>✔</td>
<td>x</td>
</tr>
<tr>
<td>1995</td>
<td>6</td>
<td>Healthy</td>
<td>2 mg/kg as either 30 minute infusion</td>
<td>FPI</td>
<td>0, 10, 20, 30, 45 min, 1, 1.25, 1.5, 2, 3, 4, 6 hrs</td>
<td>29 ± 4.6 (23 - 34)</td>
<td>55.3 ± 13.8 (45 - 75)</td>
<td>✔</td>
<td>x</td>
</tr>
<tr>
<td>2002</td>
<td>6</td>
<td>CF (CrCl ~127 ml/ml)</td>
<td>3.3 mg/kg q8h or 10 mg/kg q24h as 30 or 60 min infusions</td>
<td>FPI</td>
<td>0, 10, 20, 30, 45 min, 1, 1.25, 1.5, 2, 3, 4, 6 hrs</td>
<td>30.3 (18 - 50)</td>
<td>57.4 (36 - 88)</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>2007</td>
<td>14</td>
<td>CF</td>
<td>8 mg/kg once daily as a 10 minute infusion</td>
<td>HPLC</td>
<td>0.5, 1, 4, 6, 8</td>
<td>22 - 67</td>
<td>66 - 78.5</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>1981</td>
<td>7</td>
<td>Healthy</td>
<td>Bolus: 1 mg/kg, infusion: 2 - 3.5 mg/kg for 8 hours</td>
<td>Antibiotic Activity</td>
<td>5, 7</td>
<td>60</td>
<td>50 - 80</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>1972</td>
<td>4</td>
<td>Healthy (CrCl &gt; 80 ml/ml)</td>
<td>100 mg as 60 min infusion + 30 mg as 2 hour infusion</td>
<td>Antibiotic Activity</td>
<td>0, 1, 1.5, 2, 2.5, 3, 3.5, 4 hrs</td>
<td>64.5 (33 - 87)</td>
<td>64.5 (33 - 87)</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>1976</td>
<td>16</td>
<td>Healthy + Other</td>
<td>1 mg/kg as a 2 minute infusion</td>
<td>Antibiotic Activity</td>
<td>0, 1, 2, 5, 10, 20, 30 min, 1, 2, 4, 5 hrs</td>
<td>30</td>
<td>30</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>1976</td>
<td>30</td>
<td>Healthy + Other</td>
<td>80 mg and 1.5 mg/kg as a bolus injection (15 sec)</td>
<td>Antibiotic Activity</td>
<td>0, 1, 2.25, 2.5, 3, 4, 5, 6, 7, 8, 9 hrs</td>
<td>64</td>
<td>64</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Table 16: Identified TOB IV studies
Eight identified studies wherein TOB INH was used. Tabulated key study design elements: year of publication, number of subjects (n), body weight (kg), age (yrs), number of male participants, subject disease state (Healthy, CF – Cystic Fibrosis, or other), analytical method (LLOQ if reported), dose strength and regimen, inhalation device, availability of *Cp(t)* profile, and availability of *Cs(t)* profile.

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>Body Weight</th>
<th>Age</th>
<th>Male</th>
<th>H - CF - O</th>
<th>Analytical Method (LLOQ)</th>
<th>Dose</th>
<th>Device</th>
<th>Cp(t)</th>
<th>Cs(t)</th>
<th>Deposition</th>
<th>Exposure Metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOB 301 1997</td>
<td>6</td>
<td>57.5</td>
<td>25</td>
<td>4</td>
<td>CF</td>
<td>FPI (0.025 mg/L)</td>
<td>600 mg</td>
<td>Ultrasonic nebulizer (Wisto Senior)</td>
<td>✓</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>TOB 302 2011</td>
<td>6/7</td>
<td>--</td>
<td>33/21</td>
<td>6/4</td>
<td>H/CF</td>
<td>FPI</td>
<td>300 mg</td>
<td>Pari LC Plus / eFlow Nebuliser</td>
<td>X</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>TOB 303 2003</td>
<td>12</td>
<td>--</td>
<td>34</td>
<td>10</td>
<td>H</td>
<td>FPI (0.05 mg/L)</td>
<td>80 / 300 mg</td>
<td>PulmoSphere / TOBI</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>TOB 304 1997</td>
<td>60</td>
<td>--</td>
<td>21.5</td>
<td>32</td>
<td>CF</td>
<td>HPLC</td>
<td>600/300/300</td>
<td>UltraNeb / Sidestream/Pari-LC</td>
<td>~</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>TOB 305 1993</td>
<td>10</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>H/O/O</td>
<td>Immunoenzymatic (0.3 mg/L)</td>
<td>300</td>
<td>--</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>X</td>
</tr>
<tr>
<td>TOB 306 2002</td>
<td>258</td>
<td>--</td>
<td>21</td>
<td>149</td>
<td>CF</td>
<td>FPI (0.18 mg/L)</td>
<td>300 mg /BID</td>
<td>Jet nebulizer (PARI LC PLUS)</td>
<td>~</td>
<td>~</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>TOB 307 2003</td>
<td>49</td>
<td>64/53</td>
<td>24</td>
<td>19</td>
<td>CF</td>
<td>FPI (0.18 mg/L)</td>
<td>30/60/90/300</td>
<td>AeroDose / PARI LC PLUS</td>
<td>✓</td>
<td>✓</td>
<td>~</td>
<td>✓</td>
</tr>
<tr>
<td>TOB 308 1995</td>
<td>24</td>
<td>--</td>
<td>17</td>
<td>18</td>
<td>CF</td>
<td>Radioimmunoassay</td>
<td>666</td>
<td>DeVibis UltraNeb 99/100</td>
<td>✓</td>
<td>X</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>TOB 309 2007</td>
<td>84</td>
<td>7-50</td>
<td>43</td>
<td>CF</td>
<td>FPI (0.05 mg/L)</td>
<td>28/56/56/84/112 /300 mg</td>
<td>T-326 DPI / PARI LC PLUS</td>
<td>~</td>
<td>~</td>
<td>X</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>TOB 310 1989</td>
<td>20</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>O</td>
<td>Enzyme Immunoassay (0.1mg/L)</td>
<td>80 mg</td>
<td>Jet nebulizer</td>
<td>X</td>
<td>~</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Table 17: Identified TOB INH studies
4.4.2. Dose Linearity for IV and INH studies

The NCA results of the \(c_p(t)\) profiles from TOB 101 – TOB 104 are shown in Table 18. The estimated half-lives (\(t_{1/2}\)) for the hour studies ranged from 125 minutes to 167 minutes which was in line with reported literature values. This short half-life is known to be a consequence of the small volume of distribution which was between 16 and 18 liters in the NCA, and clearance values approaching glomerular filtration which range from 78 to 105 ml/min in the NCA performed in this study. When the administered dose was corrected for bodyweight (BW), there did not appear to be a difference in \(CL_{tot}\) or \(Vd_{ss}\) between healthy and CF subjects as shown in Figure 14. Given that there is no difference between CF patients and HV, the data from these studies can be pooled for performing a dose dependence study. When comparing \(CL_{tot}\) and \(Vd_{ss}\) as the nominal dose is increased from 1.5 mg/kg to 10 mg/kg, there is no evidence to support non-linear PK after IV infusion dosing within the tested dose range as shown in Figure 15. This is further supported by the dose escalation graph with dose (on the x-axis) plotted against AUC (on the y-axis) which had a linear model fit \(r^2\) of 0.9998 (Figure 16) and the Log-Log plot where the exponent in the power model is 1.0898 (Figure 17). These

<table>
<thead>
<tr>
<th></th>
<th>TOB 101</th>
<th>TOB 102</th>
<th>TOB 103A</th>
<th>TOB 103B</th>
<th>TOB 104</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CL_{tot}) (mL/min)</td>
<td>105</td>
<td>96</td>
<td>94</td>
<td>89</td>
<td>78</td>
</tr>
<tr>
<td>BW Normalized (CL_{tot}) (mL/min/kg)</td>
<td>1.3</td>
<td>1.2</td>
<td>1.7</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>(Vd_{ss}) (Liter)</td>
<td>18</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>BW Normalized (Vd_{ss}) (L/kg)</td>
<td>0.230</td>
<td>0.201</td>
<td>0.284</td>
<td>0.289</td>
<td>0.314</td>
</tr>
<tr>
<td>AUC (mg*min/L)</td>
<td>1120</td>
<td>1652</td>
<td>1949</td>
<td>6166</td>
<td>5913</td>
</tr>
<tr>
<td>(MRT_{sys}) (min)</td>
<td>172</td>
<td>165</td>
<td>167</td>
<td>179</td>
<td>232</td>
</tr>
<tr>
<td>(t_{1/2}) (min)</td>
<td>131</td>
<td>125</td>
<td>135</td>
<td>154</td>
<td>167</td>
</tr>
<tr>
<td>% Extrapolated</td>
<td>8.0%</td>
<td>14%</td>
<td>8.0%</td>
<td>2.9%</td>
<td>16%</td>
</tr>
<tr>
<td>(r^2, VI)</td>
<td>0.9973, (✓)</td>
<td>0.9988, (✓)</td>
<td>0.9939, (✓)</td>
<td>0.9873, (✓)</td>
<td>0.9440, (✓)</td>
</tr>
</tbody>
</table>

Table 18 NCA results for the four selected IV studies
results were in line with literature and supported the use of disease state and BW as covariates to be modeled.

Figure 14 NCA CL_{tot} and V_{dss} plotted against BW corrected doses for TOB 101 – TOB 103B

Figure 15 NCA CL_{tot} and V_{dss} plotted against none BW corrected doses for TOB 101 – TOB 103B
Similar to the IV meta-analysis, the meta-analysis incorporating plasma exposure metrics (namely AUC and $c_{\text{max}}$) after inhalation did not show any evidence of non-dose linearity and or any differences between HV and CF patients. Sample results from the meta-analysis are shown in Figures 18 and 19 where the reported AUC was plotted against the device and the calculated dose to lung respectively. A linear model and a
power model was fit to these data points to emphasize the lack of conclusive information suggesting non-linearity, and hence the assumption of a linear model.

Figure 18 Reported serum AUC plotted against the device dose.

Blue circles represent HV studies and red circles represent studies conducted in CF patients.

Figure 19 Reported serum AUC plotted against the calculated lung dose (device dose*dose to lung).

Blue circles represent HV studies and red circles represent studies conducted in CF patients.
4.4.3. IV Data Modeling Results

Successful fits were obtained for each of the four data sets from the three studies (TOB 101-103). Model selection criteria was higher than 4.1, R² values were above 0.99 for all the selected fits, correlation matrix values were all below 0.5, coefficients of variation for each of the parameter estimates were below 30%, and the visual inspection of the observed versus simulated values were all acceptable. A weighting factor of 1/y² was used for all but TOB 101 which did not utilize a weighting factor in fitting the model to the collected data. The model file for the various fits are reported in Appendix 2 as Code 1 of this chapter along with the results of all the attempted fits (Table 1 – 4) and the visual inspection plots for the various weighting factors (Figures 1 – 8).

4.4.4. IV MCS Optimization

No parameter space could be identified for models 1 and 2 that would have been able to describe all the observed data. While there was a parameter space for model 1 that accurately described the observed c_p(t) profiles, due to its lack of pulmonary distribution, it was incapable of capturing the reported c_s(t) profiles. On the other hand, model 2 was able to capture the plasma concentration time profile and some of the sputum levels, but was not able to describe the measured accumulation of the drug in sputum after repeated IV dosing as shown in TOB 104 (Figure 9 and 10 in Appendix 2). Model 3 was able to predict the observed data well as the observed c_p(t) and c_s(t) profiles data fell within the predicted 5%-95%-percentiles. The VPC plots and visual comparison of PK parameters from study TOB 104 are shown in Figure 20 below and the remaining VPCs are shown in Appendix 2 as Figures 11 – 14.
Figure 20 TOB 104 VPC and exposure metric variability comparison.

Additionally, in the figure above there are box and whisper plots that compare the reported exposure metrics to their respective simulated exposure metrics. As can be seen there is no evidence that would support a difference between the reported and simulated exposure metrics. A sample code used for both the optimization process as well as for generating these plots is listed in Appendix 2 as Code 2. The numerical comparison of the reported and simulated exposure metrics is reported in the Appendix 2 of this chapter as Tables 5 – 9. The optimized parameters for these simulations are listed with their associated variability in Table 19. For the MCS, an n = 3000 has not shown improvements in matching the reported $c_p(t)$ and $c_s(t)$ profiles or reported PK parameter variability compared to an n =300. The time interval for the simulations for TOB 101- TOB 103B was 0.5 minutes, and for TOB 104 was 1 minute. Drug amounts
in all the model compartments as well as the cumulative amount eliminated via pulmonary excretion and central compartment elimination are shown in Figure 21.

Based on this, the CC elimination accounts for 92% of the total elimination of the drug from the body while pulmonary elimination accounts for the remaining 8%.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population Value</th>
<th>Distribution calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{l_{pop}}$ (ml/min)</td>
<td>70</td>
<td>$C_{l_{tot}} = C_{l_{pop}} \cdot \left(\frac{BW_i}{BW_{pop}}\right)^{0.75} + \epsilon^{C_{l_{tot}}}$</td>
</tr>
<tr>
<td>$k_{12}$ (min$^{-1}$)</td>
<td>0.010</td>
<td>LN(0.010,15)</td>
</tr>
<tr>
<td>$k_{21}$ (min$^{-1}$)</td>
<td>0.015</td>
<td>LN(0.015,15)</td>
</tr>
<tr>
<td>$V_{0, pop}$ (l)</td>
<td>10</td>
<td>$V_0 = V_{pop} \cdot \left(\frac{BW_i}{BW_{pop}}\right)^{1} + \epsilon^{V_0}$</td>
</tr>
<tr>
<td>$\epsilon^{C_{l_{tot}}}$</td>
<td>1</td>
<td>N(1,15)</td>
</tr>
<tr>
<td>$\epsilon^{V_0}$</td>
<td>1</td>
<td>N(1,10)</td>
</tr>
<tr>
<td>$k_{pa}$ (min$^{-1}$)</td>
<td>0</td>
<td>LN(0,0)</td>
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<tr>
<td>$F_{oral}$ (%)</td>
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<td>N(0,0)</td>
</tr>
<tr>
<td>$k_{aa}$ (min$^{-1}$)</td>
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<td>LN(0.0006,30)</td>
</tr>
<tr>
<td>$k_{aa}$ (min$^{-1}$)</td>
<td>0.9</td>
<td>LN(0.9,30)</td>
</tr>
<tr>
<td>$k_{pm}$ (min$^{-1}$)</td>
<td>0.16</td>
<td>LN(0.16,20)</td>
</tr>
<tr>
<td>$k_{em}$ (min$^{-1}$)</td>
<td>0.6</td>
<td>LN(0.6,20)</td>
</tr>
<tr>
<td>$k_{pd}$ (min$^{-1}$)</td>
<td>0.0008</td>
<td>LN(0.0008,30)</td>
</tr>
<tr>
<td>$k_{ad}$ (min$^{-1}$)</td>
<td>0.001</td>
<td>LN(0.001,30)</td>
</tr>
<tr>
<td>$k_{pl1}$ (min$^{-1}$)</td>
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<td>LN(0.0005,10)</td>
</tr>
<tr>
<td>$k_{pl2}$ (min$^{-1}$)</td>
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<td>LN(0.0001,10)</td>
</tr>
<tr>
<td>$k_{sl1}$ (min$^{-1}$)</td>
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<td>LN(0.0005,10)</td>
</tr>
<tr>
<td>$k_{sl2}$ (min$^{-1}$)</td>
<td>0.0002</td>
<td>LN(0.0002,10)</td>
</tr>
<tr>
<td>TLV (ml)</td>
<td>20</td>
<td>$TLV = TLV_{pop} \cdot \left(\frac{BW_i}{BW_{pop}}\right)^{1} + \epsilon^{TLV}$</td>
</tr>
<tr>
<td>$\epsilon^{TLV}$</td>
<td>1</td>
<td>N(1,20)</td>
</tr>
<tr>
<td>CLV (ml)</td>
<td>16</td>
<td>$CLV = TLV \cdot 0.8$</td>
</tr>
</tbody>
</table>

Table 19 Model 3 parameter point and variability estimates with their respective distributions after final optimization with IV data sets
Figure 21 Amounts of drug in various compartments of Model 3 simulated over 5760 minutes after IV administration using the final optimize IV parameters
4.4.5. IV Sensitivity analysis results

With a single dose of 105 mg infused over 10 minutes, simulations run to 24 hours showed AUC in plasma was sensitive to changes in CL\textsubscript{tot}, k\textsubscript{pls1}, k\textsubscript{pls2}, such that a 25-fold change in these parameters resulted in -13, -1.3, -1.3-fold change respectively in AUC. Similarly, the fold change in $k_1$, CL\textsubscript{tot}, and $k_2$ resulted in a -1.7, -1.5, 1.1-fold change in $c_{\text{max}}$ respectively. In addition to CL\textsubscript{tot}, which was one of the top three most influential parameters on sputum AUC and $c_{\text{max}}$, $k_{ca}$ resulted in a -4.6, and -5.1-fold change to AUC and $c_{\text{max}}$ respectively, and $k_{cm}$ resulted in a -5.4, and -4.9-fold change to AUC and $c_{\text{max}}$ respectively. Similar results were obtained after a repeated dosing sensitivity analysis study where 105mg was administered as an IV infusion for three consecutive days. Plasma AUC was still predominantly altered by CL\textsubscript{tot}, k\textsubscript{pls1}, and k\textsubscript{cls1}, and $c_{\text{max}}$ was no longer influenced by $k_2$, with only CL\textsubscript{tot} and $k_1$ leading to any notable fold changes in $c_{\text{max}}$. The fold change elicited in each of the exposure metrics as a response to a 25-fold change in each of the model parameter is tabulated in Table 20 for the single dose and Table 21 for the repeated dosing experiment. The resulting plasma and sputum profiles after single dose are shown in the appendix as Figure 15 – 27 and Figures 28 – 40 for the repeated dose. The code used to perform the sensitivity analysis is shown in the appendix under Code 3.
Table 20 TOB SD sensitivity analysis results

Single IV dose sensitivity analysis results for IV optimized parameter space. Table reports the fold change (Max/Min * -1 (if lower parameter value results in larger exposure metric value)) in exposure metric given a 25-fold change in the model parameter (from 1/5 to 5 times mean optimized value). Simulation parameters were set to a BW of 70kg, Dose = 1.5mg/kg (105mg), Infusion time = 30 min, and sampling schedule from 0 to 24 hours.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Sputum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>AUC</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;tot&lt;/sub&gt;</td>
<td>-13.0 -1.3</td>
<td>-9.7 -9.9</td>
</tr>
<tr>
<td>k&lt;sub&gt;12&lt;/sub&gt;</td>
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<td>-1.3 -1.3</td>
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<td>1.3 1.4</td>
</tr>
<tr>
<td>V&lt;sub&gt;b&lt;/sub&gt;</td>
<td>1 1</td>
<td>1 1</td>
</tr>
<tr>
<td>k&lt;sub&gt;pa&lt;/sub&gt;</td>
<td>1 1</td>
<td>-1.1 -1.1</td>
</tr>
<tr>
<td>k&lt;sub&gt;c&lt;/sub&gt;</td>
<td>1 1</td>
<td>-4.6 -5.1</td>
</tr>
<tr>
<td>k&lt;sub&gt;pm&lt;/sub&gt;</td>
<td>1 1</td>
<td>1.1 1.1</td>
</tr>
<tr>
<td>k&lt;sub&gt;cm&lt;/sub&gt;</td>
<td>-1 -1</td>
<td>-5.4 -4.9</td>
</tr>
<tr>
<td>k&lt;sub&gt;pd&lt;/sub&gt;</td>
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<td>2.1 2.6</td>
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<tr>
<td>k&lt;sub&gt;cd&lt;/sub&gt;</td>
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<td>3.9 4.6</td>
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<td>k&lt;sub&gt;ph1&lt;/sub&gt;</td>
<td>-1.3 -1</td>
<td>3.7 3.6</td>
</tr>
<tr>
<td>k&lt;sub&gt;ph2&lt;/sub&gt;</td>
<td>1 1</td>
<td>-1.1 -1.1</td>
</tr>
<tr>
<td>k&lt;sub&gt;ch1&lt;/sub&gt;</td>
<td>-1.3 -1</td>
<td>4.3 4.4</td>
</tr>
<tr>
<td>k&lt;sub&gt;ch2&lt;/sub&gt;</td>
<td>1 1</td>
<td>-1.3 -1.2</td>
</tr>
</tbody>
</table>

Table 21 TOB RD sensitivity analysis results

Repeated IV dose sensitivity analysis results for IV optimized parameter space. Table reports the fold change (Max/Min * -1 (if lower parameter value results in larger exposure metric value)) in exposure metric given a 25-fold change in the model parameter (from 1/5 to 5 times mean optimized value). Simulation parameters were set to a BW of 70kg, Dose = 1.5mg/kg (105mg), Infusion time = 30 min, and sampling schedule from 0 to 24 hours.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Sputum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>AUC</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;tot&lt;/sub&gt;</td>
<td>-13.0 -1.3</td>
<td>-15 -12</td>
</tr>
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<td>k&lt;sub&gt;12&lt;/sub&gt;</td>
<td>1.0 1.2</td>
<td>-1.0 -1.2</td>
</tr>
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<td>-1.0 -1.0</td>
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<tr>
<td>k&lt;sub&gt;c&lt;/sub&gt;</td>
<td>1.0 1.0</td>
<td>-2.9 -3</td>
</tr>
<tr>
<td>k&lt;sub&gt;pm&lt;/sub&gt;</td>
<td>-1.0 -1.0</td>
<td>1.0 1.0</td>
</tr>
<tr>
<td>k&lt;sub&gt;cm&lt;/sub&gt;</td>
<td>-1.0 -1.0</td>
<td>-8.7 -7.4</td>
</tr>
<tr>
<td>k&lt;sub&gt;pd&lt;/sub&gt;</td>
<td>-1.0 -1.0</td>
<td>1.5 1.9</td>
</tr>
<tr>
<td>k&lt;sub&gt;cd&lt;/sub&gt;</td>
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<td>2.7 3.3</td>
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<tr>
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<td>4.0 3.9</td>
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<td>k&lt;sub&gt;ph2&lt;/sub&gt;</td>
<td>1.0 1.0</td>
<td>-1.2 -1.2</td>
</tr>
<tr>
<td>k&lt;sub&gt;ch1&lt;/sub&gt;</td>
<td>-1.2 -1.0</td>
<td>4.0 4.2</td>
</tr>
<tr>
<td>k&lt;sub&gt;ch2&lt;/sub&gt;</td>
<td>1.1 1.0</td>
<td>-1.8 -1.6</td>
</tr>
</tbody>
</table>
Dose = 1.5mg/kg/day (105mg), Infusion time = 30 min, and sampling schedule from 48 to 72 hours.

4.4.6. INH Modeling Approach #1 – #4 as Negative results

The first approach attempted to utilize the final IV optimized model parameters and model 3 to predict $c_p(t)$ and $c_s(t)$ from INH studies (approach #1). After repeated attempts to optimize the fraction of the dose that was deposited in the peripheral lung ($F_{pd}$) (and by consequence, the fraction deposited in the central lung-$F_{cd}$), no value of $F_{pd}$ allowed for acceptable prediction of the observed $c_p(t)$ and $c_s(t)$ profiles. As can be seen in Figure 73, there was an underprediction of the plasma concentrations, and an overprediction of sputum concentrations. In the second approach, we attempted to identify a universal parameter space that was capable of describing both the IV and INH data sets by re-optimizing the parameters to describe both the IV and INH data sets simultaneously (approach #2). This approach was also unsuccessful as it was not possible to capture both plasma profiles while at the same time capturing both sputum profiles as seen in Figure 74. Approach 1 and 2 were likely unsuccessful due to the fact that there was very limited sputum data after repeated dosing via IV administration and the available data was a clustered close to the LOQ. Given that there was no identifiable parameter space that was capable of describing all the available IV and INH data sets simultaneously, the parameter optimization was then preformed solely on the available INH data sets from study 307. Optimization was considered successful as it was capable of describing the $c_p(t)$ and $c_s(t)$ profiles for the four tested doses as shown in Figure 75. However, when attempting to predict the exposure metrics from the remaining studies, there was a systematic overprediction of
the exposure metrics across the three remaining studies (sample case shown in table 12). This systemic overprediction resulted in approach #3 being deemed unacceptable. In approach #4, data from across the four inhalation studies for the same device (Pari Nebulizer) was used to optimize the parameter space. This approach resulted in a parameter space that was able to, without bias, predict the four data sets used in the optimization process (307A, 303A, 30A, and 309F) (Table 13) but still resulted in the systemic overprediction of the plasma exposure metrics for the remaining studies (Figure 20).

**4.4.7. Approach #5 as successful results**

Initial estimates for the fifth approach for model 3 parameter optimization were taken from the final estimated IV parameters. Model parameters were optimized to match predicted with observed exposure metrics, incorporating up to 40% random variability following a log-normal distribution. Clinical PK studies with inhaled TOB (28 - 300 mg in HV and CF after single dose) were used as reference for this optimization. The four studies included in the analysis provided a total of 16 data sets (either \(c_p(t)\) profiles, \(c_s(t)\) profiles, plasma exposure metrics, or sputum exposure metrics). All sixteen data sets had plasma information while 10 of the 16 had sputum information that was utilized for validation. Across studies, doses and inhalation devices, predicted \(c_p(t)\) and \(c_s(t)\) profiles (95-percentiles) included observed profiles; predicted AUC and \(c_{max}\) were within ±20% on average as can be seen in Table 22 below. The accepted VPC plots for both \(c_p(t)\) and \(c_s(t)\), as well as the visual comparison for the reported and simulated PK parameters from study TOB 104 are shown in Figure 22 below and the remaining results are shown in Appendix 2 as Figures 41 – 55. Additionally, the
simulated exposure metric central tendency and variability was compared with those reported in each of the publications. These comparisons are shown below in Table 23 and for the remaining studies included in Appendix 2 as Tables 10 – 25. The final optimized parameter space that was deemed acceptable and used to generate the various tables and figures is listed in Table 24 below. Secondary derived model parameters that allow for a comparison between the IV and INH optimized parameters are reported in Table 25.
<table>
<thead>
<tr>
<th>Study ID</th>
<th>Group</th>
<th>Device</th>
<th>Dose (mg)</th>
<th>DTL (%)</th>
<th>Estimated DTL (mg)</th>
<th>Inhalation Time (min)</th>
<th>Plasma AUC</th>
<th>Plasma $c_{\text{max}}$</th>
<th>Sputum AUC</th>
<th>Sputum $c_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>302A</td>
<td>HV</td>
<td>Pari</td>
<td>300</td>
<td>14.8</td>
<td>44</td>
<td>16.00</td>
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<td></td>
</tr>
<tr>
<td>302B</td>
<td>HV</td>
<td>eFlow</td>
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<td>15.1</td>
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<td>8.50</td>
<td>7.9</td>
<td>-16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>302C</td>
<td>CF</td>
<td>Pari</td>
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<td>eFlow</td>
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<td>-46</td>
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<td></td>
</tr>
<tr>
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<td>CF</td>
<td>Pari</td>
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<td>18</td>
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<td>-48</td>
<td>13</td>
<td>81</td>
</tr>
<tr>
<td>307 B</td>
<td>CF</td>
<td>AeroDose</td>
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<td>35.4</td>
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<td>2.80</td>
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<td>-45</td>
<td>102</td>
<td>119</td>
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<td>-39</td>
<td>62</td>
<td>106</td>
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<tr>
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<td>AeroDose</td>
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<td>-47</td>
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<td>25</td>
</tr>
<tr>
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<td>1.70</td>
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<td>-19</td>
<td>102</td>
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</tr>
<tr>
<td>309B</td>
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<td>PulmoSphere</td>
<td>56</td>
<td>34.3</td>
<td>19</td>
<td>4.20</td>
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<td>-2.6</td>
<td>-48</td>
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<td>309C</td>
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<td>7.7</td>
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</tr>
<tr>
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<td>CF</td>
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<td>10</td>
<td>30</td>
<td>16.00</td>
<td>42</td>
<td>72</td>
<td>-52</td>
<td>-76</td>
</tr>
</tbody>
</table>

**Average percent difference (prediction error)**

-5.1  -18  20  1.1
Figure 22 TOB 307D VPC and exposure metric variability comparison.

Top left - $c_p(t)$ profile, top center – $c_s(t)$ profile, top right - $c_s$ AUC box and whisker plot (WHP), bottom left – $c_{s,max}$ WHP, bottom center- $c_p$ AUC WHP, bottom right – $c_{p,max}$ WHP

<table>
<thead>
<tr>
<th>Metric</th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum AUC$_{0.8}$ (mg*hr/L)</td>
<td>1275 ± 1359</td>
<td>1850 ± 1879</td>
<td>45%</td>
</tr>
<tr>
<td>Sputum $C_{max}$ (mg/L)</td>
<td>0.96 ± 0.95</td>
<td>1.20 ± 2.58</td>
<td>25%</td>
</tr>
<tr>
<td>Plasma AUC$_{0.8}$ (mg*hr/L)</td>
<td>3.94 ± 1.52</td>
<td>3.52 ± 1.73</td>
<td>-10%</td>
</tr>
<tr>
<td>Plasma $C_{max}$ (mg/L)</td>
<td>0.96 ± 0.4</td>
<td>0.51 ± 0.27</td>
<td>-47%</td>
</tr>
</tbody>
</table>

Table 23 Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 307D
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population Value</th>
<th>Distribution Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl_{pop}$ (ml/min)</td>
<td>70</td>
<td>$Cl_{tot} = Cl_{pop} * \left( \frac{BW_i}{BW_{pop}} \right)^{0.75} + \epsilon_i^{Cl_{tot}}$</td>
</tr>
<tr>
<td>$k_{12}$ (min⁻¹)</td>
<td>0.010</td>
<td>LN(0.010,20)</td>
</tr>
<tr>
<td>$k_{31}$ (min⁻¹)</td>
<td>0.015</td>
<td>LN(0.015,20)</td>
</tr>
<tr>
<td>$V_{0, pop}$ (l)</td>
<td>10</td>
<td>$V_0 = V_{pop} * \left( \frac{BW_i}{BW_{pop}} \right)^{1} * \epsilon_i^{V_0}$</td>
</tr>
<tr>
<td>$\epsilon_i^{Cl_{tot}}$</td>
<td>1</td>
<td>N(1,30)</td>
</tr>
<tr>
<td>$\epsilon_i^{V_0}$</td>
<td>1</td>
<td>N(1,30)</td>
</tr>
<tr>
<td>$k_{ps}$ (min⁻¹)</td>
<td>0.9</td>
<td>LN(0.9,30)</td>
</tr>
<tr>
<td>$k_{sa}$ (min⁻¹)</td>
<td>0.65</td>
<td>LN(0.65,30)</td>
</tr>
<tr>
<td>$k_{pm}$ (min⁻¹)</td>
<td>0.02</td>
<td>LN(0.02,20)</td>
</tr>
<tr>
<td>$k_{m}$ (min⁻¹)</td>
<td>0.08</td>
<td>LN(0.08,20)</td>
</tr>
<tr>
<td>$k_{pe}$ (min⁻¹)</td>
<td>0.008</td>
<td>LN(0.008,30)</td>
</tr>
<tr>
<td>$k_{sa}$ (min⁻¹)</td>
<td>0.0002</td>
<td>LN(0.0002,30)</td>
</tr>
<tr>
<td>$k_{pia}$ (min⁻¹)</td>
<td>0.015</td>
<td>LN(0.015,40)</td>
</tr>
<tr>
<td>$k_{pia2}$ (min⁻¹)</td>
<td>0.004</td>
<td>LN(0.004,40)</td>
</tr>
<tr>
<td>$k_{pia3}$ (min⁻¹)</td>
<td>0.002</td>
<td>LN(0.002,40)</td>
</tr>
<tr>
<td>$k_{pia2}$ (min⁻¹)</td>
<td>0.3</td>
<td>LN(0.3,40)</td>
</tr>
<tr>
<td>$TLV$ (ml)</td>
<td>20</td>
<td>$TLV = TLV_{pop} * \left( \frac{BW_i}{BW_{pop}} \right)^{1} * \epsilon_i^{TLV}$</td>
</tr>
<tr>
<td>$\epsilon_i^{TLV}$</td>
<td>1</td>
<td>N(1,30)</td>
</tr>
<tr>
<td>$CLV$ (ml)</td>
<td>16</td>
<td>$CLV = TLV + 0.8$</td>
</tr>
</tbody>
</table>

Table 24 Model 3 parameter point and variability estimates with their respective distributions after final optimization with INH data sets
Table 25 Model 3, secondary derived parameters based on the final optimized parameters

4.4.8. Pulmonary Bioavailability

Across the four inhalation studies analyzed, the traditional method of evaluating $F_{pul}$ via the AUC ratio method demonstrated that the mean $F_{pul}$ was 18% and ranged from 3.6% to 31%. The calculated $F_{inh}$ values (based on Equation 2) for each of the studies are shown below in Table 26. Since TOB is not orally bioavailable ($F_{oral} \sim 0\%$), the low $F_{pul}$ reflects poor absorption of deposited TOB across pulmonary epithelia.
Table 26 Calculated $F_{inh}$ values using AUC-ratio method with study TOB 103A as IV reference.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Calculated $F_{pul}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>307A</td>
<td>9.3</td>
</tr>
<tr>
<td>307B</td>
<td>27</td>
</tr>
<tr>
<td>307C</td>
<td>28</td>
</tr>
<tr>
<td>307D</td>
<td>25</td>
</tr>
<tr>
<td>303A</td>
<td>3.9</td>
</tr>
<tr>
<td>303B</td>
<td>31</td>
</tr>
<tr>
<td>309A</td>
<td>26</td>
</tr>
<tr>
<td>309B</td>
<td>28</td>
</tr>
<tr>
<td>309C</td>
<td>25</td>
</tr>
<tr>
<td>309D</td>
<td>23</td>
</tr>
<tr>
<td>309E</td>
<td>23</td>
</tr>
<tr>
<td>309F</td>
<td>9.0</td>
</tr>
<tr>
<td>302A</td>
<td>6.2</td>
</tr>
<tr>
<td>302B</td>
<td>6.8</td>
</tr>
<tr>
<td>302C</td>
<td>9.4</td>
</tr>
<tr>
<td>302D</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Using the parameters optimized with the available IV data, and performing mass balance excretion simulation after IV administration, demonstrated that 91% of the administered dose was eliminated from the central compartment while the remaining 9% of the dose was eliminated from the central unbound lung compartment through mucociliary clearance. Similarly, when the parameters optimized with the available INH data were used to perform the same IV administration simulations, 91% was eliminated from the central compartment while 1% was eliminated via the other pathway. After INH simulations, however, using both the final IV and INH parameter space, results were not as similar. 93% was eliminated from the central compartment and 7% via the other pathway after INH administration simulation using the optimized INH parameters, and 82% eliminated from the central compartment and 18% via the other pathway after INH
administration using the optimized IV parameter space. A simulation depicting the amount in each compartment after INH administration using the final optimized INH parameters is shown in Figure 23 below. The cumulative amount excreted plots for the four bioavailability simulations are reported in Appendix 2 as Figures 56 – 59.
Figure 23 Amounts of drug in various compartments of Model 3 simulated over 5760 minutes after INH administration using the final optimize INH parameters
4.4.9. INH Sensitivity analysis results

With a single dose of 300 mg inhaled over 10 minutes, simulations run to 12 hours showed AUC in both plasma was sensitive to changes in $\text{CL}_{\text{tot}}$, $k_{\text{pls1}}$, $k_{\text{pls2}}$, such that with a 25-fold change in these parameters resulted in -4.8, -3.3, 2.9-fold change respectively in AUC. Similarly, the fold change in $k_{\text{ca}}$, $k_{\text{pls1}}$, and $k_{\text{cm}}$ resulted in a 1.7, -1.5, -1.5-fold change in $c_{\text{max}}$ respectively. In addition to $k_{\text{ca}}$, which was one of the top three most influential parameters to sputum AUC, $k_{\text{cm}}$ and $k_{\text{pa}}$, resulted in a -18, -1.7 and -1.6-fold change to AUC respectively. $k_{\text{ca}}$, $k_{\text{pa}}$ and $k_{\text{pd}}$ resulted in a -118, -23, and 23-fold change to sputum $c_{\text{max}}$ respectively. The fold change elicited in each of the exposure metrics as a response to a 25-fold change in each of the model parameter is tabulated in Table 27. The resulting plasma and sputum profiles after single dose are shown in Appendix 2 as Figure 60 – 72. The code used to perform the sensitivity analysis is similar to that utilized for the IV sensitivity analysis shown in Appendix 2 under Code 3.
Table 27 INH TOB sensitivity analysis results

Single INH dose sensitivity analysis results for INH optimized parameter space. Table reports the fold change (Max/Min \* -1 (if lower parameter value results in larger exposure metric value)) in exposure metric given a 25-fold change in the model parameter (from 1/5 to 5 times mean optimized value). Simulation parameters were set to a BW of 70kg, Dose = 300mg, Inhalation time = 10 min, and sampling schedule from 0 to 12 hours.

### 4.5. Discussion and Conclusions

#### 4.5.1. Study Limitations

Confidence in the results generated by any model is highly dependent on the data used in the development of the model. As such, the data used in the development of a given model often needs to be critically evaluated. For the semi-PBPK model that was developed within this work, the data that was utilized in validating the constructed model was evaluated based on the inclusion standards described in the methods above. Of the collected IV studies listed above in Table 16, studies TOB 105 - 108 were
excluded from the data analysis for four primary reasons. First, these studies did not sample plasma concentrations frequently enough such that the reported $c_p(t)$ profiles could not have been analyzed nor used for estimating model parameters. With infrequent and/or insufficient plasma concentrations samples, as is the case with those studies, parameter identifiability is challenging. Second, the analytical method was not considered to be reliable in those four studies. The method utilized for determining the concentration of TOB in plasma was antibiotic activity using an Agar-well diffusion method specific to the bacteria *Bacillus subtilis*. This method is known to be a nonselective method for detecting TOB, and hence the concentrations reported were associated with a high level of variability. Third, if measured concentrations were below the lower limits of quantitation (LLOQ), they typically were replaced with zero. This was problematic since the reported $c_p(t)$ profile is typically a mean profile, and individual profiles are often not included. Thus, the mean plasma concentration at a given time point can be skewed to a lower value due to these zero replacements. Concentrations that are more susceptible to being below LLOQ are those that at later time points occur after drug levels have had time to decrease throughout the body due to drug elimination. The zero substitution for concentrations below LLOQ is more so problematic with older analytical detection methods as they typically had higher LLOQs. Fourth, the reported mean $c_p(t)$ data from these studies often were indistinguishable from each other given their reported variability. Using this data would lead to very poor parameter estimation as parameter identifiability would again not be possible with such data sets.
Aside from evaluating the available data to determine whether or not it was acceptable for inclusion and analysis, it is also vital to discuss the lack of availability of certain data sets. All the identified IV studies were performed as short 10 to 60-minute infusions and none where administered as IV bolus. Assuming an appropriate data collection schedule was followed, and IV bolus study would have been valuable in approximating the distribution and re-distribution parameters ($k_{12}$ and $k_{21}$) with certainty. This is not the case with IV infusion studies because while the drug is still being infused, the already administered dose will be undergoing distribution. Hence distribution and redistribution parameters become much more difficult to estimate after an IV infusion study.

In addition to the lack of IV bolus studies, there were also no IV SD studies performed in HV or CF patients that reported sputum data. Study TOB 104 was the only study that reported sputum data, namely a $c_s(t)$ profile and a $c_{\text{max}}$ in sputum, however this was only performed in CF patients and there were no HV studies available. In both IV and INH studies, there is a scarcity of repeat dose studies providing sputum concentration data. In addition, there were no repeat dosing INH studies available in either HV or CF patients, and while there were single dose studies in for both HV and CF patients, no sputum data was available for HV after single does INH. The lack of available sputum data for HV either after IV or INH administration can be explained as HV do not naturally expectorate, naturally limiting sputum data collection. This lack of data limits the validity of the pulmonary parameters of the model as well as the ultimate generalizability of the model.

4.5.2. **Dose linearity after IV and INH and compare with literature findings**
As can be seen in Figure 14, when the IV infused dose was corrected for bodyweight (BW), there was no apparent difference in $\text{CL}_{\text{tot}}$ or $\text{Vd}_{\text{ss}}$ between HV and CF subjects. Given the absence of a difference, PK exposure metric data was pooled across studies, allowing the testing of dose dependence across the IV studies. When comparing $\text{CL}_{\text{tot}}$ and $\text{Vd}_{\text{ss}}$ as the nominal dose increased from 1.5 mg/kg to 10 mg/kg, there was no evidence to support non-linear PK after IV infusion dosing within the tested dose range. This can be clearly observed on Figure 15 with no changes observed in both $\text{CL}_{\text{tot}}$ and $\text{Vd}_{\text{ss}}$ values as dose increased along the x-axis. Dose independent clearance and volumes are both key defining factors of linear PK. Furthermore, in the linear dose escalation plot shown in Figures 16 comparing the administered dose to the NCA calculated AUC, the high $r^2$ value of the linear model further suggested linear pk. Additionally, when the same data was plotted on a log-log scale as in Figure 17, and a power model was utilized for fitting the data, the coefficient of the exponential term is close to 1.0 which would also indicate suggest linear PK. After INH administration the same conclusions were reached after evaluating the calculated AUC as the administer dose increased. As seen in Figure 19, AUC increased in linear fashion as dose to lung increased. The scatter of the reported AUC on this plot is likely due to the variability associated with the INH device, INH technique and sampling of the data. This finding is in agreement with the literature as several authors have also reported that TOB experience’s linear PK across a similar dose range.$^{15,57}$ Moreover, given that there are no saturable processes (absorption, distribution, metabolism, or elimination) involved in kinetics of TOB, it is unlikely that additional data would contradict these results.
4.5.3. Final IV Parameter Space

This semi-PBPK model of I.V. administered TOB with an optimized model parameter space resulted in mechanistically plausible parameter estimates, and was able to predict both $c_p(t)$ and $c_s(t)$ profiles from clinical PK studies with HV and CF after single or repeated therapeutic doses. This, however, was only plausible after the addition of the pulmonary sequestration compartments, which was singularly driven by a repeat-dose sputum data set that clearly demonstrated TOB accumulation in sputum, but not in plasma. Based on the final optimized parameter value, and as peripheral compartments to the central compartment, these pulmonary sequestration compartments appeared to be deep and high capacity compartments. It appears that they functioned as reservoir or donor compartments maintaining sputum concentration for the repeated dosing study validation. As can be seen in Figure 21, the amount of drug in the peripheral and central sequestration lung compartments is among one of the larger levels, and remains higher than most compartments for a prolonged period of time. Previous dissertation work by Min Li has shown in-vitro evidence supporting the idea that the lung has the capacity to sequester drug and thus maintain sputum levels after drug administration. 24

Furthermore, the secondary derived model parameters shown in Table 25 also show that the sequestration compartments capacity rate constants ($k_{pl}$ and $k_{cls}$) given the final IV parameter space were greater than 1.0. These values therefore indicate that with respect to the central compartment, the sequestration compartments are high capacity compartments. Based on the capacity rate constants ($k_p$ and $k_c$),
however, the unbound peripheral and central lung compartments are relatively lower capacity compartments. Equilibration rate constants (the sum of the input and output rate constant from a given compartment) help elucidate how rapidly a compartment equilibrates with respect to adjacent compartments. Larger values denote a more rapid equilibration, while smaller values a slower equilibration. This is best observed when these rate constants are instead converted to half-lives. For the unbound peripheral and central lung compartments, the equilibration half-lives were 102 and 0.77 minutes respectively, while for the sequestration peripheral and central lung compartments both equilibration half-lives were much longer at 1155 and 990 minutes respectively. IV administered TOB is expected to be predominantly cleared through the total clearance route with nearly 93% of the dose eliminated via $\text{CL}_{\text{tot}}$ and the remaining 7% being eliminated through the mucociliary clearance ($k_{cm}$).

4.5.4. Sensitivity analysis results for IV parameter space

After a single IV administered dose, sputum exposures were found to be sensitive to $\text{CL}_{\text{tot}}$, but insensitive to all other systemic disposition parameters; with regards to pulmonary disposition, only mucociliary clearance and pulmonary absorption from the central lung appeared to influence these parameters. Unlike sputum exposures, systemic exposures are not very sensitive to PUL disposition rates. Systemic exposures were found to be insensitive to pulmonary and systemic disposition parameters, except $\text{CL}_{\text{tot}}$. Similarly, the sensitivity analysis after repeated dosing displayed similar results (Table 20) in comparison to single dosing (Table 21). These results reveal that in order to maximize pulmonary concentrations after IV administration
while minimizing systemic concentrations, an optimization process needs to occur were $\text{CL}_{\text{tot}}$ should be increased while $k_{\text{pa}}$ and $k_{\text{cm}}$ should be decreased. This supports the hypothesis listed of the study.

4.5.5. Limitation of data leading to change of IV-> INH model

As discussed above, the only study that provided sputum data after IV administration was a repeat dose study that provided a total of four mean sputum concentrations. The first of these four concentrations, was a non-zero pre-dose measurement of the concentration of TOB in sputum. This single data point was the predominate driving factor in parameter optimization and selection. It was this data point that lead to the introduction of the deep, high capacity pulmonary sequestration compartments. Despite this, after INH administration, there was no repeated dose studies available to provide sputum concentration. In addition, the data obtained from the available INH studies did not support the use of the same parameter space optimized in the IV studies (negative results shown in Appendix 2 Figure 73) nor was there a single parameter space that was capable of simultaneously describing both the available IV and INH data (negative results shown in Appendix 2 Figure 74). Therefore, a separate parameter space was required to describe the INH data.

4.5.6. Final INH Parameter space

The final parameter set (listed in Table 24) that was optimized using the available inhalation data sets indicated that with exception to PLS, the three-other remaining pulmonary compartments (namely, PLU, CLU, and CLS) were low capacity compartments as suggested by their respective secondarily derived ratio parameters.
summarized in Table 25. This can be clearly observed in the PLS plot in Figure 23 as this plot showed that the amount of drug in PLS was notably higher than the remaining compartments. In addition to being a higher capacity compartment, PLS also appears to be a slowly equilibrating compartment relative to the remaining three pulmonary compartments. This slower equilibration is evidenced by the prolonged levels in the PLS plot mentioned above as well as a prolonged equilibration half-life of 36 min ($k_{plasm} = 0.019 \text{ min}^{-1}$).

Given the available data there was no evidence of differences in the pulmonary disposition of TOB between CF patients and HV. Inspection of the reported PK data for each of the inhalation publications included in the analysis showed that when the same inhalation device was used to inhale a similar dose, no differences could be observed in plasma exposure metrics. This is clearly observed in Table 26 of Appendix 2 when comparing TOB studies 302A and 303A, as representative studies of HV using a Pari inhaler, with studies 302C and 307A where CF patients were using the same device to inhale the same 300 mg dose. Sputum information was not available for comparison between the two groups. Due to the variability associated with sputum data, even if the data were available, it is unlikely that a difference would have been detected. Likewise, by evaluating the same reported exposure metrics, no difference was observed between the various inhalation devices tested in the studies. When 300 mg was inhaled with both the Pari inhaler and the eFlow nebulizer, no difference was evident in either HV (TOB 302A vs 302B) and CF patients (TOB 302C vs 302D). While there are known physiological differences between CF and HV patients, the lack of data and the
resolution of the available data sets both lead to an inability to characterize these physiological differences.

4.5.7. Sensitivity analysis results for INH parameter space

After a single dose inhalation, sputum exposures were found to be sensitive to \( k_{ca} \), \( k_{pa} \), \( k_{cm} \), and \( k_{pd} \), but insensitive to all other systemic disposition parameters. Systemic exposures were found to be predominantly sensitive to pulmonary disposition parameters: \( k_{ca} \), \( k_{pls1} \), \( k_{pls2} \), and \( k_{cm} \), but were insensitive to systemic disposition parameters except \( CL_{tot} \). As hypothesized in Hypothesis 3.2, slowing down PUL uptake (\( k_{ca} \) and \( k_{pa} \)) and mucociliary clearance (\( k_{cm} \)) would in fact increase pulmonary exposure as characterized by AUC and \( c_{max} \). In addition to this, the results also indicated that increasing \( k_{pd} \), efflux in the peripheral lung, would also lead to increased pulmonary concentrations.

4.5.8. Reconciling IV and INH parameter spaces

While there was no parameter space that was capable of simultaneously describing the available IV and INH data, there was an identifiable parameter space to describe the IV data, and another parameter space was identified to describe the available INH data. In comparing Figure 21 and Figure 23, as well as the numerical values of the secondarily derived model parameters listed in Table 25, it becomes apparent that major differences between the IV and INH parameter sets can be observed in the CLS compartment. While the IV parameter space indicated that this compartment is a high capacity slowly equilibrating compartment, the INH parameter
space specified that it is a low capacity fast equilibrating compartment. The increase in the equilibration rate may likely be due to the route of administration. Since TOB is being administered via INH, pulmonary concentrations will likely be much higher than those after IV administration. Consequently, cellular sequestration may be saturated and hence a more rapid equilibration occurs. This therefore alters the CLS from behaving as a reservoir compartment to behaving as a pass-through compartment. The remaining three pulmonary compartments are comparable given both parameter spaces.
CHAPTER 5

SEMI-PBPK MODELING OF INH CIPROFLOXIN

5.1. Background

5.1.1. Treatment of CF with Ciprofloxacin

Discovered in 1981 as the first oral broad-spectrum antibiotic of its class, Ciprofloxacin (CIP), a fluoroquinolone antibiotic, has since been approved by the FDA for use in treatment of urinary tract infections, chronic bacterial prostatitis, chronic bacterial prostatitis, lower respiratory infections, and numerous other indications.\textsuperscript{98,99} It also has numerous off-label and investigational uses such as in the treatment of chlamydia infections, inflammatory bowel diseases, and pelvic inflammatory disease.\textsuperscript{100–102} CIP is known to exhibit bactericidal effects on both gram positive and gram-negative bacteria. \textit{Pseudomonas aeruginosa}, a gram-negative bacterium, is known to be susceptible to CIP, with CIP being very potent against it with an experimental MIC\textsubscript{50} of 0.19 mg/L and a MIC\textsubscript{90} of 32 mg/L.\textsuperscript{103} The bactericidal action of CIP results from inhibition of the enzymes topoisomerase II and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination.\textsuperscript{104}
5.1.2. Relevant Physiochemical Properties of Ciprofloxacin

Often deemed as a small molecular weight compound, CIP has molecular weight of 331.3 grams per mole. Additionally, it is moderately lipophilic with an experimentally obtained partition coefficient log(P) value of -0.13 and is categorized as a sparingly soluble compound according to the USP with a solubility than 40 mg/mL at a pH of 4. At a concentration of 50 µm in the apical compartment, CIP has shown to have an apparent permeability (p$_{app}$) of 2.99·10^{-6} ± 2.92·10^{-7} cm/s in the Caco-2 cell line.$^{105}$ When a similar study was conducted in a Calu-3 cell line with concentrations of either 10 or 20 µM, results were similar, indicating a larger P$_{app}$ of 3.52·10^{-4} cm/s. Based on its experimentally determined solubility and permeability, CIP was classified as a BCS class IV compound.$^{106}$ Additionally, it was noted that while 81% of the CIP was present at the basolateral compartment at the end of the study when the initial concentration was 10 µM, only 48% was recovered in the basolateral compartment when 20 µM was tested.$^{105}$ This indicate the possibility of transporter involvement, however, the authors noted that further studies needed to be conducted. When the permeability of CIP was compared with the permeability of fluorescein (a low permeability, paracellular marker) in studies performed in the small intestine of rats, both compounds had similar permeability values.$^{107}$ This led the authors to conclude that CIP is a low permeability drug. The same authors also tested the permeability of CIP in the same system with benzbromarone (a MRP2 inhibitor), verapamil (a Pgp/MRP2 inhibitor) and quinidine (an OCT1 inhibitor). There was no difference in the permeability of CIP when tested alone versus when tested with any of these inhibitors. Accordingly, the authors concluded that CIP was not a substrate for any of the tested transporters. However, P$_{app}$ of CIP from
the serosal to the mucosal was studied alone vs in presence of quinidine, a significant difference was found suggesting the involvement of an efflux protein in CIP secretion.

5.1.3. Summary of Known PK/ADME Properties of Ciprofloxacin

With a reported plasma protein binding 20-40%, CIP still exhibits extensive distribution throughout the body with a Vdss (after a 100 mg IV infusion dose in HV) of 2.64 ± 0.5 L/kg and a similar volume of 2.54 ±1.2 L/kg in CF patients. Total clearance values (CLtot) are also calculated after the same dosing regimens in those subjects. For HV, the reported CLtot value was 0.51 ± 0.12 L/kg/hr which was slightly lower than that of CF patients 0.61 ± 0.21 L/kg/hr, with renal clearance accounting for 53% of the total clearance in HV and 62% in CF patients. After oral administration of radiolabeled CIP, 15% of the dose was detected in feces, and an additional 15% of the dose was detected in the form of four metabolites eliminated in urine. Some authors have shown that 15% of an orally administered dose is recovered in feces, while others have indicated that recovery may be as high as 35%. Both authors, however, have indicated that this may likely be due to biliary clearance or transintestinal elimination. In their Clinical Pharmacology and Biopharmaceutics review of ®Cipro, the FDA identified body weight, creatinine clearance, and the presence of CF in a patient, as significant covariates in the Pop-PK modeling of CIP.

5.2. Objectives

The major objectives for this chapter were to:

a) Develop and validate semi-PBPK model for CIP after the INH;
b) Determine sensitivity of plasma and sputum exposure to model parameters;

c) Investigate route-dependent elimination pathways.

5.3. Methods

5.3.1. Summary and Discussion of final IV, PO, and INH studies identified

Out of the nine identified CIP IV studies, one study was eliminated from analysis inclusion because of the non-selective, poorly sensitive microbiological assay utilized for plasma concentrations. Older microbiological assays are known to have poor selectivity and have been shown to overpredict concentrations in plasma, and hence this study was not included for analysis. There was also another study that did not report individual or a mean level plasma concentration time profile, and hence was excluded from analysis.

Twelve studies were identified where CIP was administered orally to both HV and CF patients. Two of those studies were excluded due to the use of the aforementioned unreliable microbiological assay for plasma concentration analysis. One study was not included in analysis as it only reported data from a single subject. Lastly, the study by Michael et al. was also excluded from analysis as the reported exposure metrics, and \( c_p(t) \) profiles were inconsistent with each other and with the text.

All available and collected Ciprofloxacin studies satisfied inclusion criteria and hence were utilized and all the performed analysis and modeling within this study. Studies involving patients on Dialysis, or patients suffering from other medical conditions were not collected.

5.3.2. Comparative NCA Results
NCA was performed on the digitized \(c_p(t)\) profiles for each of the individual studies across the dosing routes (IV, PO, and INH). To ensure dose-linearity, AUC vs Dose and log(AUC) vs log(Dose) plots were inspected. For IV studies, \(CL_{\text{tot}}, CL_{\text{nonren}}, CL_{\text{ren}},\) and \(V_{\text{dss}}\) were plotted against administered dose, age, body weight, and disease state. This was done in an attempt to see if change in any of these potential covariates led to a significant change in any of the estimated PK parameters. Additionally, since it is known that CF patients have a lower BW on average relative to HV (due to their malnutrition), the four PK parameters were also corrected for body weight and plotted against disease state (i.e., HV vs CF patients).

5.3.3. Semi-PBPK Models Tested

An open two-compartment body model was used to fit the collected IV data. The model incorporated loss from the central compartment via \(CL_{\text{tot}}\). Initial estimates for this model were estimated obtained from the previously discussed work with TOB. Three separate fits were performed: 1) with no weighting factor applied, 2) with a \(1/y\) weighting factor, and lastly, 3) with a \(1/y^2\) weighting factor. Parameter selection was based on the highest \(r^2, MSC,\) as well as parameter certainty (lowest CV’s for the parameters). Model parameter estimates from the individual studies were averaged and used as the initial estimate for the MCS. MCS were performed to optimize the parameter space to describe all the observed \(c_p(t)\) profiles as well as minimize the percent difference for the individual exposure metrics. Parameter variability was also optimized during the MCS. CV for each of the parameters was increased in increments of 5% until the model-precited SD for the exposure metrics from each of the individual studies were comparable to those that are reported. In addition, CVs were also optimized such that
the majority of the reported \(c_p(t)\) profiles were within the 5\textsuperscript{th}-95\textsuperscript{th} percentiles of the simulated \(c_p(t)\) profiles.

Final optimized systemic disposition parameters identified using the IV data sets were fixed, and the model was expanded to include a GI compartment. Initial estimates for \(F_{\text{oral}}\) and \(k_{ga}\) were obtained from the NCA estimation. Alternatively, MCS were performed with this expanded oral administration model to optimize the central tendency and the distribution values for the oral absorption parameters.

INH studies were grouped by dose and disease state during parameter optimization and were individually simulated, once the final parameter space was obtained. Specifically, \(c_p(t)\) data from CIP 301A and CIP 303A were averaged for each of the time points for which both studies reported a concentration. Both those studies were conducted in HV, and a dose of 32.5 mg was inhaled through the same device. Data from CIP 302A and CIP 304A, as well as CIP 302B and CIP 304B were also combined as both sets of studies were conducted in CF patients where a dose of 32.5 mg and 65 mg was inhaled respectively. Data was combined due to the observed variability across \(c_p(t)\) profiles for studies at the tested the same dose in the same patient population, suggesting considerable inter-study variation.

There was no evidence of pulmonary sequestration for CIP, and hence the pulmonary sequestration rate constants \(k_{p\text{ls}1}, k_{p\text{ls}2}, k_{c\text{ls}1},\) and \(k_{c\text{ls}2}\) were all set to a value of 10,000, ie virtually instantaneous transfer. This modification to inhalation Model 3 in essence converts the sequestration compartment to high-throughput transfer compartments. MCS were performed in an attempt to optimize the six remaining pulmonary disposition parameters \((k_{pa}, k_{ca}, k_{pd}, k_{cd}, k_{pm},\) and \(k_{cm})\) with the systemic
disposition and oral parameter fixed at their previously optimized values. No acceptable parameter space was identified, primarily due to the inability to simultaneously simulate the rapid distribution phase as well as the terminal phase specifically in CF data sets after inhalation.

Therefore, a global re-optimization with IV, PO, and INH data sets was performed to simultaneously optimize the systemic disposition, oral absorption, and pulmonary disposition parameters. It was necessary to introduce a disease state specific covariate model on each of the systemic disposition and oral absorption parameters.

5.3.4. Parameter Sensitivity Analysis

As an indicator of how sensitive plasma and sputum exposures are to the final model parameters, a sensitivity analysis was performed with the final parameter estimates after IV, PO, and INH dosing. BW was set to either 70 kg or 55 kg for HV and CF patients, respectively. For the IV sensitivity analysis, a dose of 300 mg was infused over 10 minutes, for the PO sensitivity analysis a dose of 100 mg was administered, and for the INH sensitivity analysis a dose of 32.5 mg was inhaled. The models utilized for parameter optimization were the same models utilized for the sensitivity analysis (i.e., open two compartment body model, open two compartment body model with GI compartment, and the full inhalation semi-PBPK model 3). Variability across the parameters was disabled by setting all the CV’s to zero, systemic and oral absorption parameter values were set to either one-fifth or five times the mean optimized values to cover a 25-fold range around the point estimate. Pulmonary uptake, distribution and redistribution parameters were set to one-tenth or ten-times the mean optimized values to cover a 100-fold range around the point estimate. Simulations were performed for up
to 24hrs. AUC and $c_{\text{max}}$ values in plasma and sputum were calculated at each of the extremes. The fold change was calculated as the ratio between the maximum and minimum values of the simulated plasma AUC multiplied by a negative one if the maximum value was obtained from when the parameter being tested was multiplied by $1/5$. This was also done for plasma $c_{\text{max}}$, as well as sputum AUC and $c_{\text{max}}$. Both $c_p(t)$, and $c_s(t)$ profiles were generated for comparison. The top three parameters to cause the largest-fold change in the four-exposure metrics were determined at the end of the sensitivity analysis. This sensitivity analysis would then allow for a better understanding as to how to increase sputum concentrations or how to minimize systemic exposure.

5.3.5. Assessment of Pulmonary Kinetics

To gain insight of pulmonary disposition, and the contribution of pulmonary exposure on systemic exposure, pulmonary kinetics were investigated. The total fraction of the dose that was absorbed into systemic circulation was denoted as $F_{\text{INH}}$ and was calculated as the sum of the $F_{\text{pul}}$ and $F_{\text{oral}}$ as seen in Equation 12.

$$F_{\text{INH}} \times (DtL + DtGI) = F_{\text{pul}} \times DtL + F_{\text{oral}} \times DtGI$$

**Equation 12** Calculation of bioavailability after inhalation with and without charcoal block data.

The NCA estimate of $F_{\text{INH}}$ can also be described as the product of $\text{AUC}_{\text{INH}}$ and $\frac{\text{CL}_{\text{tot}}}{\text{V}}$ divided by the total dose as in Equation 13. This calculation assumes that charcoal
block does not affect CL$_{tot}$, however it does assume that the concurrent administration of charcoal black is able to prevent any oral absorption (essentially making $F_{oral} = 0$).

\[
\frac{AUC_{INH} * CL_{IV}}{DL + DG} \cdot (DtL + DtGI) = F_{pul} \cdot DtL + F_{oral} \cdot DtGI
\]

**Equation 13** NCA method for adding $F_{pul}$ and $F_{oral}$ to determine $F_{inh}$

The availability of CIP $c_p(t)$ profiles after CIP inhalation with co-administration of charcoal allows for the derivation of Equation 14 by dividing Equation 13 utilizing the no charcoal block data by the same equation with the charcoal data set. The result is shown in 5.3.5.3, is a NCA approach to estimating pulmonary bioavailability ($F_{pul}$).

\[
F_{pul} = \frac{F_{oral} \cdot DtGI}{(AUC \text{ Ratio} - 1) \cdot DtL}
\]

**Equation 14** Explicit Solution for pulmonary bioavailability after inhalation calculated using with and without charcoal block data

Equation 15 below describes the MRT$_{INH}$ as the weighted sum of pulmonary absorption and oral absorption in addition to the MRT$_{sys}$. AUC Ratio referrers to the ratio of the AUC obtained from the Charcoal study divided by the AUC obtained from the data from when charcoal was not administered. Mean pulmonary absorption time from the pulmonary compartments into systemic circulation was therefore calculated using a modified solution of Equation 15.

\[
MRT_{INH} = MAT_{pul} \left(\frac{DtL}{DtGI + DtL}\right) + MAT_{PO} \left(\frac{DtGI}{DtGI + DtL}\right) + MRT_{sys}
\]

**Equation 15** Calculating mean residency time after INH

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5.3.6. Assessment of Mass Balance

The cumulative drug eliminated systemically, and via GI Tract (not absorbed and pulmonary transfer via mucociliary clearance) was investigated after IV, PO, and INH administration. These mass balance excretion simulations were performed up to 48 hours with a resolution of 10 minutes. These simulations were performed to understand the contribution of each elimination pathway to total drug elimination since the pulmonary model allows for transfer of CIP into the GI tract.

5.3.7. Software

For the included studies, captured \( c_p(t) \) and \( c_s(t) \) profiles were digitized using the GetData® Graph Digitizer Version 2.24 software. NCA was performed on the plasma concentration data. Model parameter fitting was performed in Scientist v. 3.0.0.215 Build: 1334 using a stiff integrator. MCS and data visualization was performed in R-studio using the deSolve tool package with lsoda switching automatically between stiff and non-stiff methods to handle the ordinary differential equations associated with the model.

5.4. Results

5.4.1. Summary of Final IV, PO, and INH Studies Identified

A search of the literature on PubMed and Google Scholar resulted in the identification of 9 publications where CIP was administered as IV infusion. These publications and their respective key study design elements are listed in Table
Two studies were excluded from the analysis, specifically CIP 103 and CIP 109. The remaining seven studies enrolled between 6 and 32 volunteers including both HV and CF patients. Doses ranged from 50 mg to 400 mg and were infused over the course of 5 to 60 minutes to both HV and CF patients. These studies only provided \( c_p(t) \) profiles, and no \( c_s(t) \) profiles were available after IV administration.

Six of the included IV studies also included PO administration data, and another five studies were identified where CIP was administered orally. Only one of the five PO administration studies was acceptable. As such a total of 7 studies were included in the PO analysis and resulted in 12 \( c_p(t) \) profiles (2 of which were from CF patients). The administered doses ranged from 100 mg to 1020 mg. All but one study specified that the administrated formulation was a tablet, and for the study in which the formulation was not specified, it was assumed to be as a tablet. The included PO administration studies along with the relevant study design parameters are tabulated in Table 29.

A total of 4 studies were identified where CIP was inhaled either HV or CF patients (n=2 for both). These studies and their key study design elements, such as number of subjects (n), mean body weight, mean age, etc., are listed in Table 30. Additionally, the availability of \( c_p(t) \), \( c_s(t) \), exposure metrics for either plasma or sputum, and availability of deposition data is also tabulated. All four studies were suitable for analysis as they satisfied the inclusion criteria. Only one device was tested in these four studies, and only two doses were tested (32.5 mg or 65 mg). The available data from these studies included 8 \( c_p(t) \) profiles and matching exposure metrics, as well as 2 \( c_s(t) \)
profiles. The Novartis’ T-326 DPI was the inhalation device utilized in this study, for which the deposition data was also reported in literature.
Seven included studies wherein CIP was administered IV. Tabulated key study design elements: number of subjects (n), subject disease state (HV – Healthy, CF – cystic Fibrosis, or other), dose regimen, bioanalytical method, sampling schedule, age (yrs), body weight, cp(t) profile, cs(t) profile, and the availability of cp(t) profile.

### Table 28: Identified IV CIP studies

<table>
<thead>
<tr>
<th>Study Identifier</th>
<th>CIP 101</th>
<th>CIP 102</th>
<th>CIP 104</th>
<th>CIP 105</th>
<th>CIP 106</th>
<th>CIP 107</th>
<th>CIP 108</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>32</td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>24</td>
<td>5, 12</td>
</tr>
<tr>
<td>Subject Disease State</td>
<td>CF</td>
<td>HV</td>
<td>HV</td>
<td>HV</td>
<td>HV</td>
<td>HV</td>
<td>HV, CF</td>
</tr>
<tr>
<td>Dose Strength</td>
<td>IV: 200 mg infusion PO: 750 mg</td>
<td>IV: 50, 100, 200 mg as a (15, 15, 20 min) infusions PO: 100, 250, 500, 750 mg</td>
<td>IV: 100 as a 6 min infusions PO: 100, 250, 500, 1000 mg</td>
<td>IV: 300, and 400 as 60 min infusions PO: 500, and 750 mg</td>
<td>IV: 400 as 60 min infusions PO: 500 mg</td>
<td>IV: 400 as 60 min infusions PO: 500 mg</td>
<td>IV: HV = 4mg/kg CF=6mg/kg as 20min infusion PO:15 mg/kg</td>
</tr>
<tr>
<td>Assay method</td>
<td>HPLC/Fluorometric Detection (0.005 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.025 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.05 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
</tr>
<tr>
<td>PK Sampling (min)</td>
<td>15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720</td>
<td>16, 20, 26, 35, 45, 60, 75, 90, 105, 120, 135, 195, 255, 375, 495, 615, 735</td>
<td>6, 14, 22, 35, 50, 65, 95, 125, 185, 245, 365, 485, 605, 725</td>
<td>90, 120, 135, 150, 180, 240, 300, 360, 420, 480, 600, 780, 1020, 1260, 1500</td>
<td>105, 165, 225, 285, 345, 420, 495, 672, 1260, 1500</td>
<td>60, 75, 90, 120, 135, 150, 180, 240, 300, 420, 540, 660, 780, 1020, 1500</td>
<td>25, 30, 35, 50, 65, 80, 110, 140, 200, 260, 380, 500, 740</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.67 ± 3</td>
<td>30 (22 - 35)</td>
<td>29 ± 9</td>
<td>27.4 ± 4.3</td>
<td>(22 - 37)</td>
<td>Young = 18 - 40 Elderly &gt;65</td>
<td>HV = 27 (21-31) CF = 21 (16 - 28)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>48.17 ± 5.55</td>
<td>67 (52 - 80)</td>
<td>75 ± 11</td>
<td>73.0 ± 6.7</td>
<td>82 (73 - 96)</td>
<td>NA</td>
<td>HV = 68 (62-73) CF = 58 (41-77)</td>
</tr>
<tr>
<td>Cp(t) Profile</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Cs(t) Profile</td>
<td>✔</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Seven included studies wherein CIP was administered PO. Tabulated key study design elements: number of subjects (n), subject disease state (HV - Healthy, CF - cystic Fibrosis state), dose regimen, bioanalytical method, sampling schedule, age (yrs), body weight (kg), availability of Cp(t) profile, and the availability of Cs(t) profile.

<table>
<thead>
<tr>
<th>Study Identifier</th>
<th>101</th>
<th>102</th>
<th>104</th>
<th>105</th>
<th>106</th>
<th>108</th>
<th>204</th>
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<tr>
<td>n</td>
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<td>32</td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>5,12</td>
<td>12,12</td>
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<tr>
<td>Subject</td>
<td>CF</td>
<td>HV</td>
<td>HV</td>
<td>HV</td>
<td>HV</td>
<td>HV, CF</td>
<td>HV, CF</td>
</tr>
<tr>
<td>Disease State</td>
<td>CF</td>
<td>HV</td>
<td>HV</td>
<td>HV</td>
<td>HV</td>
<td>HV, CF</td>
<td>HV, CF</td>
</tr>
<tr>
<td>Dose Strength</td>
<td>IV: 200 mg infusion PO: 750 mg</td>
<td>IV: 50, 100, 200 mg as a (15, 15, 20 min) infusions PO: 100, 250, 500, 750 mg</td>
<td>IV: 100 as a 6 min infusions PO: 250 mg</td>
<td>IV: 300, and 400 as 60 min infusions PO: 500 mg</td>
<td>IV: 400 as 60 min infusions PO: 500, 750 mg</td>
<td>IV: HV = 4mg/kg CF=6mg/kg as 20min infusion PO: 15 mg/kg</td>
<td>PO: 500 mg Q8H</td>
</tr>
<tr>
<td>Formulation</td>
<td>NA</td>
<td>Tablet</td>
<td>Tablet</td>
<td>Tablet</td>
<td>Tablet</td>
<td>Tablet</td>
<td>Tablet</td>
</tr>
<tr>
<td>Assay method</td>
<td>HPLC/Fluorometric Detection (0.005 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.025 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
<td>HPLC/Fluorometric Detection (0.01 mg/L)</td>
</tr>
<tr>
<td>PK Sampling (min)</td>
<td>15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720</td>
<td>16, 20, 26, 35, 45, 60, 75, 90, 105, 120, 135, 195, 255, 375, 495, 615, 735</td>
<td>6, 14, 22, 35, 50, 65, 95,125, 185, 245, 365, 485, 605, 725, 90, 120, 135, 150, 180, 240, 300, 360, 420, 480, 660, 780, 1020, 1260, 1500</td>
<td>105, 165, 225, 285, 345, 420, 495, 672, 1260, 1500</td>
<td>25, 30, 35, 50, 65, 80, 110, 140, 200, 260, 380, 500, 740</td>
<td>0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 480, 600</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.67 ± 3</td>
<td>30 (22 - 35)</td>
<td>29 ± 9</td>
<td>27.4 ± 4.3</td>
<td>(22 - 37)</td>
<td>HV = 27 (21-31)</td>
<td>CF = 21 (16 - 28)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>48.17 ± 5.55</td>
<td>67 (52 - 80)</td>
<td>75 ± 11</td>
<td>73.0 ± 6.7</td>
<td>82 (73 - 96)</td>
<td>HV = 68 (62-73)</td>
<td>CF = 58 (41-77)</td>
</tr>
<tr>
<td>Cp(t) Profile</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Cs(t) Profile</td>
<td>✔</td>
<td>✔</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Table 30 Identified INH CIP studies

Four included studies wherein CIP was administered via INH. Tabulated key study design elements: number of subjects (n), subject disease state (HV - Healthy, CF – cystic Fibrosis, or other), dose regimen, bioanalytical method, sampling schedule, age (yrs), body weight, availability of \( c_p(t) \) profile, and the availability of \( c_s(t) \) profile

5.4.2. Assessment of PK Dose-Proportionality

NCA was performed on the 12 individual data sets obtained from the seven included studies. Visual inspection for each of the log-linear regression of the terminal slope used in estimating \( \lambda \) were acceptable, \( R^2 \) values were all larger than 0.9645, and the AUC percent extrapolated did not exceed 10% for each of the individual data sets analyzed. The estimated PK parameters are tabulated in Table 31.
Table 31 NCA estimated PK parameters for each of the individual data sets from the seven included studies. (CIP IV administration)

Inspection of the AUC vs IV dose plotted on a linear scale as well as on a log-log plot did not provide any evidence of deviation from linear PK after IV administration.

When a linear and a power model was fit to both plots respectively, the resulting $R^2$ value was 0.851 and the exponential coefficient of 1.44. These plots are shown in Figure 24.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Cl}_{\text{tot}}$ (mL/min)</td>
<td>579</td>
<td>603</td>
<td>737</td>
<td>844</td>
<td>678</td>
<td>327</td>
<td>436</td>
<td>270</td>
<td>424</td>
<td>316</td>
<td>643</td>
<td>467</td>
</tr>
<tr>
<td>BW Normalized $\text{Cl}_{\text{tot}}$ (mL/min/kg)</td>
<td>12.0</td>
<td>12.0</td>
<td>11.0</td>
<td>12.6</td>
<td>9.0</td>
<td>4.5</td>
<td>6.0</td>
<td>3.3</td>
<td>11.0</td>
<td>6.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Vd}_{\text{ss}}$ (L)</td>
<td>96</td>
<td>206</td>
<td>196</td>
<td>216</td>
<td>148</td>
<td>77</td>
<td>102</td>
<td>85</td>
<td>100</td>
<td>74</td>
<td>125</td>
<td>90</td>
</tr>
<tr>
<td>BW Normalized $\text{Vd}_{\text{ss}}$ (L/kg)</td>
<td>2.002</td>
<td>3.073</td>
<td>2.930</td>
<td>3.226</td>
<td>1.974</td>
<td>1.048</td>
<td>1.397</td>
<td>1.042</td>
<td>2.162</td>
<td>1.320</td>
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</tr>
<tr>
<td>$\text{AUC}$ (mg\text{-}min/L)</td>
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<td>62</td>
<td>136</td>
<td>237</td>
<td>147</td>
<td>918</td>
<td>918</td>
<td>1480</td>
<td>942</td>
<td>1267</td>
<td>541</td>
<td>552</td>
</tr>
<tr>
<td>MRT$_{sys}$ (min)</td>
<td>166</td>
<td>256</td>
<td>266</td>
<td>258</td>
<td>218</td>
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<td>234</td>
<td>316</td>
<td>236</td>
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<td>230</td>
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<td>239</td>
<td>226</td>
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<td>191</td>
</tr>
<tr>
<td>% Extrapolated</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>$r^2$</td>
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<td>0.9993</td>
<td>0.9993</td>
<td>0.9966</td>
<td>0.9972</td>
<td>0.9948</td>
<td>0.9948</td>
<td>0.9945</td>
<td>0.9978</td>
<td>0.9964</td>
<td>0.9872</td>
<td>0.9966</td>
</tr>
</tbody>
</table>

Figure 24 CIP IV dose escalation plots

IV infusion dose dependence plotting AUC with increasing IV dose on a linear plot with a linear regression through the estimated AUC values (left plot), and on a log-log plot with a power model fit to the estimated AUC values (right plot).

The same analysis was performed with the available data after PO administration. When a linear and a power model was fit to both plots respectively, the
resulting $R^2$ value was 0.854 and the exponential coefficient of 1.05. These plots are shown in Figure 25. Additionally, a linear model was fit to PO dose vs $c_{\text{max}}$ data set, and the resulting $R^2$ was 0.82. The data and linear fit model are shown in Figure 26 below.

**Figure 25 CIP PO dose escalation plots**

PO dose dependence plotting AUC with increasing PO dose on a linear plot with a linear regression through the estimated AUC values (left plot), and on a log-log plot with a power model fit to the estimated AUC values (right plot). Blue dots indicate data reported for HV while orange dots indicate data reported for CF patients.

**Figure 26 CIP $c_{\text{max}}$ with increase PO dose**

PO dose escalation plotting $c_{\text{max}}$ with increasing PO dose on a linear plot with a linear regression through the estimated $c_{\text{max}}$ values (left plot). Blue dots indicate data reported for HV while orange dots indicate data reported for CF patients.

For the available data after INH, the analysis was performed on the total dose that was deposited in the body. That deposited dose was defined as the sum of the
dose deposited in the lungs and the dose deposited in the GI tract. When a linear and a power model was fit to both plots respectively, the resulting $R^2$ value was 0.7125 and the exponential coefficient of 1.08. These plots are shown in Figure 27. Additionally, a linear model was fit to inhaled dose vs $c_{\text{max}}$ data set, and the resulting $R^2$ was 0.68. The data and linear fit model are shown in Figure 28 below. As such, there was no evidence of nonlinearity.

**Figure 27  CIP INH dose escalation plots**

Pulmonary deposition dose dependence plotting AUC with increasing total deposited dose in the lungs and the GI on a linear plot with a linear regression through the estimated AUC values (left plot), and on a log-log plot with a power model fit to the estimated AUC values (right plot). Blue dots indicate data reported for HV while orange dots indicate data reported for CF patients.
**Figure 28 CIP $c_{\text{max}}$ with increase INH dose**

INH dose escalation plotting $c_{\text{max}}$ with increasing INH dose on a linear plot with a linear regression through the estimated $c_{\text{max}}$ values (left plot). Blue dots indicate data reported for HV while orange dots indicate data reported for CF patients.

Overall, there is no evidence of deviation from dose-proportional PK after IV, PO, and INH administration.

**5.4.3. Pulmonary Bioavailability**

Across the four inhalation studies analyzed, the $F_{\text{pul}}$ via the AUC ratio method demonstrated discussed above ranged from 11% to 76%. Study 303B was the study where CIP was co-administered with charcoal. The average $F_{\text{pul}}$ for HV was 46% while the average for CF patients was 34%. It is of note however, that only two studies were available with data for HV. Similarly, the average $MAT_{\text{pul}}$ was 593 min for HV, and 268 minutes for CF patients. The individual values for each of the studies in tabulated below in Table 32.
Calculated $F_{\text{pul}}$ values using NCA estimates values and CIP 303B as the charcoal reference. MAT$_{\text{pul}}$ also used NCA estimates from IV and PO studies as a reference.

### 5.4.4. PK Covariate Exploration

$\text{CL}_{\text{tot}}$, $\text{CL}_{\text{nonren}}$, $\text{CL}_{\text{ren}}$, and $\text{Vd}_{\text{ss}}$ were plotted against administered dose, age, body weight, and disease state. Disease state appeared to be the only covariate that may be related to the PK parameters. For HV, both $\text{Vd}_{\text{ss}}$ and $\text{CL}_{\text{nonren}}$ were 129 L and 309 ml/min respectively which were notably larger than those for CF; 111 L and 207 mL/min, respectively. However, the opposite was try for $\text{CL}_{\text{tot}}$ and $\text{CL}_{\text{ren}}$ with CF patients having values of 611 mL/min and 381 mL/min, respectively, while HV had a $\text{CL}_{\text{tot}}$ value of 530 mL/min and a $\text{CL}_{\text{ren}}$ values of 279 mL/min. These comparisons are visually represented in Figures 29 through 32 below. When each of the PK parameters were body weight normalized and compared across disease state, the difference was magnified.

<table>
<thead>
<tr>
<th>Study</th>
<th>$F_{\text{pul}}$ (%)</th>
<th>MAT$_{\text{pul}}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>301A</td>
<td>17</td>
<td>678</td>
</tr>
<tr>
<td>302A</td>
<td>22</td>
<td>196</td>
</tr>
<tr>
<td>302B</td>
<td>36</td>
<td>286</td>
</tr>
<tr>
<td>303A</td>
<td>76</td>
<td>508</td>
</tr>
<tr>
<td>303B</td>
<td>46</td>
<td>377</td>
</tr>
<tr>
<td>304A</td>
<td>21</td>
<td>305</td>
</tr>
<tr>
<td>304B</td>
<td>78</td>
<td>338</td>
</tr>
<tr>
<td>304C</td>
<td>11</td>
<td>213</td>
</tr>
</tbody>
</table>

**Table 32 CIP INH NCA analysis**

Calculated $F_{\text{pul}}$ values using NCA estimates values and CIP 303B as the charcoal reference. MAT$_{\text{pul}}$ also used NCA estimates from IV and PO studies as a reference.
Figure 29 Comparison between NCA and model optimized $V_{dss}$

$V_{dss}$ obtained through NCA plotted against disease state for individual data sets within studies. Blue dots resemble individual studies while red lines represent model optimized values.

Figure 30 Comparison between NCA and model optimized $CL_{tot}$

$CL_{tot}$ obtained through NCA plotted against disease state for individual data sets within studies. Blue dots resemble individual studies while red lines represent model optimized values.
**Figure 31 Comparison between NCA and model optimized \( \text{CL}_{\text{ren}} \)**

\( \text{CL}_{\text{ren}} \) obtained through NCA plotted against disease state for individual data sets within studies. Blue dots resemble individual studies while red lines represent model optimized values.

**Figure 32 Comparison between NCA and model optimized \( \text{CL}_{\text{nonren}} \)**

\( \text{CL}_{\text{nonren}} \) obtained through NCA plotted against the disease state for individual data sets within studies. Blue dots resemble individual studies while red lines represent model optimized values.

For PO studies, exploratory plots of \( F_{\text{oral}}, \text{MAT}^{\text{PO}}, \) and \( k_{ga} \) vs dose, age, body weight, and disease state were inspected to determine if there were covariates for those PK parameters. Similar to the data shown for the IV studies, the PO PK parameters appeared to be related to BW. \( F_{\text{oral}}, \text{MAT}^{\text{po}}, \) and \( k_{ga} \) were 56\%, 53 min, and 0.028 min\(^{-1}\), respectively for HV, while 78\%, 95 min, and 0.011 min\(^{-1}\) for CF patients, respectively.
These three PK parameters are plotted against the disease state in Figures 33, 34, and 35 respectively.

**Figure 33 Comparison between NCA and model optimized $F_{oral}$**

$F_{oral}$ obtained through NCA plotted against disease state for individual data sets within studies. Blue dots resemble individual studies while red lines represent model optimized values.

**Figure 34 Comparison of MAT$^{PO}$ between HV and CF patients**

MAT$^{po}$ obtained through NCA plotted against disease state for individual data sets within studies. Blue dots resemble individual studies.
5.4.5. Modeling of IV Data

Successful fits were obtained for each of the twelve data sets from the seven studies. MSC values are higher than 4.9, $R^2$ values were above 0.9950 for all the selected fits, correlation matrix values were all below 0.5, coefficients of variation for each of the parameter estimates were below 30%, and the visual inspection of the observed versus simulated values were all acceptable. Weighting factor varied from data set to data set and was selected based on the weight factor that resulted in the most acceptable results. Table 33 shows the resulting parameter values based on the fits produced by the individual data sets. The model file for the various fits are reported in Appendix 3.
Table 33 NCA results for the seven selected CIP IV studies

Two compartment model parameter estimates and goodness of fit validation criteria results for each of the twelve data sets obtained from the final seven included IV studies.

Global MCS across studies and across dosing routes resulted in an acceptable parameter space that described observed $c_p(t)$ profiles and exposure metrics after IV administration. The five systemic disposition parameters were optimized with a binary disease state covariate model for HV or CF following a normal distribution. For each disease state, there was an optimized central tendency (mean) and a variation (CV) as shown in Table 34. Optimized parameter values indicate HV have lower $\text{CL}_{\text{ren}}$ (315 mL/min) as compared to CF (425 mL/min), however, the optimized $\text{CL}_{\text{nonren}}$ was faster (315 mL/min) compared with the CF patients (225 mL/min). $k_{12}$ and $k_{21}$ were both faster for CF patients than for HV; 0.050 and 0.020 min$^{-1}$ respectively, and 0.025 and 0.015 min$^{-1}$ respectively. Lastly, HV had a notably higher $V_0$ (75 L) than the optimized $V_0$ value for CF patients (20 L). With exception of $k_{12}$ and $k_{21}$ for HV, which followed a normal distribution with a 25% CV, all other systemic disposition parameters followed a normal distribution with a 20% CV.
Table 34 Final optimized systemic disposition model parameters for CIP with specified underlying covariate models and distributions

Table 35 indicates the acceptability of the VI of the $c_p(t)$ profiles as well as the percent difference for the exposure metrics for each of the data sets. The predicted AUC was on average 7.9% lower than the reported AUC, with a 12% standard deviation. Additionally, predicted SD for each of the individual studies were below the reported SD in 10 out of 12 of the analyzed data sets. Sample results of the VI of the $c_p(t)$ profile can be seen in Figure 36 for study CIP 104A where 100 mg was IV infused in HV. The remaining VI are included in Appendix 3.
Table 35 Predicted vs reported AUC exposure metrics for individual analyzed IV data sets using MCS approach

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Group</th>
<th>BW (kg)</th>
<th>Dose (mg)</th>
<th>Infusion Time (min)</th>
<th>(c_p(t))</th>
<th>Reported AUC (mg*hr/L)</th>
<th>AUC SD</th>
<th>Predicted AUC (mg*hr/L)</th>
<th>AUC SD</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>101A</td>
<td>CF</td>
<td>48</td>
<td>200</td>
<td>15</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>5.12</td>
<td>0.70</td>
<td>-3.0</td>
</tr>
<tr>
<td>102A</td>
<td>HV</td>
<td>67</td>
<td>50</td>
<td>15</td>
<td>✓</td>
<td>1.2</td>
<td>0.20</td>
<td>1.16</td>
<td>0.16</td>
<td>-3.9</td>
</tr>
<tr>
<td>102B</td>
<td>HV</td>
<td>67</td>
<td>100</td>
<td>1</td>
<td>✓</td>
<td>2.9</td>
<td>0.52</td>
<td>2.32</td>
<td>0.34</td>
<td>-20.0</td>
</tr>
<tr>
<td>102C</td>
<td>HV</td>
<td>67</td>
<td>200</td>
<td>202</td>
<td>✓</td>
<td>5.3</td>
<td>1.12</td>
<td>4.67</td>
<td>0.64</td>
<td>-12.1</td>
</tr>
<tr>
<td>104A</td>
<td>HV</td>
<td>75</td>
<td>100</td>
<td>5</td>
<td>✓</td>
<td>1.8</td>
<td>0.42</td>
<td>2.1</td>
<td>0.32</td>
<td>17.4</td>
</tr>
<tr>
<td>105A</td>
<td>HV</td>
<td>73</td>
<td>300</td>
<td>6</td>
<td>✓</td>
<td>8.6</td>
<td>1.5</td>
<td>6.9</td>
<td>0.97</td>
<td>-20.0</td>
</tr>
<tr>
<td>105B</td>
<td>HV</td>
<td>73</td>
<td>400</td>
<td>6</td>
<td>✓</td>
<td>11.6</td>
<td>1.6</td>
<td>9.2</td>
<td>1.29</td>
<td>-16.6</td>
</tr>
<tr>
<td>106A</td>
<td>HV</td>
<td>82</td>
<td>400</td>
<td>60</td>
<td>✓</td>
<td>12.0</td>
<td>1.15</td>
<td>10.2</td>
<td>1.29</td>
<td>-15.0</td>
</tr>
<tr>
<td>107A</td>
<td>HV</td>
<td>75</td>
<td>400</td>
<td>60</td>
<td>✓</td>
<td>10.0</td>
<td>1.8</td>
<td>9.2</td>
<td>1.29</td>
<td>-8.4</td>
</tr>
<tr>
<td>108A</td>
<td>CF</td>
<td>58</td>
<td>348</td>
<td>20</td>
<td>✓</td>
<td>8.4</td>
<td>1.76</td>
<td>9.04</td>
<td>1.34</td>
<td>8.4</td>
</tr>
<tr>
<td>108B</td>
<td>HV</td>
<td>68</td>
<td>272</td>
<td>5</td>
<td>✓</td>
<td>8.6</td>
<td>0.72</td>
<td>7.8</td>
<td>0.88</td>
<td>-9.0</td>
</tr>
</tbody>
</table>

Average percent difference (prediction error) -7.9

Standard Deviation 12

Figure 36 CIP 104 \(c_p(t)\) VPC after a 100 mg IV dose in HV

5.4.6. Modeling of PO Data

Global MCS across studies and across dosing routes resulted in an acceptable parameter space that described observed \(c_p(t)\) profiles and exposure metrics after PO administration. Both oral absorption parameters were optimized with a binary disease
state covariate model for HV or CF following a normal distribution. For each disease state, there was an optimized central tendency (mean) and a variation (CV) as shown in Table 36. $F_{oral}$ for HV was optimized to mean a point estimate of 73% with a 35% CV, and 85% with a 30% CV for CF. $k_{ga}$ was optimized to $0.020\text{ min}^{-1}$ with a 10% CV for HV, and $0.011\text{ min}^{-1}$ with a CV of 50% for CF patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population Value</th>
<th>Distribution calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{oral}$(%)</td>
<td>$F_{oral} = \frac{HV}{CF} \sim N(73.35, 0.35)$</td>
<td></td>
</tr>
<tr>
<td>$k_{ga}$(min$^{-1}$)</td>
<td>$k_{ga} = \frac{HV}{CF} \sim LN(0.020, 0.10)$</td>
<td></td>
</tr>
</tbody>
</table>

Table 36 Final optimized oral absorption model parameters for CIP with specified underlying covariate models and distributions

Table 37 indicates the acceptability of the VI of the $c_p(t)$ profiles as well as the percent difference for the exposure metrics for each of the PO data sets. The predicted AUC was on average 0.12% lower than the reported AUC, with a 17% standard deviation. Additionally, there was, on average an underprediction in $c_{max}$ by 13% with a 15% SD. The predicted SD for both AUC and $c_{max}$ for each of the individual data sets was numerically similar for all but three data sets where the predicted SD was +50% larger than the reported SD. Sample results of the VI of the $c_p(t)$ profile can be seen in Figure 37 for study CIP 204B where 500 mg was orally administered in HV. The remaining VI are included in Appendix 3.
Table 37 Predicted vs reported AUC exposure metrics for individual analyzed PO data sets using MCS approach

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Study ID</th>
<th>BW (kg)</th>
<th>Dose (mg)</th>
<th>c(t)</th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AUC (mg*a/hr/L)</td>
<td>AUC SD</td>
<td>C_{max} (mg/L)</td>
</tr>
<tr>
<td>Healthy Volunteers</td>
<td>102A</td>
<td>67</td>
<td>100</td>
<td>✓</td>
<td>1.8</td>
<td>0.6</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>102B</td>
<td>67</td>
<td>250</td>
<td>✓</td>
<td>4.2</td>
<td>1.1</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>102C</td>
<td>67</td>
<td>500</td>
<td>✓</td>
<td>6.8</td>
<td>1.3</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>102D</td>
<td>67</td>
<td>750</td>
<td>✓</td>
<td>8.8</td>
<td>1.1</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>104A</td>
<td>75</td>
<td>750</td>
<td>✓</td>
<td>3.3</td>
<td>1.22</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>105A</td>
<td>73</td>
<td>500</td>
<td>✓</td>
<td>10.7</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>105B</td>
<td>73</td>
<td>750</td>
<td>✓</td>
<td>16.8</td>
<td>4.8</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>106A</td>
<td>82</td>
<td>500</td>
<td>✓</td>
<td>9</td>
<td>1.22</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td>108B</td>
<td>68</td>
<td>1020</td>
<td>✓</td>
<td>18.6</td>
<td>4.82</td>
<td>4.47</td>
</tr>
<tr>
<td></td>
<td>204B</td>
<td>22</td>
<td>500</td>
<td>✓</td>
<td>10</td>
<td>2.78</td>
<td>2.72</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>108A</td>
<td>58</td>
<td>870</td>
<td>✓</td>
<td>17.2</td>
<td>4.25</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>204A</td>
<td>21</td>
<td>500</td>
<td>✓</td>
<td>11.07</td>
<td>3.43</td>
<td>2.84</td>
</tr>
</tbody>
</table>

Average percent difference (prediction error) = -0.12, -13

Standard Deviation

---

Figure 37 CIP 204B c(t) VPC after a 500 mg PO dose in HV
5.4.7. Modeling of INH Data

Global MCS across studies and across dosing routes resulted in an acceptable parameter space that described observed \( c_p(t) \) profiles and exposure metrics after INH administration. All 6 pulmonary disposition rate constants followed the same log-normal distributions for both HV and CF as shown in Table 38. Both mucociliary clearance values \( (k_{pm} \text{ and } k_{cm}) \) were identical at 0.02 \( \text{min}^{-1} \), while \( k_{pa} \) was half of \( k_{ca} \) (0.01 and 0.02 \( \text{min}^{-1} \), respectively). \( k_{pd} \) was optimized to a value of 0.008 \( \text{min}^{-1} \) and \( k_{cd} \) was much slower at a value of 0.0002 \( \text{min}^{-1} \). All pulmonary rate constants were associated with a variability of 20% CV.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population Value</th>
<th>Distribution calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{pa} ) (min(^{-1}))</td>
<td>0.01</td>
<td>LN(0.01,75)</td>
</tr>
<tr>
<td>( k_{ca} ) (min(^{-1}))</td>
<td>0.02</td>
<td>LN(0.02,75)</td>
</tr>
<tr>
<td>( k_{pm} ) (min(^{-1}))</td>
<td>0.02</td>
<td>LN(0.02,75)</td>
</tr>
<tr>
<td>( k_{cm} ) (min(^{-1}))</td>
<td>0.02</td>
<td>LN(0.02,75)</td>
</tr>
<tr>
<td>( k_{pd} ) (min(^{-1}))</td>
<td>0.008</td>
<td>LN(0.008,75)</td>
</tr>
<tr>
<td>( k_{cd} ) (min(^{-1}))</td>
<td>0.0002</td>
<td>LN(0.0002,75)</td>
</tr>
<tr>
<td>TLV (ml)</td>
<td>30</td>
<td>( TLV = TLV_{pop} \times \left( \frac{BW}{BW_{pop}} \right)^{1} )</td>
</tr>
<tr>
<td>CLV (ml)</td>
<td>24</td>
<td>( CLV = TLV \times 0.8 )</td>
</tr>
</tbody>
</table>

Table 38 Final optimized pulmonary disposition model parameters with specified underlying distributions

Table 39 demonstrates the acceptability of the VI of the grouped \( c_p(t) \) profiles as well as the percent difference for the exposure metrics for each of the INH data sets. Both reported \( c_s(t) \) profiles were compared with the predicted \( c_s(t) \) profiles and were deemed acceptable per the VI criteria specified in Chapter Two. No sputum exposure metrics were available for comparison. The predicted plasma AUC was on average
8.2% higher than the reported AUC, with a 26% SD. Additionally, there was on average an underprediction in $c_{\text{max}}$ by 4.9% with a 7.2% SD. Sample results of the VI of the combined $c_p(t)$ profile can be seen in Figure 38 for studies CIP 302A and 304A where 32.5 mg was INH by CF patients. A comparison of one of the two reported $c_s(t)$ profiles with the predicted $c_s(t)$ profiles after 65 mg was inhaled by CF patients is shown in Figure 39. The remaining VI are included in Appendix 3.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Group</th>
<th>Device</th>
<th>Dose (mg)</th>
<th>DTL (%)</th>
<th>Estimated DTL (mg)</th>
<th>$c_p(t)$</th>
<th>$c_s(t)$</th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AUC (ug*h/l)</td>
<td>AUC SD</td>
<td>$c_{\text{max}}$ (ug/l)</td>
</tr>
<tr>
<td>301A + 303A</td>
<td>HV</td>
<td></td>
<td>32.5</td>
<td>17</td>
<td>✓</td>
<td>482</td>
<td>79</td>
<td>432</td>
<td>139 67</td>
<td>19</td>
</tr>
<tr>
<td>302A + 304A</td>
<td>CF Novartis T-326 DP</td>
<td></td>
<td>32.5</td>
<td>53</td>
<td>17</td>
<td>✓ ✓</td>
<td>520</td>
<td>128</td>
<td>686</td>
<td>176</td>
</tr>
<tr>
<td>302B + 304B</td>
<td>CF</td>
<td></td>
<td>65</td>
<td>34</td>
<td>✓ ✓</td>
<td>1237</td>
<td>289</td>
<td>1373</td>
<td>351 265</td>
<td>78</td>
</tr>
</tbody>
</table>

Average percent difference (prediction error) 
Standard deviation of percent difference across studies

| Table 39 Predicted vs reported AUC exposure metrics for individual analyzed INH data sets using MCS approach |

**Figure 38 CIP INH $c_p(t)$ VPC Plot for CIP 302A and 304A**

VPC for model predicted $c_p(t)$ compared with the average reported $c_p(t)$ profiles from study CIP 302A and CIP 304A VPC after a 32.5 mg dose was inhaled by CF patients
VPC for model predicted $c_s(t)$ compared with the reported $c_s(t)$ profile from study CIP 302B after a 65 mg dose was inhaled by CF patients.

5.4.8. Parameter Sensitivity Analysis Results

SD sensitivity analysis results for both HV and CF patients were generated for the five systemic disposition parameters and are tabulated in Table 40. For a 25-fold change in the model parameters, AUC was most sensitive to changes in $CL_{ren}$ in HV and CF patients (3.7- and 7.2-fold, respectively), and $c_{max}$ was most sensitive to $V_0$ in both groups (21- and 14-fold respectively). Predicted $c_p(t)$ profile after the 25-fold change in $CL_{ren}$ is shown in Figure 40 for HV and CF patients.
Table 40 Single IV dose sensitivity analysis results for both HV and CF patients after a 100 mg infusion over 10 minutes.

Figure 40 Resulting $c_p(t)$ from a 25-fold change in $CL_{ren}$ after a SD IV infusion of 100 mg over 10 minutes in HV (left plot) and CF (right plot).

SD sensitivity analysis results for both HV and CF patients were generated for the five systemic disposition parameters as well as both oral absorption parameters and are tabulated in Table 41. AUC and $c_{max}$ were most sensitive to $F_{oral}$ with a 25-fold change in $F_{oral}$ directly resulting in a 25-fold change in both AUC and $c_{max}$ in HV and CF patients, as expected. $CL_{ren}$ was the second most influential model parameter on AUC exposure, while $k_{ga}$ was most influential on $c_{max}$. Predicted $c_p(t)$ profile after the 25-fold change in $CL_{ren}$ is shown in Figure 41 for HV and CF patients.
Table 41 Single PO dose sensitivity analysis results for both HV and CF patients after a 100 mg administration

<table>
<thead>
<tr>
<th>PO</th>
<th>HV</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>c&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;ren&lt;/sub&gt;</td>
<td>-3.6</td>
<td>-1.5</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;nonren&lt;/sub&gt;</td>
<td>-3.6</td>
<td>-1.5</td>
</tr>
<tr>
<td>k&lt;sub&gt;12&lt;/sub&gt;</td>
<td>-2.1</td>
<td>-3.9</td>
</tr>
<tr>
<td>k&lt;sub&gt;21&lt;/sub&gt;</td>
<td>1.9</td>
<td>1.5</td>
</tr>
<tr>
<td>V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>-2.8</td>
<td>-12</td>
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<tr>
<td>k&lt;sub&gt;ga&lt;/sub&gt;</td>
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<td>F&lt;sub&gt;oral&lt;/sub&gt;</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 41 Resulting c<sub>p(t)</sub> from a 25-fold change in k<sub>ga</sub> after a SD PO administration of 100 mg in HV (left plot) and CF (right plot).  

SD sensitivity analysis results for both HV and CF patients were generated for all model parameters (five systemic disposition, two oral absorption, and six pulmonary disposition parameters) and are tabulated in Table 42 Plasma AUC depends primarily on F<sub>oral</sub>, V<sub>0</sub>, and Cl<sub>ren</sub> in HV, and Cl<sub>ren</sub>, k<sub>12</sub>, and Cl<sub>nonren</sub> in CF patients. Plasma c<sub>max</sub> depends primarily on V<sub>0</sub>, F<sub>oral</sub>, and k<sub>12</sub> in HV, and V<sub>0</sub>, k<sub>12</sub>, and k<sub>ga</sub> in CF patients.
AUC depends primarily on $k_{cm}$, $k_{ca}$, and $k_{pd}$ in both HV and CF patients. Predicted $c_p(t)$ and $c_s(t)$ profiles after the 100-fold change in $k_{cm}$ is shown in Figure 42 and Figure 43 for HV and CF patients, respectively.

<table>
<thead>
<tr>
<th>INH</th>
<th>HV</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma AUC</td>
<td>Sputum AUC</td>
</tr>
<tr>
<td></td>
<td>$c_{max}$</td>
<td></td>
</tr>
<tr>
<td>CL_{ren}</td>
<td>-2.5  -1.3</td>
<td>-1.3</td>
</tr>
<tr>
<td>CL_{nonren}</td>
<td>-2.5  -1.3</td>
<td>-1.3</td>
</tr>
<tr>
<td>$k_{s2}$</td>
<td>-1.9  -2.3</td>
<td>-1.3</td>
</tr>
<tr>
<td>$k_{21}$</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>V_{0}</td>
<td>-5.1  -16</td>
<td>1.6</td>
</tr>
<tr>
<td>$k_{ga}$</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>F_{oral}</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>$k_{pa}$</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>$k_{ca}$</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>$k_{pm}$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$k_{cm}$</td>
<td>-1.2</td>
<td>-1.1</td>
</tr>
<tr>
<td>$k_{pd}$</td>
<td>-1.9</td>
<td>-1.8</td>
</tr>
<tr>
<td>$k_{cd}$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 42 Sensitivity analysis results for both HV and CF patients after inhalation of a single 32.5 mg dose
Figure 42 Resulting $c_p(t)$ and $c_s(t)$ (left and right plot respectively) from a 100-fold change in $k_{cm}$ after a SD INH of 32.5 mg in HV.

Figure 43 Resulting $c_p(t)$ and $c_s(t)$ (left and right plot respectively) from a 100-fold change in $k_{cm}$ after a SD INH of 32.5 mg in CF.
The resulting plasma and sputum profiles after SD are shown in Appendix 3 as Figure 15 – 27. The code used to perform the sensitivity analysis is shown in Appendix 3.

5.4.9. Assessment of Mass Balance Results

After an IV infusion in HV, 1.3% of the dose is predicted to be eliminated through distribution into the lungs and subsequently being mucociliary cleared into the GI tract for elimination. This percentage was notably larger in CF patients, with an estimated 8% of the dose being cleared through the same pathway. 98.7% and 92% respectively for HV and CF patients was cleared through either renal or nonrenal clearance after IV administration.

![Figure 44: CIP IV administration cumulative excretion](image)

**Figure 44 CIP IV administration cumulative excretion**

Cumulative excretion from the GI tract after IV administration of a 300 mg dose in HV (left plot) and CF patients (right plot).

After PO administration, 33% and 16% was eliminated from the GI tract in both HV and CF patients respectively, and the remaining portions were eliminated through systemic clearance mechanisms from the central compartment.
Cumulative excretion from the GI tract after PO administration of a 100 mg dose in HV (left plot) and CF patients (right plot)

However, after INH, 27% and 12% of the deposited dose was eliminated from the GI tract while the remaining 73% and 88% was eliminated from the central compartment in HV and CF patients, respectively.
5.5. Discussions and Conclusions

5.5.1. Study Limitations

For IV study CIP 101, the infusion time was not specified within the study, as such, a 15-minute infusion time was assumed. This was based on other studies performed during the same time period. From the nine identified IV studies, two studies were eliminated due to an inadequate analytical method, and insufficient reported data. The remaining seven studies provided a total of twelve $c_p(t)$ profiles for analysis. Out of the twelve PO studies, only 6 studies that provided twelve $c_p(t)$ profiles used in analysis. Lastly, all four INH studies identified were included in analysis and provided a total of 8 $c_p(t)$ and 2 $c_{s(t)}$ profiles. Given that CIP was developed and the majority of the clinical
investigations were performed in the early 1980s, this resulted in the limited availability of data or the reliability of some of the analytical methods that were experimentally used.

Furthermore, none of the IV and PO studies provide sputum exposure data in either HV or CF patients, and there were needed limited sputum data available for CF patients after INH and none was available for HV after INH. The availability of such data would have allowed for more confidence in the pulmonary disposition parameters. Additionally, there was no repeat dose studies available for IV, PO, or INH in either HV and CF. These studies would have been useful in studying possible drug accumulation in the various compartments as well as time dependence. We were also unable to obtain any data for the pediatric population, whether HV or CF patients. This is notable since they may often be the targeted population for this treatment. The lack of access to individual patient level data (both plasma and sputum) limited the ability to study the possible effects of covariate on both plasma and sputum exposures. Lastly, the INH data available was produced from the same device across the four studies. Additional data for various devices including pressurized inhalers and nebulizers would have been useful in studying the effect of device of plasma and sputum exposure.

5.5.2. PK Dose-Proportionality

The linear dose dependence plot after IV infusion shown in Figures 24 comparing the infused dose to the NCA calculated AUC, supported linear PK based on the high $r^2$ value for the linear model. Additionally, when the same data were plotted on a log-log scale (shown in the same Figure), and a power model was utilized for fitting the data, the exponential term is close to 1.0 which would also indicate linear PK. The PO data
available also supported linear PK as can be seen in both the linear fit as well as the power fit of the dose vs AUC data shown in Figure 2. Additionally, PO dose vs $c_{\text{max}}$ was inspected in Figure 26 and also supported the linear PK conclusion. The same conclusion can be drawn after CIP is INH based on the results shown in Figure 27 and Figure 28. This finding is in agreement with the literature as several authors have also reported that CIP follows linear PK across a similar dose range.\textsuperscript{109,124} These pieces of evidence provided a strong basis for the major modeling assumption, namely that all the rate constants were all first order rate constants.

5.5.3. Final Parameter Space after IV Infusion

An empiric two compartment open body model of IV infused CIP with an optimized model parameter space resulted in mechanistically plausible parameter estimates and was able to adequately predict the reported $c_p(t)$ profiles from clinical PK studies with HV and CF after a single dose. For both HV and CF patients, the peripheral compartment behaved as a high capacity, shallow compartment. Model optimized values indicated that CIP undergoes both renal and nonrenal clearance. The renal clearance values exceeded 120 mL/min suggesting that CIP undergoes net tubular secretion. CF patients had similar nonrenal clearance values as HV, and the numerical difference between both groups was further minimized when $\text{CL}_{\text{nonren}}$ was normalized by BW. However, for $\text{CL}_{\text{ren}}$, there was a stark difference between CF patients and HV; that difference was made more apparent when their respective values were BW normalized. Some authors have suggested that this increased renal clearance in CF patients may be due to overexpression for heightened activity of other transporters in the renal-tubule due to the mutation of the CFTR transporter.\textsuperscript{125}
When the optimized pulmonary disposition and oral absorption parameters were introduced to the empiric model, and CIP was administered via IV infusion, CIP was predominantly cleared through clearance from the central compartment with nearly 99% and 92% of the dose eliminated via systemic clearance (sum of renal or nonrenal clearances) in HV and CF patients respectively. After pulmonary excretion and transfer into GI tract, the remaining 1% for HV and 8% for CF was eliminated from the GI tract. These results may explain why none of the *in-vivo* clinic studies reported sputum exposure after IV administration. Additionally, this would also explain that IV administration is a sub-optimal dosing route when the lungs are being targeted as the site of action as in the case of CF.

### 5.5.4. Final Parameter Space after PO Administration

The final optimized oral absorption model and parameters for CIP suggested that HV and CF patients have a similar extent of absorption (73% and 85% respectively). Since it has already been shown that CF have lower nonrenal clearance (likely hepatic extraction), it is plausible that this lower nonrenal clearance would lead to higher $F_{oral}$ due to reduced hepatic first-pass effect. The difference between both groups was more evident in the rate of absorption ($k_{ga}$) where HV had an absorption rate constant that was nearly double that of CF patients (0.020 and 0.011 min$^{-1}$, respectively). Since CIP is known to be zwitterionic within the physiological pH range of the GI tract, it is likely that in CF patients (where some transporters are overexpressed to compensate for the mutated CFTR transporter) CIP may undergo increased efflux transport.

### 5.5.5. Final INH Model
The final INH model incorporated the two-compartment systemic disposition model, the oral absorption model, and two pulmonary compartments. This is model #1 as described in Chapter Two. The selection of this model was based on the lack availability of sputum data, specifically, after IV administration. The model allowed for absorption from the lungs (central and peripheral) to the central compartment through \( k_{pa} \) and \( k_{ca} \), however, the model did not show that drug can redistribute from the central compartment back to the lungs. Mucociliary clearance was possible from both the peripheral and the central lung into the central lung and the GI tract compartments respectively.

5.5.6. Final Parameter Space after Inhalation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population Value</th>
<th>Distribution calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Cl_{renal} ) (ml/min)</td>
<td>( Cl_{renal} = \frac{HV}{CF} \frac{N(315,20)}{N(425,20)} )</td>
<td></td>
</tr>
<tr>
<td>( Cl_{non-renal} ) (ml/min)</td>
<td>( Cl_{non-renal} = \frac{HV}{CF} \frac{N(315,20)}{N(225,20)} )</td>
<td></td>
</tr>
<tr>
<td>( k_{12} ) (min^{-1})</td>
<td>( k_{12} = \frac{HV}{CF} \frac{N(0,025,25)}{N(0,050,20)} )</td>
<td></td>
</tr>
<tr>
<td>( k_{21} ) (min^{-1})</td>
<td>( k_{21} = \frac{HV}{CF} \frac{N(0,015,25)}{N(0,020,20)} )</td>
<td></td>
</tr>
<tr>
<td>( V_0 ) (l)</td>
<td>( V_0 = \frac{HV}{CF} \frac{N(75,20)}{N(20,20)} )</td>
<td></td>
</tr>
<tr>
<td>( F_{vap} ) (%)</td>
<td>( F_{vap} = \frac{HV}{CF} \frac{N(73,35)}{N(85,30)} )</td>
<td></td>
</tr>
<tr>
<td>( k_{ga} ) (min^{-1})</td>
<td>( k_{ga} = \frac{HV}{CF} \frac{LN(0,020,10)}{LN(0,011,50)} )</td>
<td></td>
</tr>
<tr>
<td>( k_{pa} ) (min^{-1})</td>
<td>0.01 LN(0.01,75)</td>
<td></td>
</tr>
<tr>
<td>( k_{ca} ) (min^{-1})</td>
<td>0.02 LN(0.02,75)</td>
<td></td>
</tr>
<tr>
<td>( k_{pa} ) (min^{-1})</td>
<td>0.02 LN(0.02,75)</td>
<td></td>
</tr>
<tr>
<td>( k_{ca} ) (min^{-1})</td>
<td>0.02 LN(0.02,75)</td>
<td></td>
</tr>
<tr>
<td>( k_{ga} ) (min^{-1})</td>
<td>0.008 LN(0.008,75)</td>
<td></td>
</tr>
<tr>
<td>( k_{ga} ) (min^{-1})</td>
<td>0.0002 LN(0.0002,75)</td>
<td></td>
</tr>
<tr>
<td>TLV (ml)</td>
<td>30 ( TLV = TLV_{pop} \times \left( \frac{BW_i}{BW_{pop}} \right)^1 )</td>
<td></td>
</tr>
<tr>
<td>CLV (ml)</td>
<td>24 ( CLV = TLV + 0.8 )</td>
<td></td>
</tr>
</tbody>
</table>
Table 43 Final optimized model parameters with specified underlying covariate models and distributions

The final parameter set, listed in Table 43, that was optimized using the available inhalation data and was able to describe the available \(c_p(t)\) and \(c_s(t)\) profiles as well as the exposure metrics. A single parameter space was used to describe both HV and CF patients. While CF patients are known to have altered lung physiology (such as thicker mucus and altered airway morphology), there were insufficient data to resolve the differences between both groups. Furthermore, mucociliary clearance from both the peripheral and the central lung was numerically identical. Experimental *in-vivo* data has suggested otherwise, and similar modeling efforts with other drugs have also suggested that mucociliary clearance values are different for both compartments.\(^{126}\) However, again due to the limited sputum data the ability to resolve these parameters was limited.

Both the peripheral lung and the central lung compartments favored absorption into systemic circulation as compared to distribution from the central compartment into the lungs. This is especially evident in the central lung compartment where the \(\frac{k_{ca}}{k_{cd}}\) ratio is 100 relative to the \(\frac{k_{pa}}{k_{pd}}\) which is only 1.25. Due to the limited availability sputum data, a 75% CV was associated with all pulmonary rate constants.

5.5.7. Sensitivity Analysis

While the results of the sensitivity analysis were numerically different between HV and CF, the rank order of most influential parameters was the same: IV sensitivity analysis results showed that AUC for both HV and CF with mostly dependent on both \(CL_{ren}\) and \(CL_{nonren}\), and for \(c_{max}\), \(V_0\) was the most influential parameter.
After PO administration, a 25-fold change in $F_{\text{oral}}$ directly lead to a 25-fold change in both AUC and $c_{\text{max}}$ for both HV and CF patients, as expected. Among the remaining parameters, $CL_{\text{ren}}$ was most influential on AUC, and $V_0$ was the second most influential parameter on $c_{\text{max}}$.

After INH, both HV and CF plasma exposures were most sensitive to systemic disposition and oral absorption parameters. However, loss from the central lung compartment (whether through absorption or mucociliary clearance) was the most influential of pulmonary exposure/sputum in both HV and CF patients. This is plausible since loss from a compartment would likely directly and proportionally influence levels in that compartment.

5.5.8. Overall Conclusions

The validated IV, PO, and INH models were capable of describing the observed $c_p(t)$ and $c_s(t)$ profiles as well as the reported exposure metrics. Linear PK was validated across all three dosing routes. The data supported the application of a disease state covariate model that distinguished between HV and CF patients on the systemic disposition as well as the oral absorption parameters. This was most likely due to limited sputum data. Pulmonary disposition parameters were the same for both groups and followed log-normal distribution. The greatest parameter uncertainty was associated with the oral absorption parameters. Across all three dosing routes, elimination from the central compartment through renal or nonrenal pathways was the predominant mechanism of drug elimination. In both HV and CF patients, $CL_{\text{ren}}$ was most influential on AUC and $V_0$ was most influential on $c_{\text{max}}$ after IV administration, while $F_{\text{oral}}$ was most influential on plasma exposure after PO administration. After INH, systemic exposure
was dependent on systemic disposition and oral absorption parameters, while pulmonary exposure was dependent most on loss from the central lung compartment. Lung disposition favored systemic uptake from the pulmonary compartments over excretion from plasma into the lung compartments. CIP has a high bioavailability after INH, but pulmonary excretion is a minor elimination pathway (relative to CL\textsubscript{ren} and CL\textsubscript{nonren}). Lastly, sputum exposures are driven primarily by (rapid) pulmonary absorption and mucociliary clearances and not by systemic CIP exposure.
CHAPTER 6

OVERALL CONCLUSIONS, LIMITATIONS AND FUTURE DIRECTIONS

6.1. Available Data Used for Semi PBPK Modeling

As described in Chapter 2, three semi-PBPK models for lung disposition were developed and validated to describe the experimentally observed data from published literature, and the final parameter estimates were interpreted physiologically for each of the three drugs. This chapter compares the individual models/model parameter estimates, and prospective predictions for the three drugs.

Model #1 was used to describe the available $c_p(t)$ data for BUD, Model #2 was used to describe the available $c_p(t)$ and $c_s(t)$ data for CIP, and Model #3 was used to describe the available $c_p(t)$ and $c_s(t)$ data for TOB. In each of the three cases, the final model that was selected based on its ability to adequately describe the existing data, both mean concentration-time profiles and mean ($\pm$SD) exposure metrics as available after IV, PO (BUD and CIP only), and INH.

Note, however, that Model #1 is a sub-model of Model #2, and Model #2 is sub-model of Model #3. This does allow proper comparison of the final parameter sets: For Model #1 (BUD), $k_{pd}$ and $k_{cd}$ were set to 0 (i.e., unidirectional pulmonary uptake), and
the pulmonary sequestration compartments were assumed to equilibrate virtually instantaneously (i.e., \( k_{\text{pls1}}, k_{\text{pls2}}, k_{\text{cls1}}, \) and \( k_{\text{cls2}} = 10,000 \text{ min}^{-1} \)). For Model #2 (CIP), the pulmonary sequestration compartments equilibrate virtually instantly (i.e., \( k_{\text{pls1}}, k_{\text{pls2}}, k_{\text{cls1}}, \) and \( k_{\text{cls2}} = 10,000 \text{ min}^{-1} \)), but drug can be absorbed from and excreted into the pulmonary (unbound) compartments.

A summary of the available data after each route of administration for each of the three drugs is shown in Table 44. These collected data sets satisfied specific aim #1 listed in Chapter 2.

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Drug</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BUD ( c_p(t) )</td>
<td>BUD ( c_s(t) )</td>
<td>TOB ( c_p(t) )</td>
<td>TOB ( c_s(t) )</td>
<td>CIP ( c_p(t) )</td>
<td>CIP ( c_s(t) )</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>PO</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>INH</td>
<td>3</td>
<td>0</td>
<td>16</td>
<td>10</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>INH Devices</td>
<td>1</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 44 Number of available data sets (\( c_p(t) \) and \( c_s(t) \)) after each route of administration for each of the three drugs of interest.**

As discussed in earlier chapters, only a limited number of studies were available for modeling. Major limitations included the absence or scarcity of sputum data for BUD and CIP; this obviously impacted estimation of their respective final pulmonary disposition parameters.

Additionally, only a single repeat-dose study for TOB after IV administration, but not after INH, and none for BUD or CIP were reported in the literature. For BUD and CIP, this prevented formal assessment of drug accumulation in the lungs, that may not be discernible seen in plasma, as was the case for TOB.
In addition, all 13 sputum profiles (1 IV TOB, 10 INH TOB, and 2 INH CIP) were obtained from CF patients, and no information was available for HV; this prevented evaluation of any disease effect on pulmonary disposition.

Moreover, there were only a few studies available in the desired patient populations, including CF patients for TOB and CIP, and asthmatic patients for BUD. Finally, some of the studies had to be excluded due to the use of an insensitive analytical method, or inadequate sampling schedule.

In spite of these limitations, the available data sets were useful in generating appropriate disease and BW covariate models for systemic and GI absorption parameters. For pulmonary disposition, only TLV was assumed to be allometrically related to BW, as discussed in chapter 1 and 4. As pointed out above, disease effect could not be discussed from available data.

For BUD, Model #1 allows for unidirectional uptake of drug from the central and peripheral lung compartments into the systemic circulation. There were no sputum data available at all after IV administration of BUD, nor was there \textit{in-vitro} or other evidence data supporting pulmonary sequestration and or excretion of BUD.

For TOB, Model #3 allows for bidirectional movement, i.e., uptake from and excretion into pulmonary (unbound) compartments to account for the fact that TOB concentrations were achieved in sputum after IV administration; it also introduced pulmonary sequestration compartments to account for known \textit{in-vitro} (intracellular) accumulation as well as reported \textit{in-vivo} sputum accumulation after repeated INH dosing. Thus, the lung was considered an absorption, sequestration and excretion organ.
For CIP, Model #2 allowed for bidirectional flow of drug from the pulmonary compartments to and from the central compartment. This bidirectional flow allowed the model to adequately describe sputum concentrations profiles after INH. Thus, the lung was considered an absorption and excretion organ.

6.2. Comparison Across Parameter Spaces for the Three Drugs

6.2.1. Systemic Disposition Properties

Pertinent key physicochemical properties of the three drugs are summarized in Table 45. As can be seen from Table 45, all three drugs have similar molecular weights, however they have different experimental log(P) values, ionization within physiological pH, and different solubility. Additionally, there A to B $P_{\text{app}}$ values in both Caco-2 cell models and Calu-3 cell models are also different. The final optimized systemic disposition parameters for each of the three drugs are listed for comparison in Table 46. All parameters are for HV (BW = 75 kg).

<table>
<thead>
<tr>
<th>Property</th>
<th>BUD</th>
<th>TOB</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>$C_{25}H_{34}O_6$</td>
<td>$C_{18}H_{27}N_3O_9$</td>
<td>$C_{17}H_{18}FN_3O_3$</td>
</tr>
<tr>
<td>MW (g/mole)</td>
<td>431</td>
<td>468</td>
<td>331</td>
</tr>
<tr>
<td>log(P)</td>
<td>1.7</td>
<td>-7.3</td>
<td>-0.13</td>
</tr>
<tr>
<td>Ionization</td>
<td>Neutral</td>
<td>Polycationic</td>
<td>Zwitterionic</td>
</tr>
<tr>
<td>Aqueous Solubility (mg/mL)</td>
<td>0.019</td>
<td>1000</td>
<td>40</td>
</tr>
<tr>
<td>Caco-2 $P_{\text{app}}$ (cm/sec)</td>
<td>$11 \cdot 10^{-6}$</td>
<td>$1.78 \cdot 10^{-6}$</td>
<td>$3.0 \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Calu-3 $P_{\text{app}}$ (cm/sec)</td>
<td>$8.59 \cdot 10^{-5}$</td>
<td>Low, but not reported</td>
<td>$3.5 \cdot 10^{-4}$</td>
</tr>
</tbody>
</table>

Table 45 Physicochemical properties of the three selected drugs. (Both $P_{\text{app}}$ values are reported for A$\rightarrow$B permeability).
### Table 46 Comparison across the final optimized systemic disposition parameters for BUD, TOB, and CIP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BUD</th>
<th>TOB</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL&lt;sub&gt;tot&lt;/sub&gt; (mL/min)</td>
<td>1170</td>
<td>70</td>
<td>630</td>
</tr>
<tr>
<td>V&lt;sub&gt;0&lt;/sub&gt; (L)</td>
<td>45</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>k&lt;sub&gt;12&lt;/sub&gt; (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.100</td>
<td>0.010</td>
<td>0.025</td>
</tr>
<tr>
<td>k&lt;sub&gt;21&lt;/sub&gt; (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.031</td>
<td>0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>k&lt;sub&gt;10&lt;/sub&gt; (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.026</td>
<td>0.007</td>
<td>0.008</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt; (L)</td>
<td>190</td>
<td>17</td>
<td>200</td>
</tr>
<tr>
<td>Depth (k&lt;sub&gt;10&lt;/sub&gt;/k&lt;sub&gt;21&lt;/sub&gt;)</td>
<td>0.84</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>Capacity (k&lt;sub&gt;12&lt;/sub&gt;/k&lt;sub&gt;21&lt;/sub&gt;)</td>
<td>3.2</td>
<td>0.67</td>
<td>1.7</td>
</tr>
</tbody>
</table>

As expected, BUD shows the highest CL<sub>tot</sub>, followed by CIP, and TOB has the lowest value. Lipophilic compounds have been shown to undergo predominantly hepatic metabolism/biliary excretion.<sup>127</sup>

Based on its lipophilicity, BUD, which is the most lipophilic of the three tested drugs, has the largest total clearance approaching hepatic blood flow, indicating high hepatic extraction. This also explains its low F<sub>oral</sub> as result of high pre-systemic hepatic extraction (see below).

Based on its estimated CL<sub>tot</sub> and F<sub>oral</sub> values, its intermediate lipophilicity, as well as its zwitterionic properties, CIP is known to undergo both hepatic and renal elimination. Its renal clearance is reported in Chapter 5 and indicates net tubular secretion, involving drug transporters (OATs). Renal excretion is the major route of elimination as the fraction of the administered dose that is eliminated unchanged in urine after IV administration is between 40% to 60%.<sup>108,112</sup>
Lastly, TOB, which has the lowest CL$_{\text{tot}}$, virtually exclusively undergoes renal excretion due to its hydrophilicity; specifically, it undergoes net tubular reabsorption due to the possible involvement of some cationic transporters.$^{128}$

The peripheral body compartment for all three drugs - needed to properly model systemic disposition data - is a shallow body compartment, equilibrates rapidly with the central compartment (plasma): Final parameter estimates for both BUD and CIP indicate that these peripheral tissue compartment is a low-capacity compartment, i.e., contain only a small portion of the administered dose, while, for TOB, it is an intermediate-capacity compartment. This increased capacity noted for TOB may be due to the aforementioned intracellular tissue sequestration observed in in- vitro studies in the lung.$^{24}$

As both BUD and CIP are sufficiently lipophilic and small to cross epithelia, they both have large Vd$_{\text{ss}}$, indicating extensive extravascular distribution. CIP has a slightly smaller Vd$_{\text{ss}}$, which may be attributed to its zwitterionic charge in the physiological pH range. On the other hand, TOB has quite a small Vd$_{\text{ss}}$, indicating limited extravascular distribution. In addition to being a hydrophilic drug, TOB is highly charged and is likely to be a polycationic drug within physiological pH range.

To better emphasize their differences in systemic PK properties comparative simulations of c$_p$(t) and c$_s$(t) were performed. Using the final parameter estimates for each of the three drugs, a 10-minute IV infusion of a non-therapeutic dose of 200 mg was simulated. The results of these simulations are shown in Figure 48.
As can be seen, there are no BUD concentrations in sputum after IV administration. This is expected since the model (Model #1) does not allow distribution from the systemic circulation to the pulmonary compartments.

However, the final models (Model #2 and #3) for both TOB and CIP predict drug concentrations in sputum after IV administration. The magnitude of concentration difference between plasma and sputum for TOB is greater than that for CIP. Notably, TOB plasma concentrations are 100-fold higher than sputum concentrations due to its limited distribution to the lungs, given the minor capacity of the central lung sequestration compartment \((k_{cls1}/k_{cls2} = 0.0007)\). Although the peripheral lung sequestration compartment is a high-capacity compartment \((k_{pls1}/k_{pls2} = 3.75)\), the peripheral unbound lung compartment that moves TOB via mucociliary clearance into the central unbound lung compartment is a low-capacity compartment \((k_{pd}/k_{pa} = 0.009)\). As a result, the central unbound lung compartment/sputum concentrations are quite small, and most of TOB in the lung is trapped in the peripheral sequestration compartment, which serves as reservoir for TOB to redistribute primarily into the central compartment/plasma and, to a much lesser extent, into the central unbound lung compartment/sputum. Additionally, the terminal slopes in the plasma and sputum profiles appear to be parallel for both TOB and CIP. This indicates that the central unbound lung and central (body) compartments are in equilibrium, and the rate-limiting step for both compartments is distribution from the systemic circulation to the lungs, i.e. pulmonary excretion with some intrapulmonary recycling.
6.2.2. Oral Absorption Properties

The final optimized oral/GI absorption parameters for each of the three drugs are listed for comparison in Table 47.

For BUD is low, only about 10% due to high hepatic first-pass effect, given that it is a high hepatic extraction ratio drug.

As previously discussed, TOB is hydrophilic and highly charged. These characteristics lead to a negligible $F_{oral}$ of ~0%, predominantly due to poor GI permeability (consistent with poor Caco-2 permeability, as shown in Table 45).

For CIP was estimated to be 73% for HV and 85% for CF patients. These values are in line with a small degree of first-pass effect and/ or the possible involvement of GI efflux transporters. The difference between $F_{oral}$ for HV and CF
patients has been discussed by several authors, who have theorized that it may be due to the overexpression of GI uptake transporters to compensate for the mutated CFTR transporter.\textsuperscript{125,129}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BUD</th>
<th>TOB</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{ga}$ (min\textsuperscript{-1})</td>
<td>0.007</td>
<td>0</td>
<td>0.020</td>
</tr>
<tr>
<td>$F_{oral}$ (%)</td>
<td>10</td>
<td>0</td>
<td>73</td>
</tr>
</tbody>
</table>

Table 47 Comparison across the final GI absorption parameters for BUD, TOB, and CIP

To better understand the implications of these differences in GI absorption properties, comparative simulations of $c_p(t)$ and $c_s(t)$ were performed with a non-therapeutic dose of 1000 mg. The results of these simulations are shown in Figure 49.

Although there are concentrations of BUD in plasma, there are no sputum concentrations of BUD, as is expected since the model (Model #1) does not allow distribution from the systemic circulation to the pulmonary compartments.

Since TOB is not orally bioavailable, there are no plasma nor sputum concentrations.

Lastly, CIP is absorbed well from the GI into plasma, and distributes into the (unbound) lung compartments as shown by the sputum profile. Peak sputum concentration for lags behind the peak plasma concentration due to slow distribution from the central compartment into the (unbound) lung compartments. Additionally, the terminal slopes in plasma and sputum profiles appear to be parallel for CIP, which indicates that the (unbound) central lung compartment and the central body compartment are in equilibrium, and the rate-limiting step for is distribution from the
systemic circulation to the lungs, i.e., pulmonary excretion with some intrapulmonary recycling.

Figure 49 Simulations for BUD, TOB, and CIP after PO administration (1000 mg)

6.2.3. Pulmonary Disposition Properties

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BUD</th>
<th>TOB</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{pa}$ (min$^{-1}$)</td>
<td>0.066</td>
<td>0.900</td>
<td>0.010</td>
</tr>
<tr>
<td>$k_{ca}$ (min$^{-1}$)</td>
<td>0.033</td>
<td>0.650</td>
<td>0.020</td>
</tr>
<tr>
<td>$k_{pm}$ (min$^{-1}$)</td>
<td>0.008</td>
<td>0.020</td>
<td>0.020</td>
</tr>
<tr>
<td>$k_{cm}$ (min$^{-1}$)</td>
<td>0.016</td>
<td>0.080</td>
<td>0.020</td>
</tr>
<tr>
<td>$k_{pd}$ (min$^{-1}$)</td>
<td>0</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>$k_{cd}$ (min$^{-1}$)</td>
<td>0</td>
<td>0.0002</td>
<td>0.0002</td>
</tr>
<tr>
<td>$k_{pls1}$ (min$^{-1}$)</td>
<td>10000</td>
<td>0.015</td>
<td>10000</td>
</tr>
<tr>
<td>$k_{pls2}$ (min$^{-1}$)</td>
<td>10000</td>
<td>0.004</td>
<td>10000</td>
</tr>
<tr>
<td>$k_{cls1}$ (min$^{-1}$)</td>
<td>10000</td>
<td>0.002</td>
<td>10000</td>
</tr>
<tr>
<td>$k_{cls2}$ (min$^{-1}$)</td>
<td>10000</td>
<td>0.3</td>
<td>10000</td>
</tr>
</tbody>
</table>

Table 48 Comparison of the final, optimized pulmonary disposition parameters for BUD, TOB and CIP.

Absence of pulmonary sequestration is indicated by large values (10,000) for $k_{pls1}$, $k_{pls2}$, $k_{cls1}$, and $k_{cls2}$.
Overall, the final parameters indicate that: a) BUD undergoes pulmonary absorption only, b) TOB undergoes pulmonary absorption/sequestration and excretion, and c) CIP undergoes pulmonary absorption and excretion. Both BUD and CIP are not sequestered in the lungs and are absorbed rapidly into systemic circulation from their pulmonary compartments. Both TOB and CIP also undergo intrapulmonary recycling due bidirectional pulmonary uptake into and efflux from the central compartment, along with mucociliary clearance from the peripheral to the central (unbound) lung compartment.

Values for $k_{pa}$ and $k_{ca}$ for BUD are notably faster when compared to CIP. This is likely due to the lipophilicity as well as the neutral state of BUD, which would facilitate its pulmonary membrane permeability and rapid uptake of the deposited dose.

On the other hand, TOB, which is hydrophilic and has polycationic charges, has the fastest $k_{pa}$ and $k_{ca}$ values but, slow $k_{pls2}$ and $k_{cls2}$ values. The latter micro rate constants govern the movement of the drug from the pulmonary sequestration compartments to the central compartment. The low values associated with TOB point to a slow pulmonary absorption into systemic circulation. This again is in line with its hydrophilic and cationic nature and consistent with the low $P_{app}$ values in CaLu cell lines (see Table 45).

A study of mucociliary clearance values for inhaled substances indicated that mucociliary clearances values can depend on the pulmonary solubility of the inhaled drug. Specifically, more soluble compounds were shown to have mucociliary clearance rate constants between 0.0167 and 0.0083 min$^{-1}$, while less soluble
compounds had a mucociliary clearance rate constant of 0.0013 min\(^{-1}\). TOB, had the highest experimental solubility, with a solubility of 1000 mg/mL, followed by CIP with a solubility 40 mg/mL, and BUD with a solubility 19 \(\cdot 10^{-3}\) mg/mL. This rank order of solubility leads to the observed rank order of the estimated \(k_{cm}\) values, with TOB having the fastest, CIP having the intermediate, and BUD having the slowest \(k_{cm}\) values, respectively.

The final optimized pulmonary deposition parameters and pulmonary bioavailability estimates for each of the three drugs are listed for comparison in Table 49.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BUD</th>
<th>TOB</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DtL (%)</td>
<td>28%</td>
<td>15%</td>
<td>53%</td>
</tr>
<tr>
<td>DtGI (%)</td>
<td>58%</td>
<td>8.5%</td>
<td>40%</td>
</tr>
<tr>
<td>Device Dose (mg)</td>
<td>1</td>
<td>300</td>
<td>32.5</td>
</tr>
<tr>
<td>(F_{pul}) (%)</td>
<td>83%</td>
<td>10%</td>
<td>8%</td>
</tr>
<tr>
<td>(F_{inh}) (%)</td>
<td>34%</td>
<td>6%</td>
<td>36%</td>
</tr>
</tbody>
</table>

Table 49 Comparison across the final optimized pulmonary deposition parameters and pulmonary bioavailabilities for BUD, TOB, and CIP.

(Deosition values for BUD, TOB, and CIP are for the Turbuhaler, Pari LC Jet Nebulizer, and Novartis T-326 DPI device, respectively)

The value for \(F_{inh}\) depends on the composite of pulmonary and GI tract deposition along with the respective bioavailabilities; i.e., \(F_{pul}\) and \(F_{oral}\) (Equation 5 in Chapter 2).

On the other hand, \(F_{pul}\) depends solely on the pulmonary disposition micro rate constants.

For BUD, of the total inhalation device dose, 28% is deposited in the lungs while 58% is deposited in the GI tract. Pulmonary bioavailability (\(F_{pul}\)) for BUD is 83%,
meaning that of the 28% deposited in the lungs, 83% is subsequently absorbed into systemic circulation. On the other hand, the 58% deposited in the GI tract is available for GI absorption but limited by a $F_{oral}$ of 10% due to high pre-systemic hepatic metabolism. As such, after inhalation, the total bioavailability ($F_{inh}$) for BUD is only 34%, despite a high $F_{pul}$, but due to poor pulmonary deposition and poor oral bioavailability.

For TOB, only 15% of the nebulized dose is deposited in the lungs, and only 8.5% is deposited in the GI tract (due to the poor efficiency of the Pari Nebulizer). For TOB, $F_{pul}$ is only 10% - due to poor pulmonary permeability/sequestration-, and $F_{oral}$ is 0%, which leads to a low $F_{inh}$ of 6%.

For CIP, while a large fraction of the dose (53%) of the inhalation device dose is deposited the lung, $F_{pul}$ is only 8%. The low $F_{pul}$ value is the result of efficient mucociliary clearance and pulmonary excretion, which reduced pulmonary absorption. However, due to its high $F_{oral}$ and the fact that 40% of the dose are deposited in the GI tract, $F_{inh}$ for CIP is 36%, similar to BUD.

Overall, for both TOB and CIP $F_{pul}$ values were low (10% and 8%, respectively) due to their excretion from the central body compartment/plasma into the lungs. Specifically, TOB was excreted from the central body compartment into the peripheral lung sequestration compartment while CIP was excreted from the central body compartment into the peripheral unbound lung compartment.

The final parameter spaces for both TOB and CIP was utilized in Chapters 4 and 5, respectively, to study the contribution of pulmonary excretion to the overall excretion from the body after IV administration: For TOB and CIP, the lung contributed 7% and 1.3%, respectively, of the total dose to overall excretion. This indicates that the lung is
not a major elimination organ, however, pulmonary excretion plays a major role in pulmonary disposition and the resulting sputum concentrations.

All three models and validated model parameter estimates, satisfied specific aim #2 as described in Chapter 2.

To better understand the differences between the three drugs in their pulmonary disposition properties, comparative simulations of \( c_p(t) \) and \( c_e(t) \) were performed with a non-therapeutic INH dose of 500 mg. Deposition data for each drug was set to the values noted for the devices discussed earlier, and inhalation time was set to zero (i.e., instantaneous deposition was assumed). The results of these simulations are shown in Figure 50.

As can be seen in Figure 50, for BUD after INH, the \( c_e(t) \) profile shows a rapid decrease, which reflects rapid (unidirectional) absorption of the deposited dose from both peripheral and central (unbound) lung compartments into the central body compartment/plasma. Plasma concentrations initially increase rapidly due to pulmonary absorption, and, after reaching a maximum concentration, they decline in a first-order fashion with systemic elimination (\( k_{10} \)) as the rate-limiting step.

Concentrations of TOB in the central unbound lung compartment/sputum at first decrease very rapidly due to a fast \( k_{ca} \) (faster than BUD or CIP); i.e., rapid uptake into the central lung sequestration compartment, however, show a second peak. This second peak is likely due to intrapulmonary recirculation through the peripheral lung sequestration compartment, which behaves as a reservoir. After initial rapid pulmonary uptake into this high-capacity compartment, TOB distributes from the central body compartment/plasma into this peripheral lung sequestration compartment and is slowly
released into the peripheral unbound lung compartment and subsequently moved into 
the central unbound lung compartment/sputum via mucociliary clearance – leading to 
the second peak. This is also in agreement with published literature, showing TOB 
accumulation in sputum after repeated dosing. Accordingly, the lung compartments 
behave as absorption/excretion and sequestration compartments for TOB. Plasma 
concentrations of TOB after inhalation peak rapidly due to fast, initial pulmonary 
absorption ($k_{ca}$ and $k_{cls2}$). This is followed by a distribution phase which is likely 
governed by equilibration with the peripheral sequestration lung compartment, before 
reaching its terminal phase, which is governed by equilibration with the central lung 
compartment ($k_{cls1}$).

For CIP, sputum concentrations follow a biphasic decline after inhalation: The 
initial decline is due to rapid absorption from the lung compartments into the central 
body compartment/plasma ($k_{ca}$ and $k_{pa}$). The slower terminal decline is due to the 
subsequent distribution from the central compartment back to the central lung 
compartment ($k_{cd}$) and pulmonary excretion. This further proves that the lung 
compartments for CIP behave as absorption/excretion compartments. Plasma 
concentrations for CIP achieve their maximum later than TOB and BUD, which is 
explained by its slowest pulmonary absorption ($k_{ca}$) among the three drugs. The 
terminal phase in the plasma concentration-time profile for CIP declines in parallel with 
sputum concentrations and is governed by $k_{cd}$. 

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6.3. Comparison of Sensitivity Analysis for Each of the Three Drugs

6.3.1. Plasma Sensitivity Analysis

The results for the plasma and sputum sensitivity analyses are shown in Table 46.

<table>
<thead>
<tr>
<th>Exposure metric</th>
<th>Plasma AUC</th>
<th>Sputum AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>BUD</td>
<td>TOB</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>CL&lt;sub&gt;tot&lt;/sub&gt;</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>K&lt;sub&gt;10&lt;/sub&gt;</td>
<td>k&lt;sub&gt;lbs1&lt;/sub&gt;</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>F&lt;sub&gt;oral&lt;/sub&gt;</td>
<td>k&lt;sub&gt;lbs2&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Table 50 Top three most influential parameters for each of the exposure metrics for each of the studied drugs.
After INH, the plasma AUC for all three drugs was found to be most sensitive to changes in CL\text{tot}. For TOB, this was explicitly seen in the sensitivity analysis, with CL\text{tot} being the most influential parameter. For CIP, CL\text{ren} and CL\text{nonren}, the sum of which is CL\text{tot}, were both among the two three most influential parameters on plasma AUC. Lastly, for BUD, plasma AUC was most sensitive to V_0 and k_10, the product of which is CL\text{tot}.

Interestingly, for BUD, plasma AUC was also sensitive to F\text{oral}. This is likely due to the high GI deposition from the Turbuhaler (DtGI = 58%) combined with the low F\text{oral} of 10%. For TOB, plasma AUC was sensitive to the distribution from the central body compartment to the peripheral lung sequestration compartment (k_{pls1}), and the uptake from the peripheral lung sequestration compartment into the central body compartment (k_{pls2}). This is likely due to the high peripheral lung deposition of drug from the nebulizer combined with a low F_{pul} of 10%. For both BUD and TOB, given that a large fraction of their INH dose is deposited in the GI tract and the peripheral lung, a slight increase in F\text{oral} or F_{pul}, respectively, is expected to lead to a large increase in plasma AUC.

6.3.2. Sputum Sensitivity Analysis

A major underlying assumption of this work was that sputum concentrations correlate with pulmonary compartments, specifically, concentration in the central (unbound) lung compartment.

Since the model used for BUD (Model #1) and the final optimized parameter space were not validated against sputum data (due to absence of published data), sputum AUC sensitivity was not performed for BUD.
For TOB and CIP, sputum AUC was consistently sensitive to decreases in $k_{ca}$ and $k_{cm}$. The sum of these parameters characterizes the rate of loss from the central lung compartment (equivalent of $CL_{tot}$ from a central compartment). It is that rate of loss from the central (unbound) lung compartment that is most important for central lung (unbound) compartment concentrations, i.e., sputum exposure, for both drugs since their pulmonary absorption is slow and low (see Table 45). $k_{pm}$ and $k_{pd}$ appear also to be influential parameters with regards to sputum AUC. This is likely due to their influence on concentrations in the peripheral (unbound) lung compartment which in turn can (indirectly) influence concentrations in the central (unbound) lung compartment/sputum as long as some of the inhaled dose is deposited into the peripheral (unbound) lung compartment.

These findings are in line with the hypothesis stated in Chapter 2, specifically that, after inhalation, (a) decreasing systemic exposures can be accomplished by increasing total systemic clearance ($CL_{tot}$), but are less sensitive to pulmonary absorption, and (b) increasing pulmonary exposures can be accomplished by slowing down uptake from the lungs into systemic circulation ($k_{ca}$) and/or slowing down mucociliary clearance from the lungs into the GI tract ($k_{cm}$).
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APPENDIX 1
Matlab Script to run MCS for BUD

%Author: Bishoy Erian
%Start date: May 14th, 2015
%Purpose: To plot the 95th & 5th percentile after a Monte Carlo Simulation
%for my inhalation model based on the laplace transformed solution

clear all
close all
clc
close Figure 1
clf
tics
Tstart=tic;

digits(10)
rng('default')
rng(3)

%% User input area
n=20000; %number of simulations to run
Last_time_point=1440; %I typically think this should be in minutes
IFS=0.5; %0.5 means every half a minute; Increment for simulation

%Deposition Parameters
MDem=720; %nmol
CVDem=0; %60;
MFex=0.001175;
CVFex=0;0.000957;
MFpd=0.098;
CVFpd=0;0.012;
MFcd=0.117;
CVFcd=0;0.00495;

%Lung Parameters
Mkpm=0.0078;
CVkpm=0;
Mkcm=0.01563;
CVkcm=0;
Mkpa=0.3333;
CVkpa=0;
Mkca=0.166667;
CVkca=0;

%Oral Absorption Parameters
Mkga=0.0056;
CVkga=0.35;
MForal=0.0995;
CVForal=0.3;

%Systemic Disposition Parameters
MV0=58;
CVV0=0.15;
Mk12=0.095;
CVk12=0.2;
Mk21=0.0308;
CVk21=0.2;
Mk10=0.0273;
CVk10=0.15;

%Dosing interval
tau=2000;

SDem=(CVDem*MDem)^2;
SFex=(CVFex*MFex)^2;
SFpd=(CVFpd*MFpd)^2;
SFcd=(CVFcd*MFcd)^2;
Skpm=(CVkpm*Mkpm)^2;
Skcm=(CVkcm*Mkcm)^2;
Skpa=(CVkpa*Mkpa)^2;
Skca=(CVkca*Mkca)^2;
Skga=(CVkga*Mkga)^2;
SForal=(CVForal*MForal)^2;
SV0=(CVV0*MV0)^2;
Sk12=(CVk12*Mk12)^2;
Sk21=(CVk21*Mk21)^2;
Sk10=(CVk10*Mk10)^2;

%Dose parameters
%Dem1=MDem/5 : MDem/4166.5 : 5*MDem;
Dem1=normrnd(MDem,SDem,[1,n]);
%Fex1=MFex/5 : MFex/4166.5 : 5*MFex;
Fex1=normrnd(MFex,SFex,[1,n]);
%Fpd1=MFpd/5 : MFpd/4166.5 : 5*MFpd;
Fpd1=normrnd(MFpd,SFpd,[1,n]);
%Fcd1=MFcd/5 : MFcd/4166.5 : 5*MFcd;
Fcd1=normrnd(MFcd,SFcd,[1,n]);

%Lung parameters
%kpm1=Mkpm/5 : Mkpm/4166.5 : 5*Mkpm;
%kpm1=Mkpm/10 : Mkpm/2020.2 : 10*Mkpm;
kpm1=lognrnd(log(Mkpm^2 / sqrt(Skpm+Mkpm^2)),sqrt(log(Skpm/Mkpm^2 + 1)),[1,n]);
%kcm1=Mkcm/5 : Mkcm/4166.5 : 5*Mkcm;
kcm1=lognrnd(log(Mkcm^2 / sqrt(Skcm+Mkcm^2)),sqrt(log(Skcm/Mkcm^2 + 1)),[1,n]);
%kpa1=Mkpa/5 : Mkpa/4166.5 : 5*Mkpa;
kpa1=lognrnd(log(Mkpa^2 / sqrt(Skpa+Mkpa^2)),sqrt(log(Skpa/Mkpa^2 + 1)),[1,n]);
%kca1=Mkca/5 : Mkca/4166.5 : 5*Mkca;
kca1=lognrnd(log(Mkca^2 / sqrt(Skca+Mkca^2)),sqrt(log(Skca/Mkca^2 + 1)),[1,n]);

%Oral Deposition Parameters
%kga1=Mkga/5 : Mkga/4166.5 : 5*Mkga;
kga1=lognrnd(log(Mkga^2 / sqrt(Skga+Mkga^2)),sqrt(log(Skga/Mkga^2 + 1)),[1,n]);
%Foral1=MForal/5 : MForal/4166.5 : 5*MForal;
Foral1=lognrnd(log(MForal^2 / sqrt(SForal+MForal^2)),sqrt(log(SForal/MForal^2 + 1)),[1,n]);

%Systemic Disposition Parameters
%V01=MV0/5 : MV0/4166.5 : 5*MV0;
V01=lognrnd(log(MV0^2 / sqrt(SV0+MV0^2)),sqrt(log(SV0/MV0^2 + 1)),[1,n]);
%k121=Mk12/5 : Mk12/4166.5 : 5*Mk12;
k121=lognrnd(log(Mk12^2 / sqrt(Sk12+Mk12^2)),sqrt(log(Sk12/Mk12^2 + 1)),[1,n]);
%k211=Mk21/5 : Mk21/4166.5 : 5*Mk21;
k211=lognrnd(log(Mk21^2 / sqrt(Sk21+Mk21^2)),sqrt(log(Sk21/Mk21^2 + 1)),[1,n]);
%k101=Mk10/5 : Mk10/4166.5 : 5*Mk10;
k101=lognrnd(log(Mk10^2 / sqrt(Sk10+Mk10^2)),sqrt(log(Sk10/Mk10^2 + 1)),[1,n]);

% MU = log(M^2 / sqrt(V+M^2))
% SIGMA = sqrt(log(V/M^2 + 1))

% Matrix setup/allocation
time=0:IFS:Last_time_point;
t=0;
NoP=(Last_time_point/IFS)+1;%plus one accounts for the
DoseCounter=0;

% the zero time point
AmtPL=ones(NoP,n);
AmtCL=ones(NoP,n);
```
AmtGIT = ones(NoP, n);
AmtPC = ones(NoP, n);
AmtCC = ones(NoP, n);
Cp = ones(NoP, n);
ContPL = ones(NoP, n);  ContCL = ones(NoP, n);  ContGIT = ones(NoP, n);
ContPLCL = ones(NoP, n);  ContCLGIT = ones(NoP, n);  ContPLCLGIT = ones(NoP, n);
LastCC = ones(NoP, n);
ContPPL = ones(NoP, n);  ContPCL = ones(NoP, n);  ContPGIT = ones(NoP, n);
ContPPLCL = ones(NoP, n);  ContPCLGIT = ones(NoP, n);  ContPPLCLGIT = ones(NoP, n);
LastPC = ones(NoP, n);
cmax = ones(1, n);  tmax = ones(1, n);  Auc = ones(1, n);  thalfbet = ones(1, n);
AAUC = ones(1, n);  AUMC = ones(1, n);  MRT = ones(1, n);  CCa = ones(1, n);
CCb = ones(1, n);  CCc = ones(1, n);  CCp = ones(1, n);  CCg = ones(1, n);
CC1 = ones(1, n);  CC2 = ones(1, n);  CC3 = ones(1, n);  CC4 = ones(1, n);
CC5 = ones(1, n);  CC6 = ones(1, n);  CC7 = ones(1, n);  CC8 = ones(1, n);
CC9 = ones(1, n);  CC10 = ones(1, n);  CC11 = ones(1, n);  CC12 = ones(1, n);
CC13 = ones(1, n);  CC14 = ones(1, n);  CC15 = ones(1, n);  CC16 = ones(1, n);
CC17 = ones(1, n);  CC18 = ones(1, n);  CC19 = ones(1, n);  CC20 = ones(1, n);
CC21 = ones(1, n);  CC22 = ones(1, n);
for j = 1:1:n
    % Parameter value selection from randomly generated array
    Dem = Dem1(1, j);  Fex = Fex1(1, j);  Fpd = Fpd1(1, j);
    Fcd = Fcd1(1, j);  kpm = kpm1(1, j);  kcm = kcm1(1, j);
    kpa = kpa1(1, j);  kca = kca1(1, j);  kga = kga1(1, j);
    Foral = Foral1(1, j);  V0 = V01(1, j);  k12 = k121(1, j);
    k21 = k211(1, j);  k10 = k101(1, j);

    kp = kpm + kpa;
    kc = kcm + kca;
    alp_p_bet = k12 + k21 + k10;
    alp_m_bet = k21 * k10;
    alp = -(alp_p_bet - (alp_p_bet^2) - (4 * alp_m_bet) )^0.5)/2;
    bet = -(alp_p_bet + (alp_p_bet^2) - (4 * alp_m_bet) )^0.5)/2;
    Fgd = 1 - Fpd - Fcd - Fex;
    PL0 = Dem * Fpd;
    CL0 = Dem * Fcd;
    GIT0 = Dem * Fgd;

    % Percentage done
    if (j*100/n) == 0 || (j*100/n) == 25 || (j*100/n) == 50 || (j*100/n) == 75 || (j*100/n) == 100
        clc
        howmuch = num2str(j*100/n);
```

disp(strcat('currently',{' '},howmuch, ' % done'));
end
for i=1:1:NoP
    if(rem(time(1,i),tau)==0);
        if (time(1,i)==0);
            LastCC(i,j)=0;
            LastPC(i,j)=0;
            GIT0=Dem*Fgd;
            CL0=Dem*Fcd;
            PL0=Dem*Fpd;
        else
            GIT0=(((CL0*kcm*(exp(-kc*(IFS+t)))-\(exp(-kga * (IFS+t)))/(kga-kc))+\(PL0*kcm*kpm*((exp(-kc*(IFS+t))/((kp-kc)*(kga-kc)))+(exp(-kp*(IFS+t))/((kp-kc)*\(kga-kp)))+\(exp(-kga*(IFS+t))/((kp-kc)*(kga-kp))))))+(\(GIT0*exp(-\(kga*(IFS+t))))+(Dem*Fgd));
            CL0=(((CL0*exp(-\(kc*(t+IFS)))+(PL0*kpm*(exp(-\(kc*(t+IFS))-\(exp(-kp*(t+IFS)))/((kp-kc)*\(kga-kp)))))+(\(CL0*exp(-\(kc*(t+IFS)))+\(PL0*kpm*(exp(-\(kc*(t+IFS))-\(exp(-kp*(t+IFS)))/((kp-kc)*\(kga-kp)))))\(+(Dem*Fcd));
            LastCC(i,j)=AmtCC(i-1,j);
            LastPC(i,j)=AmtPC(i-1,j);
        end
        t=0;
        DoseCounter=DoseCounter+1;
    else
        LastCC(i,j)=LastCC(i-1,j);
        LastPC(i,j)=LastPC(i-1,j);
        GIT0=GIT0;
        CL0=CL0;
        PL0=PL0;
        t=t+IFS;
    end
    AmtPL(i,j)=PL0*exp(-kp*t);
    AmtCL(i,j)=CL0*exp(-kc*t)+PL0*kpm*(exp(-\(kc*t)-\(exp(-kp*t)))/((kp-kc));
    GIT1=GIT0*exp(-\(kga*t));
    GIT2=CL0*kcm*(exp(-\(kc*t)-\(exp(-kga*t)))/(kga-kc);
    GIT3=PL0*kpm*kcm*(\(exp(-kp*t)/((kp-kp)*\(kga-kp)))+\(exp(-kc*t)/((kp-kc)*\(kga-kc)))+\(exp(-kga*t)/((kp-kp)*\(kc-kga)))));
    AmtGIT(i,j)=GIT1+GIT2+GIT3;
    ContPL(i,j)=PL0*kpa*\((k21-kp)*exp(-\(kp*t))/((kp-kp)*(bet-kp)))+\((k21-alp)*exp(-\(alp*t))/((kp-alp)*(bet-alp)))+\((k21-bet)*exp(-\(bet*t))/((kp-bet)*(alp-bet)))};
    ContCL(i,j)=CL0*kca*\((k21-kc)*exp(-\(kc*t))/((kp-kc)*(bet-kc)))+\((k21-alp)*exp(-\(alp*t))/((kp-alp)*(bet-alp)))+\((k21-bet)*exp(-\(bet*t))/((kp-bet)*(alp-bet))))];
ContGIT(i,j)=GIT0*kga*Foral*( ((k21-kga)*exp(-kga*t)/((alp-kga)*(bet-kga)) + ((k21-alp)*exp(-alp*t)/((kga-alp)*(bet-alp)) + ((k21-bet)*exp(-bet*t)/((kga-bet)*(alp-bet))));

ContPLCL(i,j)=PL0*kpm*kca* ( ((k21-kc)*exp(-kc*t)/((alp-kc)*(bet-kc)*(kp-kc)) + ((k21-kp)*exp(-kp*t)/((alp-kp)*(bet-kp)*(kga-kc)) + ((k21-alp)*exp(-alp*t)/((kga-alp)*(alp-bet)) + ((k21-bet)*exp(-bet*t)/((kga-bet)*(alp-bet))));

ContCLGIT(i,j)=CL0*kcm*kga*Foral* ( ((k21-kc)*exp(-kc*t)/((alp-kc)*(bet-kc)*(kga-kc)) + ((k21-kga)*exp(-kga*t)/((alp-kga)*(bet-kga)*(kcg-kc)) + ((k21-alp)*exp(-alp*t)/((kga-alp)*(alp-bet)) + ((k21-bet)*exp(-bet*t)/((kga-bet)*(alp-bet))));

ContPCLGLIT(i,j)=PL0*kpm*kcm*kga*Foral* ( ((k21-kc)*exp(-kc*t)/((alp-kc)*(bet-kc)*(kga-kc)*(kp-kc)) + ((k21-kga)*exp(-kga*t)/((alp-kga)*(bet-kga)*(kcg-kc)*(kp-kc)) + ((k21-alp)*exp(-alp*t)/((kga-alp)*(alp-bet)) + ((k21-bet)*exp(-bet*t)/((kga-bet)*(alp-bet)));

ContPCLG(i,j)=CL0*kca*k12* ( exp(-kct)/((alp-kct)*bgk) + (exp(-bet)/((kp-bet)));

ContPGIT(i,j)=GIT0*kga*Foral*k12* ( exp(-kgt)/((alp-kgt)*bgk) + (exp(-alp)/((kga-alp)*bgk));

ContPCLL(i,j)=PL0*kpm*kca*k12* ( exp(-kct)/((alp-kct)*bgk) + (exp(-bet)/((kp-bet)));

ContPC(i,j)=ContP(i,j)+ContPCL(i,j)+ContPGIT(i,j)+ContPCLL(i,j)+ContPCLGLIT(i,j)+ContPLCLG(i,j)+LastPC(i,j);

end

%%

CC1(1,j)=PL0*kpa*(((k21-kp)/((alp-kp)*(bet-kp)));
CC2(1,j)=PL0*kpa*(((k21-alp)/((kp-alp)*(bet-alp))));
CC3(1,j)=PL0*kpa*(((k21-bet)/((kp-bet)*(alp-bet))));
CC4(1,j)= CL0*kca*(((k21-kc)/((alp-kc)*(bet-kc))));
CC5(1,j)=CL0*kca*(((k21-alp)/((kc-alp)*(bet-alp))));
CC6(1,j)=CL0*kca*(((k21-bet)/((kc-bet)*(alp-bet))));
CC7(1,j)=PL0*kpm*kca*(((k21-kc)/((alp-kc)*(bet-kc)*(kp-kc))));
CC8(1,j)=PL0*kpm*kca*(((k21-kp)/((alp-kp)*(bet-kp)*(kc-kp))));
CC9(1,j)=PL0*kpm*kca*(((k21-alp)/((kp-alp)*(bet-alp)*(kc-alp))));
CC10(1,j)=PL0*kpm*kca*(((k21-bet)/((kp-bet)*(alp-bet)*(kc-bet))));
CC11(1,j)=GIT0*kga*Foral*(((k21-kga)/((alp-kga)*(bet-kga))));
CC12(1,j)=GIT0*kga*Foral*(((k21-alp)/((kga-alp)*(bet-kga))));
CC13(1,j)=GIT0*kga*Foral*(((k21-bet)/((kga-bet)*(alp-kga))));
CC14(1,j)=CL0*kcm*kga*Foral*(((k21-kc)/((alp-kc)*(bet-kc)*(kga-kc))));
CC15(1,j)=CL0*kcm*kga*Foral*(((k21-kpm)/((alp-kpm)*(bet-kpm)*(kga-kpm))));
CC16(1,j)=CL0*kcm*kga*Foral*(((k21-alp)/((kpg-alp)*(bet-kpg)*(kga-kpg))));
CC17(1,j)=CL0*kcm*kga*Foral*(((k21-bet)/((kpg-bet)*(alp-kpg)*(kga-kpg))));
CC18(1,j)=PL0*kpm*kcm*kga*Foral*(((k21-kc)/((alp-kc)*(bet-kc)*(kga-kc)*(kp-kc))));
CC19(1,j)=PL0*kpm*kcm*kga*Foral*(((k21-kp)/((alp-kp)*(bet-kp)*(kga-kp)*(kp-kp))));
CC20(1,j)=PL0*kpm*kcm*kga*Foral*(((k21-kp)/((kga-kp)*(alp-kp)*(kcp-kp))));
CC21(1,j)=PL0*kpm*kcm*kga*Foral*(((k21-kc)/((kga-kc)*(alp-kc)*(kp-kc))));
CC22(1,j)=PL0*kpm*kcm*kga*Foral*(((k21-kp)/((kga-kp)*(alp-kp)*(kc-kp))));

CCa(1,j)=CC2(1,j)+CC5(1,j)+CC9(1,j)+CC12(1,j)+CC16(1,j)+CC21(1,j);
CCb(1,j)=CC3(1,j)+CC6(1,j)+CC10(1,j)+CC13(1,j)+CC17(1,j)+CC22(1,j);
CCc(1,j)=CC4(1,j)+CC7(1,j)+CC14(1,j)+CC18(1,j);
CCp(1,j)=CC1(1,j)+CC8(1,j)+CC20(1,j);
CCg(1,j)=CC11(1,j)+CC15(1,j)+CC19(1,j);

AAUC(1,j)=CCa(1,j)/alp + CCb(1,j)/bet + CCc(1,j)/kc + CCp(1,j)/kp + CCg(1,j)/kga;
Auc(1,j)=AAUC(1,j)/V0;
AUMC(1,j)=CCa(1,j)/(alp^2) + CCb(1,j)/(bet^2) + CCc(1,j)/(kc^2) + CCp(1,j)/(kp^2) + CCg(1,j)/(kga^2);
Mrt(1,j)=AUMC(1,j)/AAUC(1,j);

AUC=cell(5,1);
AUC{1,1}=num2str(mean(Auc),'%.1f');
AUC{2,1}=num2str(std(Auc),'%.1f');
AUC{3,1}=num2str(std(Auc)/sqrt(n),'%.1f');
AUC{4,1}=num2str(median(Auc),'%.1f');
AUC{5,1}=[num2str(prctile(Auc(1,:),5),'%.1f'),', -',num2str(prctile(Auc(1,:),95),'%.1f')];
AUC{6,1}=[num2str(min(Auc),'%.1f'),', -',num2str(min(Auc)+range(Auc),'%.1f')]

Cmax=cell(6,1);
Cmax{1,1}=num2str(mean(cmax),'%.1f');
Cmax{2,1}=num2str(std(cmax),'%.1f');
Cmax{3,1}=num2str(std(cmax)/sqrt(n),'%.1f');
Cmax{4,1}=num2str(median(cmax),'%.1f');
Cmax{5,1}=[num2str(prctile(cmax(1,:),5),'%.1f'),', -',num2str(prctile(cmax(1,:),95),'%.1f')];
Cmax{6,1}=[num2str(min(cmax),'%.1f'),', -',num2str(min(cmax)+range(cmax),'%.1f')]

Tmax=cell(6,1);
Tmax{1,1}=num2str(mean(tmax),'%.1f');
Tmax{2,1}=num2str(std(tmax),'%.1f');
Tmax{3,1}=num2str(std(tmax)/sqrt(n),'%.1f');
Tmax{4,1}=num2str(median(tmax),'%.1f');
Tmax{5,1}=[num2str(prctile(tmax(1,:),5),'%.1f'),', -',num2str(prctile(tmax(1,:),95),'%.1f')];
Tmax{6,1}=[num2str(min(tmax),'%.1f'),', -',num2str(min(tmax)+range(tmax),'%.1f')]

tbethalf=cell(6,1);
tbethalf{1,1}=num2str(mean(thalfbet),'%.1f');
tbethalf{2,1}=num2str(std(thalfbet),'%.1f');
tbethalf{3,1}=num2str(std(thalfbet)/sqrt(n),'%.1f');
tbethalf{4,1}=num2str(median(thalfbet),'%.1f');
tbethalf{5,1}=[num2str(prctile(thalfbet(1,:),05),'%.1f'),', -',num2str(prctile(thalfbet(1,:),95),'%.1f')];
tbethalf{6,1}=[num2str(min(thalfbet),'%.1f'),', -',num2str(min(thalfbet)+range(thalfbet),'%.1f')]

MRT=cell(6,1);
MRT{1,1}=num2str(mean(Mrt),'%.1f');
MRT{2,1}=num2str(std(Mrt),'%.1f');
MRT{3,1}=num2str(std(Mrt)/sqrt(n),'%.1f');
MRT{4,1}=num2str(median(Mrt),'%.1f');
MRT{5,1}=[num2str(prctile(Mrt(1,:),5),'%.1f'),', -',num2str(prctile(Mrt(1,:),95),'%.1f')];
MRT{6,1}=[num2str(min(Mrt),'%.1f'),', -',num2str(min(Mrt)+range(Mrt),'%.1f')]

Rowname = {'Mean';'SD';'SEM';'Median';'5 - 95 th Percentile';'Range'};
Table=table(AUC,Cmax,Tmax,tbethalf,MRT,'RowNames',Rowname)
Finding the Median and Percentiles

```matlab
mCp=zeros(size(Cp,1),1);
sdCp=zeros(size(Cp,1),1);
uci=zeros(size(Cp,1),1);
lci=zeros(size(Cp,1),1);

for i=1:1:NoP
    mCp(i,1)=median(Cp(i,:));
    sdCp(i,1)=std(Cp(i,:));
    uci(i,1)=prctile(Cp(i,:),95);
    lci(i,1)=prctile(Cp(i,:),05);
    mecp=mean(Cp(i,:));
end
```

Graphing

```matlab
set(0,'DefaultFigureWindowStyle','normal')
figure ('name','Linear','NumberTitle','off')
hold on;
ucig=area(time,uci);
set(ucig,'FaceColor',[0.8 0.8 0.8])
set(ucig,'LineStyle','---')
plot(time,uci,'--r')
lcig=area(time,lci);
set(lcig,'FaceColor',[1 1 1])
set(lcig,'LineStyle','---')
p4=plot(time,lci,'--r');
p5=plot(time,mCp,'k');

TMd90L=[0 15 30 60 120 180 240 360];
CMd90L=[0 2.28 2.59 1.53 1.00 0.68 0.46 0.18];
p6=plot(TMd90L,CMd90L,'+');
TMd45L=[0 15 30 60 120 180 240 360];
CMd45L=[0 2.83 2.44 1.94 1.33 0.73 0.45 0.16];
p7=plot(TMd45L,CMd45L,'o');
TTh60L=[0 15 30 60 120 180 240 360];
CTh60L=[0 3.22 2.99 1.91 1.19 0.84 0.61 0.25];
p8=plot(TTh60L,CTh60L,'*');
TTh30L=[0 15 30 60 120 180 240 360];
```

CTh30L=[0 1.30 1.46 1.08 0.44 0.24 0.13 0.05];
p9=plot(TTh30L,CTh30L,'x');
POT201=[0 0.30 60 90 120 180 240 300 360 480 720 1440];
POC201=[0 0.83 2.32 2.60 2.64 2.47 2.09 1.55 1.1 0.73 0.33 0.04];
%p10=plot(POT201,POC201,'x');
set(gca,'FontSize',14)
xlabel('Time (min)')
ylabel('Plasma Conc (nM)')
legend([p4 p5 p6 p7 p8 p9],{'95% & 5% percentile','Median','Md 90', 'Md 45','Th60', 'Th30'},'location','east')
%legend([p4 p5 p10],{'95% & 5% percentile','Median','Ref #201'},'location','northeast')
title('95 and 5 Percentiles of Plasma Concentration time profile after 20000 MCS ')
axis([0 Last_time_point 0 max(uci)])

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%      Log
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
figure ('name','Log
-Linear','NumberTitle','off')
hold on;
sucig=area(time,uci);
set(sucig,'FaceColor',[0.8 0.8 0.8])
set(sucig,'LineStyle','-')
plot(time,uci,'--r')
slcig=area(time,lici);
set(slcig,'FaceColor',[1 1 1])
set(slcig,'LineStyle',--)\n
s4=plot(time,lici,'--r');
s5=plot(time,mCp,'k');
s6=plot(TMd90L,CMd90L,'+');
s7=plot(TMd45L,CMd45L,'o');
s8=plot(TTh60L,CTh60L,'*');
s9=plot(TTh30L,CTh30L,'x');
set(gca,'FontSize',14)
xlabel('Time (min)')
ylabel('Plasma Conc (nM)')
legend([s4 s5 s6 s7 s8 s9],{'95% & 5% percentile','Median','Md 90', 'Md 45','Th60', 'Th30'},'location','northeast')
%legend([s4 s5],{'95% & 5% percentile','Median'},'location','east')
title('95 and 5 Percentiles of Plasma Concentration time profile after 20000 MCS ')\nset(gca,'YScale','log')
axis([0 500 0.1 10])

%close Log-Linear
toc(Tstart)
Scientist Fits of IV TOB

**Figure 1.** Simulations results of plasma concentration time profile on a linear-scale for TOB 101 based on the parameters estimated from various applied weighting factors.

**Figure 2.** Simulations results of plasma concentration time profile on a Log-scale for TOB 101 based on the parameters estimated from various applied weighting factors.
Figure 3. Simulations results of plasma concentration time profile on a linear-scale for TOB 102 based on the parameters estimated from various applied weighting factors.

Figure 4. Simulations results of plasma concentration time profile on a Log-scale for TOB 102 based on the parameters estimated from various applied weighting factors.
Figure 5. Simulations results of plasma concentration time profile on a linear-scale for TOB 103A based on the parameters estimated from various applied weighting factors.

Figure 6. Simulations results of plasma concentration time profile on a Log-scale for TOB 103A based on the parameters estimated from various applied weighting factors.
Figure 7. Simulations results of plasma concentration time profile on a linear-scale for TOB 103B based on the parameters estimated from various applied weighting factors.

Figure 8. Simulations results of plasma concentration time profile on a Log-scale for TOB 103B based on the parameters estimated from various applied weighting factors.
Table 1. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study TOB 101. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 2. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y² applied to data from study TOB 102. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.

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<th>1/y²</th>
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Visual inspection: ✓ ✓ ✓

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Table 3. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y² applied to data from study TOB 103A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 4. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y² applied to data from study TOB 103B. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.

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<tr>
<th>Time (min)</th>
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<th>Weighting Factor</th>
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Visual inspection ✓ ✓ ✓

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<tr>
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<td>k₁₂ CV</td>
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MCS Results for IV TOB
Figure 9. TOB 104 VPC predictions using model 2’s inability to capture pre-dose level on day 3.

Figure 10. TOB 104 VPC predictions using model 2’s inability to capture pre-dose level, the remaining data points would not be captured.
Figure 11. TOB 101 VPC and exposure metric variability comparison. (top left - $c_p(t)$ profile, top center – $c_s(t)$ profile, top right PLU(t) profile, bottom left – $c_{\text{max}}$ box and whisker plot (WHP), bottom center- $c_{60}$ WHP, bottom right – beta WHP)

Figure 12. TOB 102 VPC and exposure metric variability comparison. (top left -$c_p(t)$ profile, top center – $c_s(t)$ profile, top right PLU(t) profile, bottom left – $c_{\text{max}}$ box and whisker plot (WHP), bottom center- $c_{60}$ WHP, bottom right – beta WHP)
Figure 13. TOB 103A VPC and exposure metric variability comparison. (top left $c_p(t)$ profile, top center $c_s(t)$ profile, top right PLU(t) profile, bottom left $c_{\text{max}}$ box and whisker plot (WHP), bottom center $c_{60}$ WHP, bottom right beta WHP)

Figure 14. TOB 103B VPC and exposure metric variability comparison. (top left $c_p(t)$ profile, top center $c_s(t)$ profile, top right PLU(t) profile, bottom left $c_{\text{max}}$ box and whisker plot (WHP), bottom center $c_{60}$ WHP, bottom right beta WHP)
### Table 6. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 102

<table>
<thead>
<tr>
<th>102</th>
<th>Reported</th>
<th>Simulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_e$ ($hr^{-1}$)</td>
<td>$0.34 \pm 0.046$</td>
<td>$0.22 \pm 0.042$</td>
</tr>
<tr>
<td></td>
<td>$0.30 - 0.42$</td>
<td>$0.12 - 0.35$</td>
</tr>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>$11 \pm 1.5$</td>
<td>$9.5 \pm 0.88$</td>
</tr>
<tr>
<td></td>
<td>$9.2 - 13$</td>
<td>$7.7 - 12$</td>
</tr>
<tr>
<td>AUC (mg*hr/L)</td>
<td>$27 \pm 4.3$</td>
<td>$29 \pm 4.7$</td>
</tr>
<tr>
<td></td>
<td>$20 - 31$</td>
<td>$10 - 47$</td>
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</table>

### Table 7. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 103A

<table>
<thead>
<tr>
<th>103A</th>
<th>Reported</th>
<th>Simulated</th>
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</thead>
<tbody>
<tr>
<td>$t_{1/2}^\beta$ (hr) (Median, IQR)</td>
<td>$3.1$</td>
<td>$3.2$</td>
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<tr>
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<td>$2.7 - 5.8$</td>
<td>$2.8 - 3.5$</td>
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<tr>
<td>$C_{max}$ (mg/L) (Median, IQR)</td>
<td>$14$</td>
<td>$13$</td>
</tr>
<tr>
<td></td>
<td>$12 - 19$</td>
<td>$12 - 13$</td>
</tr>
<tr>
<td>AUC (mg*hr/L) (Median, IQR)</td>
<td>$97$</td>
<td>$114$</td>
</tr>
<tr>
<td></td>
<td>$78 - 123$</td>
<td>$102 - 126$</td>
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</table>

### Table 8. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 103B

<table>
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<th>Reported</th>
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</thead>
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<tr>
<td>$t_{1/2}^\beta$ (hr) (Median, IQR)</td>
<td>$2.7$</td>
<td>$3.1$</td>
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<td>$2.2 - 3.7$</td>
<td>$2.7 - 3.5$</td>
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<tr>
<td>$C_{max}$ (mg/L) (Median, IQR)</td>
<td>$36$</td>
<td>$36$</td>
</tr>
<tr>
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<td>$33 - 46$</td>
<td>$35 - 39$</td>
</tr>
<tr>
<td>AUC (mg*hr/L) (Median, IQR)</td>
<td>$108$</td>
<td>$133$</td>
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<td>$96 - 123$</td>
<td>$119 - 149$</td>
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### Table 9. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 104

<table>
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<th>104</th>
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<td>$k_e$ ($hr^{-1}$) Mean (range)</td>
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<td>$0.12$</td>
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<tr>
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<td>$0.22 - 0.38$</td>
<td>$0.12 - 0.36$</td>
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<tr>
<td>$C_{30}$ (mg/L) Mean (range)</td>
<td>$29$</td>
<td>$31$</td>
</tr>
<tr>
<td></td>
<td>$23 - 36$</td>
<td>$25 - 39$</td>
</tr>
<tr>
<td>AUC (mg*hr/L) Mean (range)</td>
<td>$82$</td>
<td>$112$</td>
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<td></td>
<td>$60 - 115$</td>
<td>$77 - 184$</td>
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SD and RD Sensitivity Analysis Plots for IV TOB

**Figure 15.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $CL_{tot}$ after a SD IV administration of 105 mg

**Figure 16.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{12}$ after a SD IV administration of 105 mg
Figure 17. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{21}$ after a SD IV administration of 105 mg

Figure 18. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pa}$ after a SD IV administration of 105 mg
Figure 19. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{ca}$ after a SD IV administration of 105 mg

Figure 20. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pm}$ after a SD IV administration of 105 mg
Figure 21. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cm}$ after a SD IV administration of 105 mg

Figure 22. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pd}$ after a SD IV administration of 105 mg
Figure 23. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cd}$ after a SD IV administration of 105 mg

Figure 24. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pls1}$ after a SD IV administration of 105 mg
Figure 25. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pls2}$ after a SD IV administration of 105 mg

Figure 26. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cls1}$ after a SD IV administration of 105 mg
Figure 27. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cls2}$ after a SD IV administration of 105 mg

Figure 28. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $CL_{tot}$ after a three-day RD IV administration of 105 mg
Figure 29. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{12}$ after a three-day RD IV administration of 105 mg

Figure 30. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{21}$ after a three-day RD IV administration of 105 mg
Figure 31. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pa}$ after a three-day RD IV administration of 105 mg

Figure 32. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{ca}$ after a three-day RD IV administration of 105 mg
**Figure 33.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pm}$ after a three-day RD IV administration of 105 mg

**Figure 34.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cm}$ after a three-day RD IV administration of 105 mg
Figure 35. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pd}$ after a three-day RD IV administration of 105 mg

Figure 36. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cd}$ after a three-day RD IV administration of 105 mg
Figure 37. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pls1}$ after a three-day RD IV administration of 105 mg

Figure 38. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pls2}$ after a three-day RD IV administration of 105 mg
Figure 39. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cls1}$ after a three-day RD IV administration of 105 mg

Figure 40. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cls2}$ after a three-day RD IV administration of 105 mg

VPC Plots for INH TOB
Figure 41. TOB 302A exposure metric variability comparison. (left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), right - Reported vs simulated $c_{p,max}$ WHP)

Figure 42. TOB 302B exposure metric variability comparison. (left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), right - Reported vs simulated $c_{p,max}$ WHP)
Figure 43. TOB 302C exposure metric variability comparison. (left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), right - Reported vs simulated $c_p,_{max}$ WHP)

Figure 44. TOB 302D exposure metric variability comparison. (left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), right - Reported vs simulated $c_p,_{max}$ WHP)
Figure 45. TOB 303A VPC and exposure metric variability comparison. (left - $c_p(t)$ VPC profile, center – Reported vs simulated $c_p$ AUC box and whisker plot (WHP), right - Reported vs simulated $c_{p, max}$ WHP)

Figure 46. TOB 303B VPC and exposure metric variability comparison. (left - $c_p(t)$ VPC profile, center – Reported vs simulated $c_p$ AUC box and whisker plot (WHP), right - Reported vs simulated $c_{p, max}$ WHP)
Figure 47. TOB 307A VPC and exposure metric variability comparison. (Top left – $c_p(t)$ VPC profile, top center – $c_s(t)$ VPC profile, top right - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), bottom left - Reported vs simulated $c_p, \text{max}$ WHP, bottom center - Reported vs simulated $c_s$ AUC WHP, bottom right - Reported vs simulated $c_p, \text{max}$ WHP)

Figure 48. TOB 307B VPC and exposure metric variability comparison. (Top left – $c_p(t)$ VPC profile, top center – $c_s(t)$ VPC profile, top right - Reported vs simulated $c_p$ AUC box
Figure 49. TOB 307C VPC and exposure metric variability comparison. (Top left - $c_p(t)$ VPC profile, top center – $c_s(t)$ VPC profile, top right - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), bottom left - Reported vs simulated $c_{p,max}$ WHP, bottom center - Reported vs simulated $c_s$ AUC WHP, bottom right - Reported vs simulated $c_{s,max}$ WHP)
**Figure 50.** TOB 309A VPC and exposure metric variability comparison. (Top left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), top right - Reported vs simulated $c_{p,max}$ WHP bottom left - Reported vs simulated $c_s$ AUC WHP, bottom right - Reported vs simulated $c_{s,max}$ WHP)

**Figure 51.** TOB 309b VPC and exposure metric variability comparison. (Top left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), top right - Reported vs simulated $c_{p,max}$ WHP bottom left - Reported vs simulated $c_s$ AUC WHP, bottom right - Reported vs simulated $c_{s,max}$ WHP)
Figure 52. TOB 309C VPC and exposure metric variability comparison. (Top left - Reported vs simulated \( c_p \) AUC box and whisker plot (WHP), top right - Reported vs simulated \( c_{p,\text{max}} \) WHP bottom left - Reported vs simulated \( c_s \) AUC WHP, bottom right - Reported vs simulated \( c_{s,\text{max}} \) WHP)

Figure 53. TOB 309D VPC and exposure metric variability comparison. (Top left - Reported vs simulated \( c_p \) AUC box and whisker plot (WHP), top right - Reported vs simulated \( c_{p,\text{max}} \) WHP bottom left - Reported vs simulated \( c_s \) AUC WHP, bottom right - Reported vs simulated \( c_{s,\text{max}} \) WHP)
Figure 54. TOB 309E VPC and exposure metric variability comparison. (Top left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), top right - Reported vs simulated $c_{p,\text{max}}$ WHP bottom left - Reported vs simulated $c_s$ AUC WHP, bottom right - Reported vs simulated $c_{s,\text{max}}$ WHP)

Figure 55. TOB 309F VPC and exposure metric variability comparison. (Top left - Reported vs simulated $c_p$ AUC box and whisker plot (WHP), top right - Reported vs simulated $c_{p,\text{max}}$ WHP bottom left - Reported vs simulated $c_s$ AUC WHP, bottom right - Reported vs simulated $c_{s,\text{max}}$ WHP)
SD and RD Sensitivity Analysis Plots for INH TOB

**Figure 60.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in CL$_{tot}$ after a SD INH administration of 300 mg

**Figure 61.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{12}$ after a SD INH administration of 300 mg
Figure 62. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{21}$ after a SD INH administration of 300 mg

Figure 63. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pa}$ after a SD INH administration of 300 mg
**Figure 64.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cb}$ after a SD INH administration of 300 mg

**Figure 65.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pm}$ after a SD INH administration of 300 mg
**Figure 66.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cm}$ after a SD INH administration of 300 mg

**Figure 67.** Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pd}$ after a SD INH administration of 300 mg
Figure 68. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cd}$ after a SD INH administration of 300 mg

Figure 69. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pls1}$ after a SD INH administration of 300 mg
Figure 70. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{pls2}$ after a SD INH administration of 300 mg

Figure 71. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cls1}$ after a SD INH administration of 300 mg
Figure 72. Resulting $c_p(t)$ and $c_s(t)$ from a 25-fold change in $k_{cls2}$ after a SD INH administration of 300 mg.

Figure 73. Representative predictions of $c_p(t)$ and $c_s(t)$ profiles for study 307A after attempts to optimize Fpd using Model 3 and IV optimized systemic and pulmonary disposition parameters.
Figure 74. Predictions of both TOB 104 and 307 after simultaneous parameter optimization with both data sets.

Figure 75. Simultaneous predictions of the four $c_p(t)$ and $c_v(t)$ profiles from TOB 307.
<table>
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<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AUC (mg*hr/L)</td>
<td>3.3 ± 0.7</td>
<td>3.8 ± 2.0</td>
<td>14%</td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (mg/L)</td>
<td>0.6 ± 0.2</td>
<td>0.57 ± 0.32</td>
<td>-5.4%</td>
</tr>
</tbody>
</table>

**Table 10.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 302A

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AUC (mg*hr/L)</td>
<td>3.6 ± 1.9</td>
<td>3.9 ± 2.0</td>
<td>7.9%</td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (mg/L)</td>
<td>0.7 ± 0.4</td>
<td>0.59 ± 0.32</td>
<td>-16%</td>
</tr>
</tbody>
</table>

**Table 11.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 302B

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AUC (mg*hr/L)</td>
<td>5.0 ± 3.0</td>
<td>3.4 ± 1.79</td>
<td>-32%</td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (mg/L)</td>
<td>1.2 ± 0.6</td>
<td>0.51 ± 0.29</td>
<td>-57%</td>
</tr>
</tbody>
</table>

**Table 12.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 302C

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AUC (mg*hr/L)</td>
<td>1.9 ± 0.8</td>
<td>2.32 ± 1.22</td>
<td>22%</td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (mg/L)</td>
<td>0.5 ± 0.2</td>
<td>0.35 ± 0.19</td>
<td>-30%</td>
</tr>
</tbody>
</table>

**Table 13.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 302D

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AUC (mg*hr/L)</td>
<td>2.1 ± 0.75</td>
<td>1.9 ± 0.96</td>
<td>-7.7%</td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (mg/L)</td>
<td>0.28 ± 0.09</td>
<td>0.18 ± 0.09</td>
<td>-37%</td>
</tr>
</tbody>
</table>

**Table 14.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 303A
**Table 15.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 303B

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum AUC_{0.8} (mg*hr/L)</td>
<td>1471 ± 1278</td>
<td>1656 ± 1661</td>
<td>13%</td>
</tr>
<tr>
<td>Sputum C_{max}   (mg/L)</td>
<td>0.99 ± 0.84</td>
<td>1.8 ± 3.6</td>
<td>81%</td>
</tr>
<tr>
<td>Plasma AUC_{0.8} (mg*hr/L)</td>
<td>4.96 ± 2.24</td>
<td>3.00 ± 1.47</td>
<td>-39%</td>
</tr>
<tr>
<td>Plasma C_{max}   (mg/L)</td>
<td>1.12 ± 0.44</td>
<td>0.58 ± 0.31</td>
<td>-48%</td>
</tr>
</tbody>
</table>

**Table 16.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 307A

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum AUC_{0.8} (mg*hr/L)</td>
<td>361 ± 422</td>
<td>730 ± 742</td>
<td>102%</td>
</tr>
<tr>
<td>Sputum C_{max}   (mg/L)</td>
<td>0.33 ± 0.31</td>
<td>0.73 ± 1.69</td>
<td>119%</td>
</tr>
<tr>
<td>Plasma AUC_{0.8} (mg*hr/L)</td>
<td>1.43 ± 1.43</td>
<td>1.17 ± 0.57</td>
<td>-17%</td>
</tr>
<tr>
<td>Plasma C_{max}   (mg/L)</td>
<td>0.38 ± 0.17</td>
<td>0.21 ± 0.11</td>
<td>-45%</td>
</tr>
</tbody>
</table>

**Table 17.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 307B

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum AUC_{0.8} (mg*hr/L)</td>
<td>805 ± 723</td>
<td>1306 ± 1304</td>
<td>62%</td>
</tr>
<tr>
<td>Sputum C_{max}   (mg/L)</td>
<td>0.58 ± 0.54</td>
<td>1.20 ± 2.72</td>
<td>106%</td>
</tr>
<tr>
<td>Plasma AUC_{0.8} (mg*hr/L)</td>
<td>2.98 ± 1.92</td>
<td>2.35 ± 1.15</td>
<td>-21%</td>
</tr>
<tr>
<td>Plasma C_{max}   (mg/L)</td>
<td>0.69 ± 0.34</td>
<td>0.42 ± 0.23</td>
<td>-39%</td>
</tr>
</tbody>
</table>

**Table 18.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 307C

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Predicted</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum AUC_{0.8} (mg*hr/L)</td>
<td>1275 ± 1359</td>
<td>1850 ± 1879</td>
<td>45%</td>
</tr>
<tr>
<td>Sputum C_{max}   (mg/L)</td>
<td>0.96 ± 0.95</td>
<td>1.20 ± 2.58</td>
<td>25%</td>
</tr>
<tr>
<td>Plasma AUC_{0.8} (mg*hr/L)</td>
<td>3.94 ± 1.52</td>
<td>3.52 ± 1.73</td>
<td>-10%</td>
</tr>
<tr>
<td>Plasma C_{max}   (mg/L)</td>
<td>0.96 ± 0.4</td>
<td>0.51 ± 0.27</td>
<td>-47%</td>
</tr>
</tbody>
</table>

**Table 19.** Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 307D
<table>
<thead>
<tr>
<th>Table 20. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 309A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sputum AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Sputum C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 21. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 309B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sputum AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Sputum C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 22. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 309C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sputum AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Sputum C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 23. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 309D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sputum AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Sputum C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma AUC (mg*hr/L)</strong></td>
</tr>
<tr>
<td><strong>Plasma C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</strong></td>
</tr>
</tbody>
</table>
### Table 24. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 309E

<table>
<thead>
<tr>
<th>Metric</th>
<th>Reported</th>
<th>Simulated</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum AUC (mg*hr/L)</td>
<td>1307 ± 978</td>
<td>1081 ± 1920</td>
<td>-17%</td>
</tr>
<tr>
<td>Sputum C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>1048 ± 1080</td>
<td>417 ± 745</td>
<td>-60%</td>
</tr>
<tr>
<td>Plasma AUC (mg*hr/L)</td>
<td>4.6 ± 2.0</td>
<td>4.8 ± 2.37</td>
<td>4.4%</td>
</tr>
<tr>
<td>Plasma C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>1.02 ± 0.53</td>
<td>1.10 ± 0.65</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

### Table 25. Reported exposure metric central tendency and dispersion compared to simulated values for the same metrics for TOB 309F

<table>
<thead>
<tr>
<th>Metric</th>
<th>Reported</th>
<th>Simulated</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum AUC (mg*hr/L)</td>
<td>974 ± 1143</td>
<td>466 ± 255</td>
<td>-52%</td>
</tr>
<tr>
<td>Sputum C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>737 ± 1028</td>
<td>170 ± 95</td>
<td>-76%</td>
</tr>
<tr>
<td>Plasma AUC (mg*hr/L)</td>
<td>4.8 ± 2.5</td>
<td>6.8 ± 2.79</td>
<td>42%</td>
</tr>
<tr>
<td>Plasma C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>1.04 ± 0.58</td>
<td>1.79 ± 0.90</td>
<td>72%</td>
</tr>
</tbody>
</table>
Table 26. Reported plasma and sputum exposure metrics and relevant study design components for the analyzed inhalation studies.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>CF</td>
<td>CF</td>
<td>CF</td>
<td>CF</td>
<td>HV</td>
<td>HV</td>
<td>CF</td>
<td>CF</td>
<td>CF</td>
<td>CF</td>
<td>CF</td>
<td>CF</td>
<td>HV</td>
<td>HV</td>
<td>CF</td>
<td>CF</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>300</td>
<td>30</td>
<td>60</td>
<td>90</td>
<td>60</td>
<td>90</td>
<td>60</td>
<td>90</td>
<td>60</td>
<td>90</td>
<td>60</td>
<td>90</td>
<td>60</td>
<td>90</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>DT (%</td>
<td>9.1</td>
<td>35.4</td>
<td>35.4</td>
<td>35.4</td>
<td>5.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
<td>34.3</td>
</tr>
<tr>
<td>Estimated Dose (%</td>
<td>102</td>
<td>76</td>
<td>79</td>
<td>70</td>
<td>74</td>
<td>90</td>
<td>76</td>
<td>82</td>
<td>73</td>
<td>68</td>
<td>67</td>
<td>90</td>
<td>42</td>
<td>45</td>
<td>71</td>
<td>40</td>
</tr>
</tbody>
</table>

Note: The table includes columns for study ID, group, device, dose, DTL (%), estimated DTL (mg), inhalation time (min), sputum Tmax (h), sputum Cmax (ug/g), sputum AUC (ug*h/g), sputum t1/2 (h), serum Tmax (h), serum Cmax (ug/ml), serum AUC (ug*h/ml), serum t1/2 (h), and calculated Finh (%).
Figure 56. Mass balance drug excretion IV administration simulation results based on the final optimized IV parameter space. Left plot represents cumulative drug eliminated from the central unbound lung compartment via $k_{cm}$. Right plot represents cumulative drug eliminated from the central compartment via $CL_{tot}$.

Figure 57. Mass balance drug excretion IV administration simulation results based on the final optimized INH parameter space. Left plot represents cumulative drug eliminated from
the central unbound lung compartment via $k_{cm}$. Right plot represents cumulative drug eliminated from the central compartment via $CL_{tot}$.

**Figure 58.** Mass balance drug excretion INH administration simulation results based on the final optimized IV parameter space. Left plot represents cumulative drug eliminated from the central unbound lung compartment via $k_{cm}$. Right plot represents cumulative drug eliminated from the central compartment via $CL_{tot}$.

**Figure 59.** Mass balance drug excretion INH administration simulation results based on the final optimized IV parameter space. Left plot represents cumulative drug eliminated from the central unbound lung compartment via $k_{cm}$. Right plot represents cumulative drug eliminated from the central compartment via $CL_{tot}$.
Various codes utilized for TOB analysis

IndVars: T
DepVars: CP1
Params: D1, Tinf1, V0, k12, k21, Cltot

//
// dose in mg
// conc in mg/L
// time in min
//
// Input
ARATE11=D1/Tinf1
FLAG1=UNIT(T-Tinf1)
ARATE=ARATE11*(1-FLAG1)

//
// PK-Model
AP1'=ARATE+k21*AT1-((Cltot/(V0*1000))+k12)*AP1
AT1'=k12*AP1-k21*AT1
CP1=AP1/V0

//
// Initial Conditions
T=0
AP1=0
AT1=0

***

Code 1. Two compartment open body model describing TOB disposition after IV infusion administration
rm(list=ls()); cat("014"); dev.off(); set.seed(12301991); Start.time <- proc.time() # start a
timer
BW.me <- 57.4
BW.cv <- 13*100/BW.me
n <- 300
PerKgDose <- 8 #mg/kg
tinf <- 10
t <- seq(0,3360,10)
Clpop <- 70
Clprop.me <- 1
k12.me <- 0.010
k21.me <- 0.015
V0pop <- 10
V0prop.me <- 1
Clprop.cv <- 20
k12.cv <- 30
k21.cv <- 30
V0prop.cv <- 20
kga.me <- 1
Foral.me <- 0
kga.cv <- 0
Foral.cv <- 0
kpa.me <- 0.006
kca.me <- 0.9
kpm.me <- 0.16
kcm.me <- 0.6
kpd.me <- 0.0008
kcd.me <- 0.001
TLV.me <- 20 #ml
kpls1.me <- 0.0005
kpls2.me <- 0.0001
kcls1.me <- 0.0005
kcls2.me <- 0.0002
kpa.cv <- 30
kca.cv <- 30
kpm.cv <- 20
kcm.cv <- 20
kpd.cv <- 30
kcd.cv <- 30
kpls1.cv <- 30
kpls2.cv <- 30
kcls1.cv <- 30
kcls2.cv <- 30
TLVprop.me <- 1
TLVprop.cv <- 20
kin.me <- 0
Fex.me <- 0.009
Fpd.me <- 0.21
Fcd.me <- 0.069
Din.me <- 0
kin.cv <- 0
Fex.cv <- 57
Fpd.cv <- 30
Fcd.cv <- 30
Din.cv <- 9

# Normal Distribution Generator

NormGen <- function(simulation.n, samp.m, samp.cv) {
  samp.sd <- samp.cv*samp.m/100
  out <- rnorm(simulation.n, samp.m, samp.sd)
  return(out)
}

LogNormGen <- function(n, Mean, PCV) {
  CV <- PCV/100
  SD <- (CV*Mean)
  Variance <- SD^2
  mean.in <- log(Mean^2 / sqrt(Variance+Mean^2))
  SD.in <- sqrt(log(Variance/Mean^2 + 1))
  x <- rlnorm(n, mean.in, SD.in)
  return(x)
}

# Generation of numbers

BW.n <- NormGen(n, BW.me, BW.cv)
Clprop.n <- NormGen(n, Clprop.me, Clprop.cv)
k12.n <- LogNormGen(n, k12.me, k12.cv)
k21.n <- LogNormGen(n, k21.me, k21.cv)
V0prop.n <- NormGen(n, V0prop.me, V0prop.cv)
kga.n <- LogNormGen(n, kga.me, kga.cv)
if (Foral.me==0){Foral.n<-matrix(0,1,n)} else if (Foral.me>0){Foral.n<-LogNormGen(n,Foral.me,Foral.cv)}

kpa.n <- LogNormGen(n, kpa.me, kpa.cv)
kca.n <- LogNormGen(n, kca.me, kca.cv)
kpm.n <- LogNormGen(n, kpm.me, kpm.cv)
```r
kcm.n <- LogNormGen(n, kcm.me, kcm.cv)
if (kpd.me==0){kpd.n<-rep(0,n)} else if (kpd.me>0){kpd.n<-LogNormGen(n, kpd.me, kpd.cv)}
if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n, kcd.me, kcd.cv)}
if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n, kpls1.me, kpls1.cv)}
if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n, kpls2.me, kpls2.cv)}
if (kcls1.me==0){kcls1.n<-rep(0,n)} else if (kcls1.me>0){kcls1.n<-LogNormGen(n, kcls1.me, kcls1.cv)}
if (kcls2.me==0){kcls2.n<-rep(0,n)} else if (kcls2.me>0){kcls2.n<-LogNormGen(n, kcls2.me, kcls2.cv)}

TLVprop.n <- NormGen(n, TLVprop.me,TLVprop.cv)
if (kin.me==0){kin.n<-rep(0,n)} else if (kin.me>0){kin.n<-NormGen(n, kin.me,kin.cv)}
  Fex.n <- NormGen(n, Fex.me,Fex.cv)
  Fpd.n <- NormGen(n, Fpd.me,Fpd.cv)
  Fcd.n <- NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n<-rep(0,n)} else if (Din.me>0){Din.n<-NormGen(n, Din.me,Din.cv)}

####################################################################
##########         Set up the ODE Based model function
####################################################################
TCBM <- function(t,Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, Dose, tinf ){

  Dose.Value <- (1-Din)*Dose
  Fgd        <- 1-Fex-Fpd-Fcd
  ED         <- data.frame(var = c("DOSING"), time = c(t[1],tinf,1440,1450,2880,2890), value = c(Dose.Value/tinf,0), method = c("rep"))

  Model.Function <- function(t,IC,Parm) {
    with(as.list(IC, Parm), {
      dDOSING <- 0
      dIn <- -ln*kin*(Fex+Fpd+Fcd+Fgd)
      dPLU <- ln*kin*Fpd + PLS*kpd - PLU*(kpa + kpm)
      dCLU <- ln*kin*Fcd + CLS*kcd - CLU*(kca + kcm) + PLU* kpm
    })
  }

```

```r
return(list(c(dIn,dPLU,dCLU,dPLS,dCLS,dGI,dCC,dPC,dEx,dFe,dEl,dDOSING)))
```

```r
IC <- c(In=0, PLU=0*Fpd, CLU=0*Fcd, PLS=0, CLS=0, GI=0*Fgd, CC=0, PC=0, Ex=0*Fex, Fe=0, El=0,DOSING=0)
Parm <- c(Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Fgd)
out <- ode(IC, t,Model.Function,Parm,events = list(data=ED))
return(out)
```
CP.Data <- matrix(0,length(t),(n))
CS.Data <- matrix(0,length(t),(n))
PL.Data <- matrix(0,length(t),(n))
Cmax.n=rep(0,(n))
C30.n=rep(0,(n))
C60.n=matrix(0,1,n)
Smax.n=rep(0,n)
AUC.n=Dose.n*1000/Cltot.n/60
k10.n <- Cltot.n/V0.n/1000
a.p.b.n <- k10.n+k12.n+k21.n
a.t.b.n <- k10.n*k21.n
beta.n <- (a.p.b.n - ( a.p.b.n^2 - 4*a.t.b.n )^0.5 )^0.5
beta.n <- beta.n*60
t0.5.n <- 0.693/beta.n
for (i in 1:n) {
  Dose   <- Dose.n[i]
  Cltot  <- Cltot.n[i]
  k12    <- k12.n[i]
  k21    <- k21.n[i]
  V0     <- V0.n[i]
  kga    <- kga.n[i]
  Foral  <- Foral.n[i]
  kpa    <- kpa.n[i]
  kca    <- kca.n[i]
  kpm    <- kpm.n[i]
  kcm    <- kcm.n[i]
  kpd    <- kpd.n[i]
  kcd    <- kcd.n[i]
  kpls1  <- kpls1.n[i]
  kpls2  <- kpls2.n[i]
  kcls1  <- kcls1.n[i]
  kcls2  <- kcls2.n[i]
  CLV    <- CLV.n[i]
  PLV    <- PLV.n[i]
  kin    <- kin.n[i]
  Fex    <- Fex.n[i]
  Fpd    <- Fpd.n[i]
  Fcd    <- Fcd.n[i]
  Din    <- Din.n[i]
  Current.Iteration.Data <- TCBM(t,Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd,
                                 kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, Dose, tinf)
  In.Data[,i] <- Current.Iteration.Data[,2]
  PLU.Data[,i] <- Current.Iteration.Data[,3]
  PLS.Data[,i] <- Current.Iteration.Data[,5]
GI.Data[,i] <- Current.Iteration.Data[,7]
CC.Data[,i] <- Current.Iteration.Data[,8]
PC.Data[,i] <- Current.Iteration.Data[,9]

CP.Data[,i] <- Current.Iteration.Data[,8]/V0
CS.Data[,i] <- Current.Iteration.Data[,4]/CLV
PL.Data[,i] <- Current.Iteration.Data[,3]/PLV

Cmax.n[i]<- max(CP.Data[,i])
C30.n[i] <- CP.Data[292,i]
#C60.n[i]=CP.Data[61,i]
Smax.n [i] <- max(CS.Data[,i])

##############################################################
### VPC Plotting Function for TS DATA  ##########################
##############################################################
VPCP <- function(Data,Color,YMin,YMax,XMin,XMax,Title,Ytitle,LogAxis){
  Size.of.Data<-dim(Data)
  PlottingData  <- matrix(0,Size.of.Data[1],4)
  PlottingData[,1] <- Current.Iteration.Data[,1]
  for(i in 1:length(t)){
    PlottingData[i,2] <- quantile(Data[i,],0.05);
    PlottingData[i,3] <- quantile(Data[i,],0.50)
    PlottingData[i,4] <- quantile(Data[i,],0.95)  }
  Time    <- PlottingData[,1];   Bottom  <- PlottingData[,2]
  Top     <- PlottingData[,4];   Middle  <- PlottingData[,3]
  ColNumbers <- col2rgb(Color); par(TRUE)
  mycol4 <- rgb(ColNumbers[1],ColNumbers[2], ColNumbers[3], max = 255, alpha = 90)
  plot(Time, Bottom, type= "n", col=Color, main=Title,
       ylim= c(YMin,YMax), log=LogAxis, xlim=c(XMin,XMax),
       ylab =Ytitle, xlab = 'Time (minutes)', font=2,font.lab =2)
  polygon(c(Time, rev(Time)), c(Top, rev(Bottom)),col=mycol4,border=NA)
  lines(Time, Middle, type= "l", col=Color, lwd=2.8)
  return()
}

TOB104.Time <- c(0, 30, 60, 240, 360, 480)+2880
TOB104.Conc <- c(0, 29.5, 20.7, 7.8, 4.4, 3.9)
TOB104.Conc.upper <- c(0, 33.3, 23.5, 9.5, 5.6, 4.5)
TOB104.Conc.lower <- c(0, 25.5, 17.9, 5.9, 3.2, 2.8)
TOB104.STime <- c(2880, 3000, 3120,3300)
TOB104.SConc <- c(2.2, 3.1, 3.9, 2.5)
TOB104.SConc.upper <- c(3.6, 4.3, 5.4, 4.0)
TOB104.SConc.lower <- c(0.9, 1.7, 2.4, 0.9)

#mean(beta.n);max(beta.n);min(beta.n)
#mean(C30.n);max(C30.n);min(C30.n)
#mean(AUC.n);max(AUC.n);min(AUC.n)
#mean(Smax.n);max(Smax.n);min(Smax.n);

#dev.off();par(mfrow=c(1,1));hist(TLV.n)
#mean(TLV.n);quantile(TLV.n,0.5);sd(TLV.n);max(TLV.n);min(TLV.n)
End.time <- proc.time();Total.time <- End.time-Start.time;Total.time

AUC.BX<-boxplot(AUC.n,AUC.n, names=c("Rep AUC","Sim AUC")) #, ylim=c(20,100)
AUC.BX$out<-0

AUC.BX$stats[1,1]  <- 60
AUC.BX$stats[2,1]  <- 82
AUC.BX$stats[3,1]  <- 82
AUC.BX$stats[4,1]  <- 82
AUC.BX$stats[5,1]  <--115
bxp(AUC.BX,ylim=c(40,120))

C30.BX<-boxplot(C30.n,C30.n, names=c("Rep C30","Sim C30"))
C30.BX$out<-0

C30.BX$stats[1,1]  <- 23
C30.BX$stats[2,1]  <- 29.4
C30.BX$stats[3,1]  <- 29.4
C30.BX$stats[4,1]  <- 29.4
C30.BX$stats[5,1]  <- 36

BET.BX<-boxplot(beta.n,beta.n, names=c("Rep Beta","Sim Beta"))
BET.BX$out<-0

BET.BX$stats[1,1]  <- 0.22
BET.BX$stats[2,1]  <- 0.29
BET.BX$stats[3,1]  <- 0.29
BET.BX$stats[4,1]  <- 0.29
BET.BX$stats[5,1]  <- 0.38

dev.off();par(mfrow=c(2,3)); par(TRUE)
VPCP(CP.Data,'green',1,100,2880,3360,'Cp vs t','Plasma Conc (ug/ml)' , 'y')
yLine <- seq(0.001,2000,100); xLine1 <- rep(0,length(yLine))
xLine2 <- rep(1440,length(yLine)); xLine3 <- rep(2880,length(yLine))
lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red');lines(xLine3,yLine,lty =2,lwd=3,col='red')
p
points(TOB104.Time,TOB104.Conc,col='red',pch=20,cex=2.5)
p
points(TOB104.Time,TOB104.Conc.upper,col='red',cex=2.5,pch='-
')
p
points(TOB104.Time,TOB104.Conc.lower,col='red',cex=2.5,pch='-')

VPCP(CS.Data,'cyan',0.5,10, 2880,3360,'Cs vs t','Sputum Conc (ug/ml)', 'y')
p
points(TOB104.STime,TOB104.SConc,col='black',pch=20,cex=2.5)
p
points(TOB104.STime,TOB104.SConc.upper,col='black',cex=2.5,pch='-')
p
points(TOB104.STime,TOB104.SConc.lower,col='black',cex=2.5,pch='-')
p
lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')

VPCP(PL.Data,'grey',5,90, 2880,3360,'PL Conc vs t','PL Conc (ug/ml)', 'y')
p
lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')

bxp(AUC.BX,ylim=c(60,180), main='AUC',ylab='AUC (mg*hr/l)')

bxp(C30.BX,ylim=c(22,50),main='C30',ylab='Plasma Conc at t=2910 min (mg/L)')

bxp(BET.BX,ylim=c(0.1,0.4),main='Beta',ylab='Beta (hr-1)')

########################################################################
# Key under the hood plots
########################################################################

dev.off();par(mfrow=c(2,3)); par(TRUE)

TL <- function(x) {x< max(x);X< 10^ceiling(log10(x));return(X)}

LL <- function(x) {x< max(x);X< 10^ceiling(log10(x));X< X/100;return(X)}

yLine <- seq(0.0001,10000.0001,100); xLine1 <- rep(0,length(yLine))

xLine2 <- rep(1440,length(yLine)); xLine3 <- rep(2880,length(yLine))

lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')

VPCP(PLU.Data,'blue',LL(PLU.Data),TL(PLU.Data), 0,3360,'PLU','PLU (mg)','y')

lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')

VPCP(CLU.Data,'blue',LL(CLU.Data),TL(CLU.Data), 0,3360,'CLU','CLU (mg)','y')

lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')

VPCP(PLS.Data,'blue',LL(PLS.Data),TL(PLS.Data), 0,3360,'PLS','PLS (mg)','y')

lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')

VPCP(CLS.Data,'blue',LL(CLS.Data),TL(CLS.Data), 0,3360,'CLS','CLS (mg)','y')

lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')

VPCP(CC.Data,'blue',LL(CC.Data),TL(CC.Data), 0,3360,'CC','CC (mg)','y')

lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red')
VPCP2 <-
function(Data1,Data2,Color1,Color2,YMin,YMax,XMin,XMax,Title,Ytitle,LogAxis){
  Size.of.Data<-dim(Data1); PlottingData  <- matrix(0,Size.of.Data[1],4)
  PlottingData[,1] <-Current.Iteration.Data[,1]
  for(i in 1:length(t)){
    PlottingData[i,2] <- quantile(Data1[i,],0.05); PlottingData[i,3] <- quantile(Data1[i,],0.50)
    PlottingData[i,4] <- quantile(Data1[i,],0.95) 
  }
  Time  <- PlottingData[,1]; Bottom  <- PlottingData[,2]
  Top  <- PlottingData[,4]; Middle  <- PlottingData[,3]
  ColNumbers <- col2rgb(Color1); par(TRUE)
  mycol4 <- (rgb(ColNumbers[1],ColNumbers[2], ColNumbers[3], max = 255, alpha = 90)
  plot(Time, Bottom, type= "n", col=Color1, main=Title,
    ylim= c(YMin,YMax), log=LogAxis, xlim=c(XMin,XMax),
    ylab =Ytitle, xlab = 'Time (minute'), font=2,font.lab =2)
  polygon(c(Time, rev(Time)), c(Top, rev(Bottom)),col=mycol4,border=NA)
  lines(Time, Middle, type= "l", col=Color1, lwd=2.8)
  Size.of.Data<-dim(Data2); PlottingData  <- matrix(0,Size.of.Data[1],4)
  PlottingData[,1] <-Current.Iteration.Data[,1]
  for(i in 1:length(t)){
    PlottingData[i,2] <- quantile(Data2[i,],0.05); PlottingData[i,3] <- quantile(Data2[i,],0.50)
    PlottingData[i,4] <- quantile(Data2[i,],0.95) 
  }
  Time  <- PlottingData[,1]; Bottom  <- PlottingData[,2]
  Top  <- PlottingData[,4]; Middle  <- PlottingData[,3]
  ColNumbers <- col2rgb(Color2); par(TRUE)
  mycol4 <- (rgb(ColNumbers[1],ColNumbers[2], ColNumbers[3], max = 255, alpha = 90)
  polygon(c(Time, rev(Time)), c(Top, rev(Bottom)),col=mycol4,border=NA)
  lines(Time, Middle, type= "l", col=Color2, lwd=2.8)
  return()
}

VPCP2(CP.Data,CS.Data,'green','cyan',0,50, 2880,3360,'Cp(t) & Cs(t)','Concentrations (ug/ml)"')
lines(xLine1,yLine,lty =2, lwd=3,col='red');lines(xLine2,yLine,lty =2,lwd=3,col='red');lines(xLine3,yLine,lty =2, lwd=3,col='red')
points(TOB104.Time,TOB104.Conc,col='red',pch=20)
points(TOB104.Time,TOB104.Conc.upper,col='red',cex=2,pch='-')
points(TOB104.Time,TOB104.Conc.lower,col='red',cex=2,pch='-')
points(TOB104.STime,TOB104.SConc,col='black',pch=20)
points(TOB104.STime,TOB104.SConc.upper,col='black',cex=2,pch='-')
points(TOB104.STime,TOB104.SConc.lower,col='black',cex=2,pch='-')

#for(i in 1:length(t)) {print(sum(Current.Iteration.Data[i,seq(2,12)]))}

#Dose.n[n]*3

#Slope <- lm(Current.Iteration.Data[seq(317,337,1),1]~CP.Data[seq(317,337,1),1])
(CPRes1+CPRes2+CPRes3+CPRes4+CPRes5)
(Res1+Res2+Res3+Res4)*10

Code 2 Rscript utilized for performing optimization and plotting of MCS
rm(list=ls()); cat("\014"); dev.off(); set.seed(12301991); Start.time <- proc.time() # start a
timer
BW.me <- 70
BW.cv <- 0*100/BW.me
n <- 3
PerKgDose <- 1.5 #mg/kg
tinf <- 10
t <-
c(0.5,10,15,20,30,45,60,90,120,180,240,300,360,420,480,540,600,660,720,780,840,90,0,960,1020,1080,1140,1200,1260,1320,1380,1440,1450,1500,1560,1620,1680,1740,1800,1860,1920,1980,2040,2100,2160,2220,2280,2340,2400,2460,2520,2580,2640,2700,2760,2820,2880,2890,seq(49,72)*60)
Clpop <- 70
Clprop.me <- 1
k12.me <- 0.010
k21.me <- 0.015
V0pop <- 10
V0prop.me <- 1
Clprop.cv <- 0
k12.cv <- 0
k21.cv <- 0
V0prop.cv <- 0
kga.me <- 1
Foral.me <- 0
kga.cv <- 0
Foral.cv <- 0
kpa.me <- 0.006
kca.me <- 0.9
kpm.me <- 0.16
kcm.me <- 0.6
kpd.me <- 0.0008
kcd.me <- 0.001
TLV.me <- 20 #ml
kpls1.me <- 0.0005
kpls2.me <- 0.0001
kcls1.me <- 0.0005
kcls2.me <- 0.0002
kpa.cv <- 0
kca.cv <- 0
kpm.cv <- 0
kcm.cv <- 0
kpd.cv <- 0
kcd.cv <- 0
kpls1.cv <- 0
kpls2.cv <- 0
kcls1.cv <- 0
kcls2.cv <- 0
TLVprop.me <- 1
TLVprop.cv <- 0
kin.me <- 0
Fex.me <- 0.009
Fpd.me <- 0.21
Fcd.me <- 0.069
Din.me <- 0
kin.cv <- 0
Fex.cv <- 0
Fpd.cv <- 0
Fcd.cv <- 0
Din.cv <- 0

NormGen <- function(simulation.n, samp.m, samp.cv) {
  samp.sd <- samp.cv * samp.m / 100
  out <- rnorm(simulation.n, samp.m, samp sd)
  return(out)
}

LogNormGen <- function(n, Mean, PCV) {
  CV <- PCV / 100
  SD <- (CV * Mean)
  Variance <- SD^2
  mean.in <- log(Mean^2 / sqrt(Variance + Mean^2))
  SD.in <- sqrt(log(Variance / Mean^2 + 1))
  x <- rlnorm(n, mean.in, SD.in)
  return(x)
}

BW.n <- NormGen(n, BW.me, BW.cv)
Clprop.n <- NormGen(n, Clprop.me, Clprop.cv)
Cltot.n <- Clpop * (BW.n / mean(BW.n))^0.75 * Clprop.n
#Cltot.n <- c(Clpop/5,Clpop,5*Clpop)
k12.n <- LogNormGen(n, k12.me, k12.cv)
#k12.n <- c(k12.me/5,k12.me,5*k12.me) 
k21.n <- LogNormGen(n, k21.me,k21.cv) 
#k21.n <- c(k21.me/5,k21.me,5*k21.me) 

V0prop.n <- NormGen(n, V0prop.me,V0prop.cv) 
V0.n <- V0prop*(BW.n/mean(BW.n))^1 * V0prop.n 
#V0.n <- c(V0prop/5,V0prop,5*V0prop) 

kga.n <- LogNormGen(n, kga.me,kga.cv) 
if (Foral.me==0){Foral.n<-matrix(0,1,n)} else if (Foral.me>0){Foral.n<-LogNormGen(n,Foral.me,Foral.cv)} 

kpa.n <- LogNormGen(n, kpa.me,kpa.cv) 
#kpa.n <- c(kpa.me/5,kpa.me,5*kpa.me) 

kca.n <- LogNormGen(n, kca.me,kca.cv) 
#kca.n <- c(kca.me/5,kca.me,5*kca.me) 

kpm.n <- LogNormGen(n, kpm.me,kpm.cv) 
#kpm.n <- c(kpm.me/5,kpm.me,5*kpm.me) 

kcm.n <- LogNormGen(n, kcm.me,kcm.cv) 
#kcm.n <- c(kcm.me/5,kcm.me,5*kcm.me) 

if (kpd.me==0){kpd.n<-rep(0,n)} else if (kpd.me>0){kpd.n<-LogNormGen(n, kpd.me,kpd.cv)} 
kpd.n <- c(kpd.me/5,kpd.me,5*kpd.me) 
kpd.n <-c(5*kpd.me,5*kpd.me,5*kpd.me) 
kpd.n <-c(kpd.me,kpd.me,kpd.me) 
#kpd.n <- c(kpd.me/5,kpd.me/5,kpd.me/5) 
if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n, kcd.me,kcd.cv)} 
#kcd.n <- c(kcd.me/5,kcd.me,5*kcd.me) 

if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n, kpls1.me,kpls1.cv)} 
#kpls1.n <- c(kpls1.me/5,kpls1.me,5*kpls1.me) 

if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n, kpls2.me,kpls2.cv)} 
#kpls2.n <- c(kpls2.me/5,kpls2.me,5*kpls2.me) 

if (kcls1.me==0){kcls1.n<-rep(0,n)} else if (kcls1.me>0){kcls1.n<-LogNormGen(n, kcls1.me,kcls1.cv)}
#kcls1.n <- c(kcls1.me/5,kcls1.me,5*kcls1.me)

if (kcls2.me==0){kcls2.n<-rep(0,n)} else if (kcls2.me>0){kcls2.n<-LogNormGen(n, kcls2.me,kcls2.cv)}
#kcls2.n <- c(kcls2.me/5,kcls2.me,5*kcls2.me)

TLVprop.n <- NormGen(n, TLVprop.me, TLVprop.cv)

if (kin.me==0){kin.n<-rep(0,n)} else if (kin.me>0){kin.n<-NormGen(n, kin.me,kin.cv)}
  Fex.n <- NormGen(n, Fex.me,Fex.cv)
  Fpd.n <- NormGen(n, Fpd.me,Fpd.cv)
  Fcd.n <- NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n<-rep(0,n)} else if (Din.me>0){Din.n<-LogNormGen(n, Din.me,Din.cv)}

########################################################################
###########         Set up the ODE Based model function
#######################
#############################################################
TCBM <- function(t,Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, 
    kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, Dose, tinf ){
    Dose.Value <- (1-Din)*Dose
    Fgd <- 1-Fex-Fpd-Fcd
    ED <- data.frame(var = c("DOSING"), time = c(t[1], tinf, 1440, 1450, 2880, 2890), value = c(Dose.Value/tinf, 0), method = c("rep"))

    Model.Function <- function(t,IC,Parm) {
        with(as.list(IC, Parm), {
            dDOSING <- 0
            dIn <- -ln*kin*(Fex+Fpd+Fcd+Fgd)
            dPLU <- ln*kin*Fpd + PLS*kpd - PLU*(kpa + kpm)
            dCLU <- ln*kin*Fcd + CLS*kcd - CLU*(kca + kcm) + PLU* kpm
            dGI <- ln*kin*Fgd + CLU* kcm - GI*kga
            dPLS <- PLU*kpa + CC*kpls1 - PLS *(kpd+kpls2)
            dCLS <- CLU*kca + CC*kcls1 - CLS *(kcd+kcls2)
            dCC <- PLS*kpls2 + CLS*kcls2 + GI*kga*Foral + PC*k21 - CC*(kpls1+kcls1 + k12 + (Cltot/(V0*1000)))+ DOSING
            dPC <- CC* k12 - PC *k21
            dEx <- ln*kin*Fex
            dFe <- GI*kga*(1-Foral)
            dEI <- CC*(Cltot/(V0*1000))
        })
    }
}

246
return(list(c(dIn,dPLU,dCLU,dPLS,dGI,dCC,dPC,dEx,dFe,dEl,dDOSING)))

IC <- c(In=0, PLU=0*Fpd, CLU=0*Fcd, PLS=0, CLS=0, GI=0*Fgd, CC=0, PC=0, Ex=0*Fex, Fe=0, El=0, DOSING=0)
Parm <- c(Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Fgd)
out <- ode(IC, t,Model.Function,Parm,events = list(data=ED))
return(out)

Dose.n <- BW.n*PerKgDose
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n*0.8/1000
PLV.n <- TLV.n*0.2/1000

In.Data <- matrix(0,length(t),(n))
PLU.Data <- matrix(0,length(t),(n))
CLU.Data <- matrix(0,length(t),(n))
PLS.Data <- matrix(0,length(t),(n))
CLS.Data <- matrix(0,length(t),(n))
GI.Data <- matrix(0,length(t),(n))
CC.Data <- matrix(0,length(t),(n))
PC.Data <- matrix(0,length(t),(n))
Ex.Data <- matrix(0,length(t),(n))
Fe.Data <- matrix(0,length(t),(n))
El.Data <- matrix(0,length(t),(n))
CP.Data <- matrix(0,length(t),(n))
CS.Data <- matrix(0,length(t),(n))
PL.Data <- matrix(0,length(t),(n))

Cmax.n=rep(0,n)
tmax.n=rep(0,n)
Smax.n=rep(0,n)
tsmx.n=rep(0,n)

AUC.n=Dose.n*1000/Cltot.n/60
for (i in 1:n) {
  Dose  <- Dose.n[i]
Current.Iteration.Data <- TCBM(t, Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, Dose, tinf)

In.Data[,i] <- Current.Iteration.Data[,2]
PLU.Data[,i] <- Current.Iteration.Data[,3]
PLS.Data[,i] <- Current.Iteration.Data[,5]
GI.Data[,i] <- Current.Iteration.Data[,7]
CC.Data[,i] <- Current.Iteration.Data[,8]
PC.Data[,i] <- Current.Iteration.Data[,9]

CP.Data[,i] <- Current.Iteration.Data[,8]/V0
CS.Data[,i] <- Current.Iteration.Data[,4]/CLV
PL.Data[,i] <- Current.Iteration.Data[,3]/PLV
Cmax.n[i]<- max(CP.Data[57:82,i])
tmax.n[i]<-t[which.max(CP.Data[57:82,i])]
Smax.n[i]<- max(CS.Data[57:82,i])
tsmax.n[i]<-t[which.max(CS.Data[57:82,i])]

VPCP <- function(Data,Color,YMin,YMax,XMin,XMax,Title,Ytitle,LogAxis){
  Size.of.Data<-dim(Data); PlottingData <- matrix(0,Size.of.Data[1],4)
  PlottingData[,1] <- Current.Iteration.Data[,1]
  for(i in 1:length(t)){
    PlottingData[i,2] <- quantile(Data[,i],0.05); PlottingData[i,3] <- quantile(Data[,i],0.50)
    PlottingData[i,4] <- quantile(Data[,i],0.95) 
  }
  Time <- PlottingData[,1]; Bottom <- PlottingData[,2]
  Top <- PlottingData[,4]; Middle <- PlottingData[,3]
  ColNumbers <- col2rgb(Color); par(TRUE)
  mycol4 <- rgb(ColNumbers[1],ColNumbers[2], ColNumbers[3], max = 255, alpha = 90)
  plot(Time, Bottom, type= "n", col=Color, main=Title,
       ylim= c(YMin,YMax), log=LogAxis, xlim=c(XMin,XMax),
       ylab =Ytitle, xlab = 'Time (minutes)', font=2,font.lab =2)
  polygon(c(Time, rev(Time)), c(Top, rev(Bottom)),col=mycol4,border=NA)
  lines(Time, Middle, type= "l", col=Color, lwd=2.8)
  return()
}

TL <- function(x) {x<-max(x);X<-10^ceiling(log10(x));return(X)}
LL <- function(x) {x<-max(x);X<-10^ceiling(log10(x));X<-X/100;return(X)}

plot(t)
dev.off();par(mfrow=c(2,3)); par(TRUE)
dev.off();par(mfrow=c(1,2)); par(TRUE)
VPCP(CP.Data,'green',LL(CP.Data),TL(CP.Data), 0,max(t),'Cp(t)','Plasma Conc (ug/ml)',"y")
VPCP(CS.Data,'blue',LL(CS.Data),TL(CS.Data), 0,max(t),'Cs(t)',"Sputum Conc (ug/ml)',"y")
dev.off();par(mfrow=c(2,4)); par(TRUE)
VPCP(PLU.Data,'grey',LL(PLU.Data),TL(PLU.Data), 0,max(t),'PLU(t)',"Peripheral Lung Unbound (ug)',"y")
VPCP(CLU.Data,'grey',LL(CLU.Data),TL(CLU.Data), 0,max(t),'CLU(t)',"Central Lung Unbound (ug)',"y")
VPCP(PLS.Data,'grey',LL(PLS.Data),TL(PLS.Data), 0,max(t),'PLS(t)',"Peripheral Lung Sequestered (ug)',"y")
VPCP(CLS.Data,'grey',LL(CLS.Data),TL(CLS.Data), 0,max(t),'CLS(t)','Central Lung Sequestered (ug)','y')
VPCP(CC.Data,'grey',LL(CC.Data),TL(CC.Data), 0,max(t),'CC(t)','Central Comp (ug)','y')
VPCP(PC.Data,'grey',LL(PC.Data),TL(PC.Data), 0,max(t),'PC(t)','Peripheral Comp (ug)','y')
VPCP(CP.Data,'green',LL(CP.Data),TL(CP.Data), 0,max(t),'Cp(t)','Plasma Conc (ug/ml)','y')
VPCP(CS.Data,'blue',LL(CS.Data),TL(CS.Data), 0,max(t),'Cs(t)','Sputum Conc (ug/ml)','y')

AUC Trap.Calc <- function(Time, CP){
  n <- length(Time);  AUC.Values<-rep(0,n-1)
  for (i in 1:(n-1)){
    if (i==1){ AUC.Values[i]<-(Time[i+1]-Time[i])*(CP[i]+CP[i+1])/2   }
    if (i>1){ AUC.Values[i]<-(Time[i+1]-Time[i])*(CP[i]+CP[i+1])/2 +AUC.Values[i-1]  }  
  };  return(AUC.Values[i])
}

AUCLowerParmValue <- AUC.Trap.Calc(t[57:82], CP.Data[57:82,1])
AUCHigherParmValue <- AUC.Trap.Calc(t[57:82], CP.Data[57:82,3])
AUCS <- c(AUCLowerParmValue,AUCHigherParmValue)
AUCMultiplier=1
if (AUCHigherParmValue<AUCLowerParmValue){AUCMultiplier=-1}
AUCMultiplier*max(AUCS)/min(AUCS)

LCP.Data<-log10(CP.Data)
LowerDataSet<- LCP.Data[70:82,1]
LowerSlope<-lm(LowerDataSet~t[70:82])
thalflower<-log(2)/(LowerSlope$coefficients[2]*-2.303)
HigherDataSet<- LCP.Data[70:82,3]
HighSlope<-lm(HigherDataSet~t[70:82])
thalfhigher<-log(2)/(HighSlope$coefficients[2]*-2.303)
thalf<-c(thalfhigher,thalflower)
thalfmultiplier<-1
if (thalfhigher<thalflower){thalfmultiplier=-1}
thalfmultiplier*max(thalf)/min(thalf)

Cmaxlower <- Cmax.n[1]
Cmaxhigher<- Cmax.n[3]
Cmaxmultiplier<-1
if (Cmaxhigher<Cmaxlower){Cmaxmultiplier=-1}
Cmaxmultiplier*max(Cmax.n)/min(Cmax.n)
tmaxlower <- tmax.n[1]
tmaxhigher <- tmax.n[3]
tmaxmultiplier<-1
if (tmaxhigher<tmaxlower){tmaxmultiplier=-1}
tmaxmultiplier*max(tmax.n)/min(tmax.n)

SAUCLowerParmValue <- AUC.Trap.Calc(t[57:82], CS.Data[57:82,1])
SAUCHigherParmValue <- AUC.Trap.Calc(t[57:82], CS.Data[57:82,3])
SAUCS <- c(SAUCLowerParmValue,SAUCHigherParmValue)
SAUCMultiplier=1
if (SAUCHigherParmValue<SAUCLowerParmValue){SAUCMultiplier=-1}
SAUCMultiplier*max(SAUCS)/min(SAUCS)

SLCP.Data<-log10(CS.Data)
SLowerDataSet<- SLCP.Data[70:82,1]
SLowerSlope<-lm(SLowerDataSet~t[70:82])
Sthalflower<-log(2)/(SLowerSlope$coefficients[2]*2.303)

SHigherDataSet<- SLCP.Data[70:82,3]
SHighSlope<-lm(SHigherDataSet~t[70:82])
Sthalfhigher<-log(2)/(SHighSlope$coefficients[2]*2.303)
Sthalf<-c(Sthalfhigher,Sthalflower)
Sthalfmultiplier<-1
if (Sthalfhigher<Sthalflower){Sthalfmultiplier=-1}
Sthalfmultiplier*max(Sthalf)/min(Sthalf)

Smaxlower <- Smax.n[1]
Smaxhigher<- Smax.n[3]
Smaxmultiplier<-1
if (Smaxhigher<Smaxlower){Smaxmultiplier=-1}
Smaxmultiplier*max(Smax.n)/min(Smax.n)
tsmaxlower <- tsmax.n[1]
tsmaxhigher <- tsmax.n[3]
tsmaxmultiplier<-1
if (tsmaxhigher<tsmaxlower){tsmaxmultiplier=-1}
tsmaxmultiplier*max(tmax.n)/min(tmax.n)

AUCMultiplier*max(AUCS)/min(AUCS)
Cmaxmultiplier*max(Cmax.n)/min(Cmax.n)
tmaxmultiplier*max(tmax.n)/min(tmax.n)
thalffmultiplier*max(thalf)/min(thalf)
SAUCMultiplier*max(SAUCS)/min(SAUCS)
Smaxmultiplier*max(Smax.n)/min(Smax.n)
tsmaxmultiplier*max(tsmax.n)/min(tsmax.n)
Sthalfmultiplier*max(Sthalf)/min(Sthalf)

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**Figure 1.** Simulations results of plasma concentration time profile on a linear-scale for CIP 101A based on the parameters estimated from various applied weighting factors.

**Figure 2.** Simulations results of plasma concentration time profile on a linear-scale for CIP 102A based on the parameters estimated from various applied weighting factors.
Figure 3. Simulations results of plasma concentration time profile on a linear-scale for CIP 102B based on the parameters estimated from various applied weighting factors.

Figure 4. Simulations results of plasma concentration time profile on a linear-scale for CIP 102C based on the parameters estimated from various applied weighting factors.
Figure 5. Simulations results of plasma concentration time profile on a linear-scale for CIP 104A based on the parameters estimated from various applied weighting factors.

Figure 6. Simulations results of plasma concentration time profile on a linear-scale for CIP 105A based on the parameters estimated from various applied weighting factors.
Figure 7. Simulations results of plasma concentration time profile on a linear-scale for CIP 105B based on the parameters estimated from various applied weighting factors.

Figure 8. Simulations results of plasma concentration time profile on a linear-scale for CIP 106A based on the parameters estimated from various applied weighting factors.
Figure 9. Simulations results of plasma concentration time profile on a linear-scale for CIP 107A based on the parameters estimated from various applied weighting factors.

Figure 10. Simulations results of plasma concentration time profile on a linear-scale for CIP 107C based on the parameters estimated from various applied weighting factors.
Figure 11. Simulations results of plasma concentration time profile on a linear-scale for CIP 108A based on the parameters estimated from various applied weighting factors.

Figure 12. Simulations results of plasma concentration time profile on a linear-scale for CIP 108B based on the parameters estimated from various applied weighting factors.
Table 1. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 101A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 2. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 102A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.

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<th>1/y^2</th>
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Visual inspection

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Table 3. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y² applied to data from study CIP 102B. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 4. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 102C. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.

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Table 4. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 102C. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 5. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y² applied to data from study CIP 104A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 6. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 105A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 7. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 105B. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
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Visual inspection ✓ ✓ ✓

$R^2$ 0.99926 0.99783 0.99709
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$V_o$ SD 3.18 4.2319 6.2531
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$k_{12}$ SD 0.001 0.0011 0.001104
$k_{12}$ CV 9.43 11.9085 14.03498
$k_{21}$ (min⁻¹) 0.003 0.0030 0.00283
$k_{21}$ SD 0.0004 0.0003 0.000237
$k_{21}$ CV 12.20 10.0579 8.369258
$Cl_{tot}$ (ml/min⁻¹) 205.150 210.8500 222.83
$Cl_{tot}$ SD 12.7100 13.7570 16.092
$Cl_{tot}$ CV 6.20 6.5245 7.221649

**Table 8.** Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y² applied to data from study CIP 106A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 9. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y2 applied to data from study CIP 107A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 10. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y2 applied to data from study CIP 107C. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conc. In Plasma (mg/L)</th>
<th>None</th>
<th>1/y</th>
<th>1/y²</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>4.45978</td>
<td>4.3914</td>
<td>4</td>
<td>4.2723</td>
</tr>
<tr>
<td>60</td>
<td>5.66</td>
<td>5.7165</td>
<td>5.7211</td>
<td>5.696</td>
</tr>
<tr>
<td>75</td>
<td>3.02</td>
<td>3.1039</td>
<td>3.1949</td>
<td>3.3223</td>
</tr>
<tr>
<td>90</td>
<td>2.55</td>
<td>2.3277</td>
<td>2.3060</td>
<td>2.3338</td>
</tr>
<tr>
<td>120</td>
<td>1.88</td>
<td>1.8846</td>
<td>1.7819</td>
<td>1.6934</td>
</tr>
<tr>
<td>180</td>
<td>1.38</td>
<td>1.487</td>
<td>1.4285</td>
<td>1.3394</td>
</tr>
<tr>
<td>240</td>
<td>1.11</td>
<td>1.1834</td>
<td>1.1713</td>
<td>1.1158</td>
</tr>
<tr>
<td>360</td>
<td>0.71</td>
<td>0.74947</td>
<td>0.7880</td>
<td>0.77683</td>
</tr>
<tr>
<td>480</td>
<td>0.52</td>
<td>0.47468</td>
<td>0.5301</td>
<td>0.54085</td>
</tr>
<tr>
<td>600</td>
<td>0.37</td>
<td>0.30064</td>
<td>0.3566</td>
<td>0.37656</td>
</tr>
<tr>
<td>720</td>
<td>0.26</td>
<td>0.19041</td>
<td>0.2399</td>
<td>0.26217</td>
</tr>
<tr>
<td>960</td>
<td>0.14</td>
<td>0.076375</td>
<td>0.1086</td>
<td>0.12708</td>
</tr>
</tbody>
</table>

Visual inspection: ✓ ✓ ✓

- R²: 0.99867 0.99715 0.99615
- MSC: 5.1818 5.0873 5.2099
- V₀ (Liter): 19.32 21.4630 24.212
- V₀ SD: 1.72 0.0039 3.0937
- V₀ CV: 8.91 0.0182 12.77755
- k₁₂ (min⁻¹): 0.055 0.0456 0.036815
- k₁₂ SD: 0.008 0.0065 0.005968
- k₁₂ CV: 14.89 14.2330 16.21051
- k₂₁ (min⁻¹): 0.015 0.0123 0.010531
- k₂₁ SD: 0.0016 0.0011 0.000872
- k₂₁ CV: 10.91 9.0675 8.281835
- Cl₁₀₀ (ml*min⁻¹): 447.910 431.6000 431.03
- Cl₁₀₀ SD: 20.2550 11.9210 10.265
- Cl₁₀₀ CV: 4.52 2.7620 2.381505
Table 1

Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 108A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conc. In Plasma (mg/L)</th>
<th>None</th>
<th>1/y</th>
<th>1/y^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>4.247037843</td>
<td>4.198</td>
<td>4</td>
<td>3.8746</td>
</tr>
<tr>
<td>30</td>
<td>3.19</td>
<td>3.3159</td>
<td>3.3485</td>
<td>3.3304</td>
</tr>
<tr>
<td>35.32251</td>
<td>2.77</td>
<td>2.7076</td>
<td>2.7716</td>
<td>2.8728</td>
</tr>
<tr>
<td>50</td>
<td>1.96</td>
<td>1.911</td>
<td>1.9268</td>
<td>2.0529</td>
</tr>
<tr>
<td>65</td>
<td>1.62</td>
<td>1.6076</td>
<td>1.5718</td>
<td>1.6068</td>
</tr>
<tr>
<td>80</td>
<td>1.44</td>
<td>1.4462</td>
<td>1.3931</td>
<td>1.3559</td>
</tr>
<tr>
<td>110</td>
<td>1.15</td>
<td>1.2262</td>
<td>1.1848</td>
<td>1.0957</td>
</tr>
<tr>
<td>140</td>
<td>1.09</td>
<td>1.0497</td>
<td>1.0313</td>
<td>0.94871</td>
</tr>
<tr>
<td>200</td>
<td>0.71</td>
<td>0.77043</td>
<td>0.7862</td>
<td>0.74366</td>
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<tr>
<td>260</td>
<td>0.55</td>
<td>0.56549</td>
<td>0.5996</td>
<td>0.58791</td>
</tr>
<tr>
<td>380</td>
<td>0.34</td>
<td>0.30466</td>
<td>0.3488</td>
<td>0.36788</td>
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<tr>
<td>500</td>
<td>0.22</td>
<td>0.16413</td>
<td>0.2029</td>
<td>0.23021</td>
</tr>
<tr>
<td>740</td>
<td>0.10</td>
<td>0.047639</td>
<td>0.0687</td>
<td>0.090147</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual inspection</th>
<th>✓</th>
<th>✓</th>
<th>✓</th>
</tr>
</thead>
<tbody>
<tr>
<td>R^2</td>
<td>0.99913</td>
<td>0.99779</td>
<td>0.99518</td>
</tr>
<tr>
<td>MSC</td>
<td>5.5089</td>
<td>5.3782</td>
<td>5.3129</td>
</tr>
<tr>
<td>Vo (Liter)</td>
<td>35.38</td>
<td>41.1400</td>
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</tr>
<tr>
<td>Vo SD</td>
<td>2.98</td>
<td>4.7043</td>
<td>7.9321</td>
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<tr>
<td>Vo CV</td>
<td>8.42</td>
<td>11.4349</td>
<td>14.43407</td>
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<tr>
<td>k_12 (min^-1)</td>
<td>0.052</td>
<td>0.0416</td>
<td>0.024672</td>
</tr>
<tr>
<td>k_12 SD</td>
<td>0.006</td>
<td>0.0071</td>
<td>0.006129</td>
</tr>
<tr>
<td>k_12 CV</td>
<td>11.72</td>
<td>17.0164</td>
<td>24.8403</td>
</tr>
<tr>
<td>k_21 (min^-1)</td>
<td>0.029</td>
<td>0.0197</td>
<td>0.014854</td>
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<tr>
<td>k_21 SD</td>
<td>0.0018</td>
<td>0.0017</td>
<td>0.001716</td>
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<tr>
<td>k_21 CV</td>
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<td>8.4818</td>
<td>11.55464</td>
</tr>
<tr>
<td>Cl_{tot} (ml*min^-1)</td>
<td>718.670</td>
<td>695.7700</td>
<td>698.44</td>
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<tr>
<td>Cl_{tot} SD</td>
<td>27.4360</td>
<td>19.3410</td>
<td>17.959</td>
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<tr>
<td>Cl_{tot} CV</td>
<td>3.82</td>
<td>2.7798</td>
<td>2.571302</td>
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</table>

Table 11. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 108A. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
Table 1. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 108B. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conc. In Plasma (mg/L)</th>
<th>Weighting Factor</th>
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<tr>
<td>10</td>
<td>10.1236947</td>
<td>10.097</td>
</tr>
<tr>
<td>15</td>
<td>5.72</td>
<td>5.8016</td>
</tr>
<tr>
<td>20.32251</td>
<td>3.73</td>
<td>3.6883</td>
</tr>
<tr>
<td>35</td>
<td>1.99</td>
<td>1.8277</td>
</tr>
<tr>
<td>50</td>
<td>1.51</td>
<td>1.5133</td>
</tr>
<tr>
<td>65</td>
<td>1.34</td>
<td>1.3845</td>
</tr>
<tr>
<td>95</td>
<td>1.07</td>
<td>1.1923</td>
</tr>
<tr>
<td>125</td>
<td>0.97</td>
<td>1.029</td>
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<tr>
<td>185</td>
<td>0.78</td>
<td>0.7664</td>
</tr>
<tr>
<td>245</td>
<td>0.57</td>
<td>0.57083</td>
</tr>
<tr>
<td>365</td>
<td>0.38</td>
<td>0.31667</td>
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<tr>
<td>485</td>
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<td>0.17567</td>
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<td>725</td>
<td>0.11</td>
<td>0.054063</td>
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<table>
<thead>
<tr>
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<th>✓</th>
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</thead>
<tbody>
<tr>
<td>R^2</td>
<td>0.99959</td>
<td>0.99424</td>
<td>0.99625</td>
<td></td>
</tr>
<tr>
<td>MSC</td>
<td>6.6979</td>
<td>5.0964</td>
<td>5.5275</td>
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<tr>
<td>Vo (Liter)</td>
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</tr>
<tr>
<td>Vo SD</td>
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<td>2.2866</td>
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</tr>
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<td>Vo CV</td>
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<td>19.5269</td>
<td>13.41311</td>
<td></td>
</tr>
<tr>
<td>k_{12} (min^{-1})</td>
<td>0.087</td>
<td>0.0868</td>
<td>0.07049</td>
<td></td>
</tr>
<tr>
<td>k_{12} SD</td>
<td>0.003</td>
<td>0.0118</td>
<td>0.007542</td>
<td></td>
</tr>
<tr>
<td>k_{12} CV</td>
<td>3.51</td>
<td>13.5638</td>
<td>10.69953</td>
<td></td>
</tr>
<tr>
<td>k_{21} (min^{-1})</td>
<td>0.015</td>
<td>0.0131</td>
<td>0.012522</td>
<td></td>
</tr>
<tr>
<td>k_{21} SD</td>
<td>0.0009</td>
<td>0.0010</td>
<td>0.000687</td>
<td></td>
</tr>
<tr>
<td>k_{21} CV</td>
<td>6.19</td>
<td>7.4610</td>
<td>5.487622</td>
<td></td>
</tr>
<tr>
<td>Cl_{tot} (ml*min^{-1})</td>
<td>496.110</td>
<td>463.6500</td>
<td>475.23</td>
<td></td>
</tr>
<tr>
<td>Cl_{tot} SD</td>
<td>21.4480</td>
<td>16.1260</td>
<td>12.28</td>
<td></td>
</tr>
<tr>
<td>Cl_{tot} CV</td>
<td>4.32</td>
<td>3.4781</td>
<td>2.584012</td>
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</tbody>
</table>

Table 12. Parameter estimates based on simplex fit with either no weighting factor, a weighting factor of 1/y or a weighting factor of 1/y^2 applied to data from study CIP 108B. Column highlighted in green represented the weighting factor deemed acceptable for this specific data set.
MCS Results for IV CIP

CIP 101A Cp(t) -- CF, Intravenous Dose = 200mg

Figure 13 MCS results of CIP 101A
Figure 14 MCS results of CIP 102A
Figure 15 MCS results of CIP 102B
Figure 16 MCS results of CIP 102C
Figure 17 MCS results of CIP 104A
Figure 18 MCS results of CIP 105A
Figure 19 MCS results of CIP 105B
Figure 20 MCS results of CIP 106A
Figure 21 MCS results of CIP 107A
Figure 22 MCS results of CIP 108A
Figure 23 MCS results of CIP 108B
Figure 24 MCS for PO administration in CIP 102A
Figure 25 MCS for PO administration in CIP 102B
Figure 26 MCS for PO administration in CIP 102C
Figure 27 MCS for PO administration in CIP 102D
Figure 28 MCS for PO administration in CIP 104A
Figure 29 MCS for PO administration in CIP 105A
Figure 30 MCS for PO administration in CIP 105B
Figure 31 MCS for PO administration in CIP 106A
Figure 32 MCS for PO administration in CIP 108A
Figure 33 MCS for PO administration in CIP 108B
Figure 34 MCS for PO administration in CIP 204A
Figure 35 MCS for PO administration in CIP 204B
MCS Results for INH CIP

CIP 301A Cp(t) -- HV, Inhaler Dose = 32.5mg

Figure 36 c_p(t) MSC for INH study CIP 301A
Figure 37 $c_s(t)$ MSC for INH study CIP 302A
Figure 38 $c_p(t)$ MSC for INH study CIP 302A
Figure 39 $c_s(t)$ MSC for INH study CIP 302B
Figure 40 $c_p(t)$ MSC for INH study CIP 302B
Figure 41 \( c_p(t) \) MSC for INH study CIP 303A
Figure 42 $c_p(t)$ MSC for INH study CIP 303B
Figure 43 $c_p(t)$ MSC for INH study CIP 304A
Figure 44 $c_p(t)$ MSC for INH study CIP 304B
Figure 45 $c_p(t)$ MSC for INH study CIP 304C
Sensitivity Analysis Plots for INH CIP

Sensitivity of $c_p(t)$ (left plot) and $c_s(t)$ (right plot) to Clonrenal after INH Dosing in CF

Sensitivity of $c_p(t)$ (left plot) and $c_s(t)$ (right plot) to Clrenal after INH Dosing in CF
Sensitivity of cp(t) (left plot) and cs(t) (right plot) to Foral after INH Dosing in CF

Sensitivity of cp(t) (left plot) and cs(t) (right plot) to k12 after INH Dosing in CF
Sensitivity of cp(t) (left plot) and cs(t) (right plot) to k21 after INH Dosing in CF

Sensitivity of cp(t) (left plot) and cs(t) (right plot) to kca after INH Dosing in CF
Sensitivity of \( cp(t) \) (left plot) and \( cs(t) \) (right plot) to \( kcd \) after INH Dosing in CF

Sensitivity of \( cp(t) \) (left plot) and \( cs(t) \) (right plot) to \( kcm \) after INH Dosing in CF
Sensitivity of cp(t) (left plot) and cs(t) (right plot) to kga after INH Dosing in CF

Sensitivity of cp(t) (left plot) and cs(t) (right plot) to kpa after INH Dosing in CF
Sensitivity of cp(t) (left plot) and cs(t) (right plot) to kpd after INH Dosing in CF

Sensitivity of cp(t) (left plot) and cs(t) (right plot) to kpm after INH Dosing in CF
Sensitivity of $cp(t)$ (left plot) and $cs(t)$ (right plot) to V0 after INH Dosing in CF

Sensitivity of $cp(t)$ (left plot) and $cs(t)$ (right plot) to C1nonrenal after INH Dosing in HV
Sensitivity of cp(t) (left plot) and cs(t) (right plot) to k12 after INH Dosing in HV

Sensitivity of cp(t) (left plot) and cs(t) (right plot) to k21 after INH Dosing in HV
Sensitivity of cp(t) (left plot) and cs(t) (right plot) to kcm after INH Dosing in HV

Sensitivity of cp(t) (left plot) and cs(t) (right plot) to kga after INH Dosing in HV
Sensitivity of $c_p(t)$ (left plot) and $c_s(t)$ (right plot) to $k_{pa}$ after INH Dosing in HV

Sensitivity of $c_p(t)$ (left plot) and $c_s(t)$ (right plot) to $k_{pd}$ after INH Dosing in HV
Sensitivity of $c_p(t)$ (left plot) and $c_s(t)$ (right plot) to $k_{pm}$ after INH Dosing in HV

Sensitivity of $c_p(t)$ (left plot) and $c_s(t)$ (right plot) to $V_{0}$ after INH Dosing in HV
Code Utilized for CIP MSC After INH

```r
rm(list=ls()); cat("\014"); dev.off(); set.seed(123091); Start.time <- proc.time() # start a timer

BW.me <- 80.2
BW.cv  <- 20
n       <- 300
Disease <- 1       # 1= Healthy   2= CF

InhalerDose<-32.5#mg
DtL   <- 0.53
DtGI  <- 0.43
t<-seq(0,720,5)

Clren.1.me <- 315    #Healthy
Clren.2.me <- 425    #CF
Clnonren.1.me <- 315    #Healthy
Clnonren.2.me <- 225    #CF
k12.1.me <- 0.025    #Healthy
k12.2.me <- 0.050    #CF
k21.1.me <- 0.015    #Healthy
k21.2.me <- 0.020    #CF
V0.1.me <- 75        #Healthy
V0.2.me <- 20        #CF

Clnonren.1.cv <- 20    #Healthy
Clnonren.2.cv <- 20    #CF
Clren.1.cv <- 20    #Healthy
Clren.2.cv <- 20    #CF
k12.1.cv <- 25        #Healthy
k12.2.cv <- 20        #CF
k21.1.cv <- 25        #Healthy
k21.2.cv <- 20        #CF
V0.1.cv <- 20        #Healthy
V0.2.cv <- 20        #CF

Foral.1.me  <- 73
Foral.2.me  <- 85
kga.1.me    <- 0.020
kga.2.me    <- 0.011
Foral.1.cv<-35;  Foral.2.cv<-30;  kga.1.cv<-10;  kga.2.cv<-50

kpa.me    <- 0.010
kca.me    <- 0.020
```

kpm.me <- 0.020
kcm.me <- 0.02
kpd.me <- 0.008
kcd.me <- 0.0002
kpls1.me <- 10000
kpls2.me <- 10000
kcls1.me <- 10000
kcls2.me <- 10000
TLV.me <- 30
kpa.cv <- 75; kca.cv <- 75; kpm.cv <- 75; kcm.cv <- 75; kpd.cv <- 75; kcd.cv <- 75
kpls1.cv <- 0; kpls2.cv <- 0; kcls1.cv <- 0; kcls2.cv <- 0
TLVprop.me <- 1
TLVprop.cv <- 0
kin.me <- 0
Fex.me <- 0
Fpd.me <- 0.25
Fcd.me <- 0.75
Din.me <- 0
kin.cv <- 0; Fex.cv <- 0; Fpd.cv <- 0; Fcd.cv <- 0; Din.cv <- 0

# Normal Distribution Generator

NormGen <- function(simulation.n, samp.m, samp.cv)
{  samp.sd <- samp.cv*samp.m/100
   out <- rnorm(simulation.n, samp.m, samp.sd)
   return(out)
}

LogNormGen <- function(n, Mean, PCV)
{  CV <- PCV/100; SD <- (CV*Mean); Variance <- SD^2; mean.in <-
    log(Mean^2/sqrt(Variance+Mean^2));
    SD.in <- sqrt(log(Variance/Mean^2 + 1));
    x = rlnorm(n, mean.in, SD.in)
   return(x)
}

AUC.Trap.Calc <- function(Time, CP)
{  n <- length(Time); AUC.Values <- rep(0, n-1)
    for (i in 1:(n-1))
      if (i==1) {AUC.Values[i] <- (Time[i+1]-Time[i])*(CP[i]+CP[i+1])/2}
      if (i>1) {AUC.Values[i] <- (Time[i+1]-Time[i])*(CP[i]+CP[i+1])/2 + AUC.Values[i-1]}
    return(AUC.Values[i])
}
Cmax.301A.n=rep(0,n); Cmax.302A.n=rep(0,n); Cmax.302B.n=rep(0,n); Cmax.303A.n=rep(0,n);
Cmax.303B.n=rep(0,n); Cmax.304A.n=rep(0,n); Cmax.304B.n=rep(0,n); Cmax.304C.n=rep(0,n);
CpAUC.301A.n=rep(0,n); CpAUC.302A.n=rep(0,n); CpAUC.302B.n=rep(0,n); CpAUC.303A.n=rep(0,n);
CpAUC.303B.n=rep(0,n); CpAUC.304A.n=rep(0,n); CpAUC.304B.n=rep(0,n); CpAUC.304C.n=rep(0,n);

BW.n <- NormGen(n, BW.me, BW.cv)
if (Disease==1) {Clononren.n <- NormGen(n, Clononren.1.me, Clononren.1.cv)} else if (Disease==2)
{Clononren.n <- NormGen(n, Clononren.2.me, Clononren.2.cv)}
if (Disease==1) {Clren.n <- NormGen(n, Clren.1.me, Clren.1.cv)} else if (Disease==2)
{Clren.n <- NormGen(n, Clren.2.me, Clren.2.cv)}
if (Disease==1) {k12.n <- NormGen(n, k12.1.me, k12.1.cv)} else if (Disease==2)
{k12.n <- NormGen(n, k12.2.me, k12.2.cv)}
if (Disease==1) {k21.n <- NormGen(n, k21.1.me, k21.1.cv)} else if (Disease==2)
{k21.n <- NormGen(n, k21.2.me, k21.2.cv)}
if (Disease==1) {V0.n <- NormGen(n, V0.1.me, V0.1.cv)} else if (Disease==2)
{V0.n <- NormGen(n, V0.2.me, V0.2.cv)}
if (Disease==1) {kga.n <- LogNormGen(n, kga.1.me, kga.1.cv)} else if (Disease==2)
{kga.n <- LogNormGen(n, kga.2.me, kga.2.cv)}
if (Disease==1) {Foral.n <- NormGen(n, Foral.1.me, Foral.1.cv)/100} else if (Disease==2)
{Foral.n <- NormGen(n, Foral.2.me, Foral.2.cv)/100}
kpa.n <- LogNormGen(n, kpa.me, kpa.cv); kca.n <- LogNormGen(n, kca.me, kca.cv)
kpm.n <- LogNormGen(n, kpm.me, kpm.cv); kcm.n <- LogNormGen(n, kcm.me, kcm.cv)
if (kpd.me==0) {kpd.n <- rep(0,n)} else if (kpd.me>0) {kpd.n <- LogNormGen(n, kpd.me, kpd.cv)}
if (kcd.me==0) {kcd.n <- rep(0,n)} else if (kcd.me>0) {kcd.n <- LogNormGen(n, kcd.me, kcd.cv)}
if (kpls1.me==0) {kpls1.n <- rep(0,n)} else if (kpls1.me>0) {kpls1.n <- LogNormGen(n, kpls1.me, kpls1.cv)}
if (kpls2.me==0) {kpls2.n <- rep(0,n)} else if (kpls2.me>0) {kpls2.n <- LogNormGen(n, kpls2.me, kpls2.cv)}
if (kcls1.me==0) {kcls1.n <- rep(0,n)} else if (kcls1.me>0) {kcls1.n <- LogNormGen(n, kcls1.me, kcls1.cv)}
if (kcls2.me==0) {kcls2.n <- rep(0,n)} else if (kcls2.me>0) {kcls2.n <- LogNormGen(n, kcls2.me, kcls2.cv)}
TLVprop.n <- NormGen(n, TLVprop.me, TLVprop.cv)
if (kin.me==0) {kin.n <- rep(0,n)} else if (kin.me>0) {kin.n <- NormGen(n, kin.me, kin.cv)}
Fex.n <- NormGen(n, Fex.me, Fex.cv); Fpd.n <- NormGen(n, Fpd.me, Fpd.cv); Fcd.n <- NormGen(n, Fcd.me, Fcd.cv)
if (Din.me==0) {Din.n<-rep(0,n)} else if (Din.me>0) {Din.n<-LogNormGen(n, Din.me,Din.cv)}

TCBM <- function(t,Cltot, k12, k21, V0, kpa, kca, kpm, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, LDose,GIDose, tinf ){
  Fgd <- 1-Fex-Fpd-Fcd
  ED <- data.frame(var = c("DOSING"), time = c(0,1), value = c(0,0), method = c("rep"))
  Model.Function <- function(t,IC,Parm) {
    with(as.list(IC, Parm), {
      dDOSING <- 0
      dln <- -ln*kin*(Fex+Fpd+Fcd+Fgd);dPLU<-ln*kin*Fpd + PLS*kpd - PLU*(kpa + kpm)
      dCLU <-ln*kin*Fcd + CLS*kcd - CLU*(kca + kcm) + PLU* kpm
      dGI <-ln*kin*Fgd + CLU* km - GL*kga; dPLS<-PLU*kpa + CC*kpls1 - PLS *
      (kpd+kpls2)
      dCLS <-CLU*kca + CC*kcls1 - CLS *(kcd+kcls2);dPC <-CC* k12 - PC *(k21
      dCC <-PLS*kpls2 + CLS*kcls2 + GL*kga*Foral + PC*k21 - CC*(kpls1+kcls1 + k12 +
      (Cltot/(V0*1000)))+ DOSING
      dEx <-ln*kin*Fex; dFe <- GI*kga*(1-Foral); dEl <-
      CC*(Cltot/(V0*1000))
      return(list(c(dln,dPLU,dCLU,dPLS,dCLS,dGI,dCC,dPC,dEx,dFe,dEl,dDOSING))
    })
  }
  IC <- c(In=0, PLU=LDose*Fpd, CLU=LDose*Fcd, PLS=0, CLS=0, GI=GIDose,
  CC=0, PC=0, Ex=0*Fex, Fe=0, El=0,DOSING=0)
  Parm <- c(Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Fgd)
  out <- ode(IC, t,Model.Function,Parm)
  return(out)
}

Cltot.n<-Clren.n+Clnonren.n
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n^0.8/1000
PLV.n <- TLV.n^0.2/1000
In.Data <- matrix(0,length(t),(n));PL.Data <- In.Data
PLU.Data <- In.Data;CLU.Data <- In.Data;PLS.Data <- In.Data;CLS.Data <- In.Data
Gl.Data <- In.Data;CC.Data <- In.Data;PC.Data <- In.Data;Ex.Data <- In.Data

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Fe.Data <- In.Data; El.Data <- In.Data; CP.Data <- In.Data; CS.Data <- In.Data
for (i in 1:n)
  Cltot <- Cltot.n[i]; k12 <- k12.n[i]; k21 <- k21.n[i]; V0 <- V0.n[i]; kga <- kga.n[i]
  Foral <- Foral.n[i]; kpa <- kpa.n[i]; kca <- kca.n[i]; kpm <- kpm.n[i]; kcm <- kcm.n[i]; kpd <- kpd.n[i]
  kcd <- kcd.n[i]; kpls1 <- kpls1.n[i]; kpls2 <- kpls2.n[i]; kcls1 <- kcls1.n[i]; kcls2 <- kcls2.n[i];
  CLV <- CLV.n[i]
  PLV <- PLV.n[i]; kin <- kin.n[i]; Fex <- Fex.n[i]; Fpd <- Fpd.n[i]; Fcd <- Fcd.n[i]; Din <- Din.n[i]
  LDose <- InhalerDose * DtL; GDose <- InhalerDose * DtG I
  CID <- TCBM(t, Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kcls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, LDose, GDose, tinf)
  CS.Data[, i] <- CID[4]/CLV; PL.Data[, i] <- CID[3]/PLV
  Cmax.301A.n[i] <- CP.Data[13, i] * 1000
  CP.AUC.301A.n[i] <- AUC.Trap.Calc(t, CP.Data[, i])/60*1000
}

VPC <- function(Data, Color, YMin, YMax, XMin, XMax, Title, Ytitle, LogAxis)
{
  Size.of.Data <- dim(Data); PlottingData <- matrix(0, Size.of.Data[1], 4)
  PlottingData[, 1] <- CID[, 1]
  for (i in 1:length(t)) {
    PlottingData[i, 2] <- quantile(Data[i, ], 0.05); PlottingData[i, 3] <- quantile(Data[i, ], 0.50)
    PlottingData[i, 4] <- quantile(Data[i, ], 0.95)
  }
  Time <- PlottingData[, 1]; Bottom <- PlottingData[, 2]; Top <- PlottingData[, 4]; Middle <- PlottingData[, 3]
  ColNumbers <- col2rgb(Color); par(TRUE)
  mycol4 <- rgb(ColNumbers[1], ColNumbers[2], ColNumbers[3], max = 255, alpha = 90)
  plot(Time, Bottom, type = "n", col = Color, main = Title, ylim = c(YMin, YMax), log = LogAxis, xlab = "Time (minutes)", ylab = "n", col = mycol4, border = NA)
  lines(Time, Middle, type = "l", col = Color, lwd = 2.8)
  lines(Time, Bottom, type = "l", col = Color, lwd = 2.8)
  lines(Time, Top, type = "l", col = Color, lwd = 2.8)
  return()
}

T301A <- c(15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 720, 960, 1440)
C301A <- c(0.0498, 0.0448, 0.0444, 0.0430, 0.0398, 0.0320, 0.0294, 0.0256, 0.0212, 0.0172, 0.0132, 0.0076, 0.0052, 0.0030)
VPCP(CP.Data,'green',0.005,0.5,0,720,'CIP 301A Cp(t)  -- HV, Inhaler Dose = 32.5mg,'Plasma Conc (mg/l)', 'y')
points(T301A,C301A,col='red',pch=20,cex=2.5)

dev.off();set.seed(123091)
BW.me <- 59
Disease <- 2
InhalerDose<-32.5#mg
t<-seq(0,720,5)
######################################################################
###################
###################     Generation of numbers
###################
######################################################################
BW.n     <- NormGen(n, BW.me,BW.cv)
if (Disease==1){Clnonren.n<-NormGen(n, Clnonren.1.me,Clnonren.1.cv)} else if (Disease==2)
{Clnonren.n<-NormGen(n, Clnonren.2.me,Clnonren.2.cv)}
if (Disease==1){Clren.n<-NormGen(n, Clren.1.me,Clren.1.cv)} else if (Disease==2)
{Clren.n<-NormGen(n, Clren.2.me,Clren.2.cv)}
if (Disease==1){k12.n<-NormGen(n, k12.1.me,k12.1.cv)} else if (Disease==2)
{k12.n<-NormGen(n, k12.2.me,k12.2.cv)}
if (Disease==1){k21.n<-NormGen(n, k21.1.me,k21.1.cv)} else if (Disease==2)
{k21.n<-NormGen(n, k21.2.me,k21.2.cv)}
if (Disease==1){V0.n<-NormGen(n, V0.1.me,V0.1.cv)} else if (Disease==2)
{V0.n<-NormGen(n, V0.2.me,V0.2.cv)}
if (Disease==1){kga.n<-LogNormGen(n, kga.1.me,kga.1.cv)} else if (Disease==2)
{kga.n<-LogNormGen(n, kga.2.me,kga.2.cv)}
if (Disease==1){Foral.n<-NormGen(n,Foral.1.me,Foral.1.cv)/100} else if (Disease==2)
{Foral.n<-NormGen(n,Foral.2.me,Foral.2.cv)/100}
kpa.n<- LogNormGen(n, kpa.me,kpa.cv);kca.n<- LogNormGen(n, kca.me,kca.cv)
kpm.n <- LogNormGen(n, kpm.me,kpm.cv);kcm.n<- LogNormGen(n, kcm.me,kcm.cv)
if (kpd.me==0){kpd.n<-rep(0,n)} else if (kpd.me>0){kpd.n<-LogNormGen(n, kpd.me,kpd.cv)}
if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n, kcd.me,kcd.cv)}
if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n, kpls1.me,kpls1.cv)}
if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n, kpls2.me,kpls2.cv)}
if (kcls1.me==0){kcls1.n<-rep(0,n)} else if (kcls1.me>0){kcls1.n<-LogNormGen(n, kcls1.me,kcls1.cv)}
if (kcls2.me==0){kcls2.n<-rep(0,n)} else if (kcls2.me>0){kcls2.n<-LogNormGen(n, kcls2.me,kcls2.cv)}
TLVprop.n <- NormGen(n, TLVprop.me,TLVprop.cv)
if (kin.me==0){kin.n<-rep(0,n)} else if (kin.me>0){kin.n<-NormGen(n, kin.me,kin.cv)}
Fex.n<- NormGen(n, Fex.me,Fex.cv);Fpd.n<- NormGen(n, Fpd.me,Fpd.cv);Fcd.n<-NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n<-rep(0,n)} else if (Din.me>0){Din.n<-LogNormGen(n, Din.me,Din.cv)}

# PBPK Model iterations

Cltot.n <- Clren.n+Clnonren.n
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n*0.8/1000
PLV.n <- TLV.n*0.2/1000
for (i in 1:n) {
  Cltot<-Cltot[n];k12<-k12.n; V0<-V0.n; kga<-kga.n
  Foral<-Foral.n;kpa<-kpa.n;kca<-kca.n;kpm<-kpm.n;kcm<-kcm.n;kpd<-kpd.n
  kcd<-kcd.n;kpls1<-kpls1.n;kpls2<-kpls2.n;kcls1<-kcls1.n; kcls2<-kcls2.n
  CLV<-CLV.n[1];PLV<-PLV.n[1];kin<-kin.n[Fex<-Fex.n];Fpd<-Fpd.n;Fcd<-Fcd.n; Din<-Din.n
  LDose <- InhalerDose*DtL; GIDose <- InhalerDose*DtGI
  CID<- TCBM(t,Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcls1, 
kcls2, k1, kin, Fex, Fpd, Fcd, Din, LDose, GIDose, tinf)
  CS.Data[i] <- CID[4];CLV;PL.Data[i] <- CID[3]/PLV
  Cmax.302A.n[i] <- CP.Data[13,i]*1000
  CpAUC.302A.n[i] <- AUC.Trap.Calc(t, CP.Data[i])/60*1000
}
T302A <- c(5,15,30,45,60,90,120,150,180,240,360,480,720,960,1440)
C302A <- c(0.008,0.020,0.051,0.060,0.065,0.071,0.065,0.055,0.051,0.043,0.021,0.014,0.006,0.004,0.001)
VPCP(CP.Data,'green',0.005,0.5,0,720,'CIP 302A Cp(t) -- CF, Inhaler Dose = 32.5mg','Plasma Conc (mg/l)','y')
points(T302A,C302A,col='red',pch=20,cex=2.5)
dev.off()

T302A <- c(5,15,30,45,60,90,120,150,180,240,360,480,720,960,1440)
C302A <- c(0.008,0.020,0.051,0.060,0.065,0.071,0.065,0.055,0.051,0.043,0.021,0.014,0.006,0.004,0.001)
VPCP(CS.Data,'cyan',0.001,100,0,720,'CIP 302A Cs(t) -- CF, Inhaler Dose = 32.5mg','Sputum Conc (mg/l)','y')
points(ST302A,SC302A,col='red',pch=20,cex=2.5)
dev.off();set.seed(123091)

BW.me <- 65
Disease <- 2
InhalerDose <- 65#mg
t<-seq(0,720,5)
BW.n <- NormGen(n, BW.me,BW.cv)
if (Disease==1){Clren.n<-NormGen(n, Clren.1.me,Clren.1.cv)} else if (Disease==2)
{Clren.n<-NormGen(n, Clren.2.me,Clren.2.cv)}
if (Disease==1){k12.n<-NormGen(n, k12.1.me,k12.1.cv)} else if (Disease==2)
{k12.n<-NormGen(n, k12.2.me,k12.2.cv)}
if (Disease==1){k21.n<-NormGen(n, k21.1.me,k21.1.cv)} else if (Disease==2)
{k21.n<-NormGen(n, k21.2.me,k21.2.cv)}
if (Disease==1){V0.n<-NormGen(n, V0.1.me,V0.1.cv)} else if (Disease==2)
{V0.n<-NormGen(n, V0.2.me,V0.2.cv)}
if (Disease==1){kga.n<-LogNormGen(n, kga.1.me,kga.1.cv)} else if (Disease==2)
{kga.n<-LogNormGen(n, kga.2.me,kga.2.cv)}
if (Disease==1){Foral.n<-NormGen(n,Foral.1.me,Foral.1.cv)/100} else if (Disease==2)
{Foral.n<-NormGen(n, Foral.2.me,Foral.2.cv)/100}
kpa.n<- LogNormGen(n, kpa.me,kpa.cv); kca.n<- LogNormGen(n, kca.me,kca.cv) kpm.n<- LogNormGen(n, kpm.me,kpm.cv); kcm.n<- LogNormGen(n, kcm.me,kcm.cv) if (kpd.me==0){kpd.n<-rep(0,n)} else if (kpd.me>0){kpd.n<-LogNormGen(n, kpd.me,kpd.cv)} if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n, kcd.me,kcd.cv)} if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n, kpls1.me,kpls1.cv)} if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n, kpls2.me,kpls2.cv)} if (kcls1.me==0){kcls1.n<-rep(0,n)} else if (kcls1.me>0){kcls1.n<-LogNormGen(n, kcls1.me,kcls1.cv)} if (kcls2.me==0){kcls2.n<-rep(0,n)} else if (kcls2.me>0){kcls2.n<-LogNormGen(n, kcls2.me,kcls2.cv)} TLVprop.n <- NormGen(n, TLVprop.me,TLVprop.cv)
if (kin.me==0){kin.n<-rep(0,n)} else if (kin.me>0){kin.n<-NormGen(n, kin.me,kin.cv)}
Fex.n<- NormGen(n, Fex.me,Fex.cv); Fpd.n<- NormGen(n, Fpd.me,Fpd.cv); Fcd.n<-NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n<-rep(0,n)} else if (Din.me>0){Din.n<-LogNormGen(n, Din.me,Din.cv)}
Cltot <- Clren + Clnonren
TLV.n <- TLV.me * (BW.n / mean(BW.n))^0.8 * TLVprop.n
CLV.n <- TLV.n * 0.8 / 1000
PLV.n <- TLV.n * 0.2 / 1000
for (i in 1:n) {
  Cltot <- Cltot.n[i]; k12 <- k12.n[i]; k21 <- k21.n[i]; V0 <- V0.n[i]; kga <- kga.n[i]
  Foral <- Foral.n[i]; kpa <- kpa.n[i]; kca <- kca.n[i]; kpm <- kpm.n[i]; kcm <- kcm.n[i]; kpd <- kpd.n[i]
  kcd <- kcd.n[i]; kpls1 <- kpls1.n[i]; kpls2 <- kpls2.n[i]; kcls1 <- kcls1.n[i]; kcls2 <- kcls2.n[i];
  CLV <- CLV.n[i]; PLV <- PLV.n[i]; kin <- kin.n[i]; Fex <- Fex.n[i]; Fpd <- Fpd.n[i]; Fcd <- Fcd.n[i]; Din <- Din.n[i]
  LDose <- InhalerDose * DtL; GIDose <- InhalerDose * DtGI
  CID <- TCBM(t, Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kpl,
  kin, Fex, Fpd, Fcd, Din, LDose, GIDose, tinf)
  In.Data[,i] <- CID[,2]; PLU.Data[,i] <- CID[,3]; CLU.Data[,i] <- CID[,4]; PLS.Data[,i] <- CID[,5]
  CLS.Data[,i] <- CID[,6]; GL.Data[,i] <- CID[,7]; CC.Data[,i] <- CID[,8]; PC.Data[,i] <- CID[,9]
  Ex.Data[,i] <- CID[,10]; Fe.Data[,i] <- CID[,11]; El.Data[,i] <- CID[,12]; CP.Data[,i] <-
  CID[,8] / V0
  Cmax.302B.n[i] <- CP.Data[10,i] * 1000
  CpAUC.302B.n[i] <- AUC.Trap.Calc(t, CP.Data[,i]) / 60 * 1000
}
png(width=10, height=7, units='in', res=1200, filename="C:/Users/Laptop/Desktop/Cip302B.png")
T302B <- c(5, 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 720, 960, 1440)
C302B <- c(0.020, 0.050, 0.111, 0.155, 0.137, 0.152, 0.119, 0.104, 0.115, 0.089, 0.053, 0.036, 0.018, 0.010, 0.004)
VPCP(CP.Data, 'green', 0.005, 0.5, 720, 'CIP 302B Cp(t) -- CF, Inhaler Dose = 65mg', 'Plasma Conc (mg/l)', 'y')
points(T302B, C302B, col='red', pch=20, cex=2.5)
dev.off()

png(width=10, height=7, units='in', res=1200, filename="C:/Users/Laptop/Desktop/Cip302B Sputum.png")
ST302B <- c(0.75, 2.00, 4.00, 8.00, 24.00) * 60
SC302B <- c(138.95, 208.30, 55.88, 5.73, 0.59)
VPCP(CS.Data, 'cyan', 0.5, 500, 720, 'CIP 302B Cs(t) -- CF, Inhaler Dose = 65mg', 'Sputum Conc (mg/l)', 'y')
points(ST302B, SC302B, col='red', pch=20, cex=2.5)
dev.off()
dev.off();set.seed(123091)
BW.me <- 75
Disease <- 1
InhalerDose <- 32.5#mg
t <- seq(0, 720, 5)

Generates the numbers:

BW.me <- NormGen(n, BW.me, BW.cv)
if (Disease==1){Clnonren.n <- NormGen(n, Clnonren.1.me, Clnonren.1.cv)}
else if (Disease==2)
{Clnonren.n <- NormGen(n, Clnonren.2.me, Clnonren.2.cv)}

if (Disease==1){Clren.n <- NormGen(n, Clren.1.me, Clren.1.cv)}
else if (Disease==2)
{Clren.n <- NormGen(n, Clren.2.me, Clren.2.cv)}

if (Disease==1){k12.n <- NormGen(n, k12.1.me, k12.1.cv)}
else if (Disease==2)
{k12.n <- NormGen(n, k12.2.me, k12.2.cv)}

if (Disease==1){V0.n <- NormGen(n, V0.1.me, V0.1.cv)}
else if (Disease==2)
{V0.n <- NormGen(n, V0.2.me, V0.2.cv)}

if (Disease==1){kga.n <- LogNormGen(n, kga.1.me, kga.1.cv)}
else if (Disease==2)
{kga.n <- LogNormGen(n, kga.2.me, kga.2.cv)}

if (Disease==1){Foral.n <- NormGen(n, Foral.1.me, Foral.1.cv)/100}
else if (Disease==2)
{Foral.n <- NormGen(n, Foral.2.me, Foral.2.cv)/100}

kpa.n <- LogNormGen(n, kpa.me, kpa.cv); kca.n <- LogNormGen(n, kca.me, kca.cv)
kpm.n <- LogNormGen(n, kpm.me, kpm.cv); kcm.n <- LogNormGen(n, kcm.me, kcm.cv)

if (kpd.me==0){kpd.n <- rep(0, n)}
else if (kpd.me>0){kpd.n <- LogNormGen(n, kpd.me, kpd.cv)}
if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n, kcd.me,kcd.cv)}
if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n, kpls1.me,kpls1.cv)}
if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n, kpls2.me,kpls2.cv)}
if (kcls1.me==0){kcls1.n<-rep(0,n)} else if (kcls1.me>0){kcls1.n<-LogNormGen(n, kcls1.me,kcls1.cv)}
if (kcls2.me==0){kcls2.n<-rep(0,n)} else if (kcls2.me>0){kcls2.n<-LogNormGen(n, kcls2.me,kcls2.cv)}
TLVprop.n <- NormGen(n, TLVprop.me,TLVprop.cv)
if (kin.me==0){kin.n<-rep(0,n)} else if (kin.me>0){kin.n<-NormGen(n, kin.me,kin.cv)}
Fex.n<- NormGen(n, Fex.me,Fex.cv);Fpd.n<- NormGen(n, Fpd.me,Fpd.cv);Fcd.n<-NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n<-rep(0,n)} else if (Din.me>0){Din.n<-LogNormGen(n, Din.me,Din.cv)}

#***********************************************************************
#                          PBPK Model iterations
#***********************************************************************

Cltot.n<-Clren.n+Clnonren.n
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n*0.8/1000
PLV.n <- TLV.n*0.2/1000
for (i in 1:n) {
  Cltot<-Cltot.n[i];k12<-k12.n[i];k21<-k21.n[i]; V0<-V0.n[i]; kga<-kga.n[i]
  Foral<-Foral.n[i];kpa<-kpa.n[i];kca<-kca.n[i];kpm<-kpm.n[i];kcm<-kcm.n[i];kpd<-kpd.n[i]
  kcd<-kcd.n[i];kpls1<- kpls1.n[i];kpls2<-kpls2.n[i];kcls1<-kcls1.n[i];kcls2<-kcls2.n[i];CLV<-CLV.n[i]
  PLV<-PLV.n[i];kin<-kin.n[i];Fex<-Fex.n[i];Fpd<-Fpd.n[i];Fcd<-Fcd.n[i];Din<-Din.n[i]
  LDose <- InhalerDose*DtL; GIDose <-InhalerDose*DtGI
  CID<- TCBM(t,Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1,
  kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, LDose,GIDose, tinf)
  CS.Data[i]<-CID[4]/CLV;PL.Data[i]<-CID[3]/PLV
  Cmax.302A.n[i]<-CP.Data[13,i]*1000
  CpAUC.302A.n[i]<-AUC.Trap.Calc(t, CP.Data[i])/60*1000
}

png(width=10,height=7,units='in',res=1200,filename="C:/Users/Laptop/Desktop/Cip303A.png")
T303A <- c(30,60,90,120,150,180,240,360,480,720,1440)
C303A <- c(0.077, 0.095, 0.083, 0.073, 0.065, 0.059, 0.049, 0.034, 0.027, 0.014, 0.004)
VPCP(CP.Data,'green',0.005,0.5,0,720,'CIP 303A Cp(t) -- HV, Inhaler Dose = 32.5mg','Plasma Conc (mg/l)', 'y')
points(T303A,C303A,col='red',pch=20,cex=2.5)

dev.off();set.seed(123091)
BW.me <- 75
Disease <- 1
InhalerDose<-32.5#mg
t<-seq(0,720,5)

# Generation of numbers
BW.n <- NormGen(n, BW.me,BW.cv)
if (Disease==1){Clnonren.n<-NormGen(n, Clnonren.1.me,Clnonren.1.cv)} else if (Disease==2)
{Clnonren.n<-NormGen(n, Clnonren.2.me,Clnonren.2.cv)}
if (Disease==1){Clren.n<-NormGen(n, Clren.1.me,Clren.1.cv)} else if (Disease==2)
{Clren.n<-NormGen(n, Clren.2.me,Clren.2.cv)}
if (Disease==1){k12.n<-NormGen(n, k12.1.me,k12.1.cv)} else if (Disease==2)
{k12.n<-NormGen(n, k12.2.me,k12.2.cv)}
if (Disease==1){k21.n<-NormGen(n, k21.1.me,k21.1.cv)} else if (Disease==2)
{k21.n<-NormGen(n, k21.2.me,k21.2.cv)}
if (Disease==1) {V0.n <- NormGen(n, V0.1.me, V0.1.cv)} else if (Disease==2) {V0.n <- NormGen(n, V0.2.me, V0.2.cv)}
if (Disease==1) {kga.n <- LogNormGen(n, kga.1.me, kga.1.cv)} else if (Disease==2) {kga.n <- LogNormGen(n, kga.2.me, kga.2.cv)}
if (Disease==1) {Foral.n <- NormGen(n, Foral.1.me, Foral.1.cv)/100} else if (Disease==2) {Foral.n <- NormGen(n, Foral.2.me, Foral.2.cv)/100}
kpa.n <- LogNormGen(n, kpa.me, kpa.cv); kca.n <- LogNormGen(n, kca.me, kca.cv)
kpm.n <- LogNormGen(n, kpm.me, kpm.cv); kcm.n <- LogNormGen(n, kcm.me, kcm.cv)
if (kpd.me==0){kpd.n <- rep(0,n)} else if (kpd.me>0){kpd.n <- LogNormGen(n, kpd.me, kpd.cv)}
if (kcd.me==0){kcd.n <- rep(0,n)} else if (kcd.me>0){kcd.n <- LogNormGen(n, kcd.me, kcd.cv)}
if (kpls1.me==0){kpls1.n <- rep(0,n)} else if (kpls1.me>0){kpls1.n <- LogNormGen(n, kpls1.me, kpls1.cv)}
if (kpls2.me==0){kpls2.n <- rep(0,n)} else if (kpls2.me>0){kpls2.n <- LogNormGen(n, kpls2.me, kpls2.cv)}
if (kcls1.me==0){kcls1.n <- rep(0,n)} else if (kcls1.me>0){kcls1.n <- LogNormGen(n, kcls1.me, kcls1.cv)}
if (kcls2.me==0){kcls2.n <- rep(0,n)} else if (kcls2.me>0){kcls2.n <- LogNormGen(n, kcls2.me, kcls2.cv)}
if (kin.me==0){kin.n <- rep(0,n)} else if (kin.me>0){kin.n <- NormGen(n, kin.me, kin.cv)}
Fex.n <- NormGen(n, Fex.me,Fex.cv); Fpd.n <- NormGen(n, Fpd.me,Fpd.cv); Fcd.n <- NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n <- rep(0,n)} else if (Din.me>0){Din.n <- LogNormGen(n, Din.me,Din.cv)}
############################################################
####
###############
#####################        PBPK Model iterations
################################
######################################################################

if (Disease==1) {Cltot.n <- C1ren.n+C1nonren.n}
if (Disease==2) {Cltot.n <- C1ren.n+C1nonren.n}
if (Disease==1) {TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n}
if (Disease==2) {TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n}
if (Disease==1) {CLV.n <- TLV.n*0.8/1000}
if (Disease==2) {CLV.n <- TLV.n*0.8/1000}
if (Disease==1) {PLV.n <- TLV.n*0.2/1000}
if (Disease==2) {PLV.n <- TLV.n*0.2/1000}

for (i in 1:n) {
  Cltot<-Cltot.n[i]; k12<-k12.n[i]; k21<-k21.n[i]; V0<-V0.n[i]; kga<-kga.n[i]
  Foral<-Foral.n[i]; kpa<-kpa.n[i]; kca<-kca.n[i]; kpm<-kpm.n[i]; kcm<-kcm.n[i]; kpd<-kpd.n[i]
  kcd<-kcd.n[i]; kpls1<-kpls1.n[i]; kpls2<-kpls2.n[i]; kcls1<-kcls1.n[i]; kcls2<-kcls2.n[i]
  CLV<-CLV.n[i]; PLV<-PLV.n[i]; kin<-kin.n[i]; Fex<-Fex.n[i]; Fpd<-Fpd.n[i]; Fcd<-Fcd.n[i]; Din<-Din.n[i]
  LDose <- InhalerDose*DtL; GDose <- InhalerDose*DtG
  CID<- TCBM(t, Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, LDose,0, tinf)
CLS.Data[,i]<-CID[,6];Gl.Data[,i]<-CID[,7];CC.Data[,i]<-CID[,8];PC.Data[,i]<-CID[,9];Ex.Data[,i]<-CID[,10];Fe.Data[,i]<-CID[,11];El.Data[,i]<-CID[,12];CP.Data[,i]<-CID[,8]/V0;CS.Data[,i]<-CID[,4]/CLV;PL.Data[,i]<-CID[,3]/PLV;Cmax.302A.n[i]<-CP.Data[13,i]*1000;CpAUC.302A.n[i]<-AUC.Trap.Calc(t, CP.Data[,i])/60*1000

png(width=10,height=7,units='in',res=1200,filename="C:/Users/Laptop/Desktop/Cip303B.png")
T303B <- c(30,60,90,120,150,180,240,360,480,720,1440)
C303B <- c(0.040,0.032,0.027,0.024,0.022,0.021,0.018,0.013,0.010,0.006,0.001)
VPCP(CP.Data,'green',0.001,0.1,0,720,'CIP 303B Cp(t) -- HV, Inhaler Dose = 32.5mg','Plasma Conc (mg/l)','y')
points(T303B,C303B,col='red',pch=20,cex=2.5)

dev.off();set.seed(123091)
BW.me <- 58
Disease <- 2
InhalerDose<-32.5#mg
t<-seq(0,720,5)

### Generation of numbers

```r
BW.n <- NormGen(n, BW.me,BW.cv) if (Disease==1){Clnonren.n<-NormGen(n, Clnonren.1.me,Clnonren.1.cv)} else if (Disease==2) {Clnonren.n<-NormGen(n, Clnonren.2.me,Clnonren.2.cv)} if (Disease==1){Clren.n<-NormGen(n, Clren.1.me,Clren.1.cv)} else if (Disease==2) {Clren.n<-NormGen(n, Clren.2.me,Clren.2.cv)} if (Disease==1){k12.n<-NormGen(n, k12.1.me,k12.1.cv)} else if (Disease==2) {k12.n<-NormGen(n, k12.2.me,k12.2.cv)} if (Disease==1){k21.n<-NormGen(n, k21.1.me,k21.1.cv)} else if (Disease==2) {k21.n<-NormGen(n, k21.2.me,k21.2.cv)} if (Disease==1){V0.n<-NormGen(n, V0.1.me,V0.1.cv)} else if (Disease==2) {V0.n<-NormGen(n, V0.2.me,V0.2.cv)} if (Disease==1){kga.n<-LogNormGen(n, kga.1.me,kga.1.cv)} else if (Disease==2) {kga.n<-LogNormGen(n, kga.2.me,kga.2.cv)} if (Disease==1){Foral.n<-NormGen(n,Foral.1.me,Foral.1.cv)/100} else if (Disease==2) {Foral.n<-NormGen(n,Foral.2.me,Foral.2.cv)/100} kpa.n<- LogNormGen(n, kpa.me,kpa.cv);kca.n<- LogNormGen(n, kca.me,kca.cv) kpm.n<- LogNormGen(n, kpm.me,kpm.cv);kcm.n<- LogNormGen(n, kcm.me,kcm.cv) if (kpd.me==0){kpd.n<-rep(0,n)} else if (kpd.me>0){kpd.n<-LogNormGen(n, kpd.me,kpd.cv)} if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n, kcd.me,kcd.cv)} if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n, kpls1.me,kpls1.cv)} if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n, kpls2.me,kpls2.cv)} if (kcls1.me==0){kcls1.n<-rep(0,n)} else if (kcls1.me>0){kcls1.n<-LogNormGen(n, kcls1.me,kcls1.cv)} if (kcls2.me==0){kcls2.n<-rep(0,n)} else if (kcls2.me>0){kcls2.n<-LogNormGen(n, kcls2.me,kcls2.cv)} TLVprop.n <- NormGen(n, TLVprop.me,TLVprop.cv) if (kin.me==0){kin.n<-rep(0,n)} else if (kin.me>0){kin.n<-NormGen(n, kin.me,kin.cv)} Fex.n<- NormGen(n, Fex.me,Fex.cv);Fpd.n<- NormGen(n, Fpd.me,Fpd.cv);Fcd.n<- NormGen(n, Fcd.me,Fcd.cv) if (Din.me==0){Din.n<-rep(0,n)} else if (Din.me>0){Din.n<-LogNormGen(n, Din.me,Din.cv)}
```

### PBPK Model iterations

```r
```
Cltot.n <- Clren.n + Clinonren.n
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n*0.8/1000
PLV.n <- TLV.n*0.2/1000
for (i in 1:n) {
  Cltot <- Cltot.n[i]; k12 <- k12.n[i]; k21 <- k21.n[i]; V0 <- V0.n[i]; kga <- kga.n[i]
  Foral <- Foral.n[i]; kpa <- kpa.n[i]; kca <- kca.n[i]; kpm <- kpm.n[i]; kcm <- kcm.n[i]; kpd <- kpd.n[i]
  kcd <- kcd.n[i]; kpls1 <- kpls1.n[i]; kpls2 <- kpls2.n[i]; kcls1 <- kcls1.n[i]; kcls2 <- kcls2.n[i]; CLV <- CLV.n[i]
  PLV <- PLV.n[i]; kin <- kin.n[i]; Fex <- Fex.n[i]; Fpd <- Fpd.n[i]; Fcd <- Fcd.n[i]; Din <- Din.n[i]
  LDose <- InhalerDose*DtL; GIDose <- InhalerDose*DtGI
  CID <- TCBM(t, Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, LDose, GIDose, tinf)
  In.Data[,i] <- CID[,2]; PLU.Data[,i] <- CID[,3]; CLU.Data[,i] <- CID[,4]; PLS.Data[,i] <- CID[,5]
  CLS.Data[,i] <- CID[,6]; Gl.Data[,i] <- CID[,7]; CC.Data[,i] <- CID[,8]; PC.Data[,i] <- CID[,9]
  Ex.Data[,i] <- CID[,10]; Fe.Data[,i] <- CID[,11]; El.Data[,i] <- CID[,12]; CP.Data[,i] <- CID[,8]/V0
  CS.Data[,i] <- CID[,4]/CLV; PL.Data[,i] <- CID[,3]/PLV
  Cmax.302A.n[i] <- CP.Data[13,i]*1000
  CpAUC.302A.n[i] <- AUC.Trap.Calc(t, CP.Data[,i])/60*1000
}
png(width=10, height=7, units='in', res=1200, filename="C:/Users/Laptop/Desktop/Cip304A.png")
T304A <- c(5,15,60,90,120,150,180,240,360,480,720,960,1440)
C304A <- c(0.017, 0.024, 0.085, 0.072, 0.062, 0.046, 0.036, 0.021, 0.013, 0.006, 0.003, 0.002)
VPCP(CP.Data, 'green', 0.005, 0.5, 0.720, 'CIP 304A Cp(t) -- CF, Inhaler Dose = 32.5mg', 'Plasma Conc (mg/l)', 'y')
points(T304A, C304A, col='red', pch=20, cex=2.5)
dev.off();set.seed(123091)
BW.me <- 60
Disease <- 2
InhalerDose<-65#mg
t<-seq(0,720,5)
#---------------------------------------------------------------------------------
#                          Generation of numbers
#---------------------------------------------------------------------------------
#---------------------------------------------------------------------------------
BW.n <- NormGen(n, BW.me,BW.cv)
if (Disease==1){Clonren.n<-NormGen(n, Clonren.1.me,Clonren.1.cv)} else if
(Disease==2)
{Clonren.n<-NormGen(n, Clonren.2.me,Clonren.2.cv)}
if (Disease==1){Clren.n<-NormGen(n, Clren.1.me,Clren.1.cv)} else if (Disease==2)
{Clren.n<-NormGen(n, Clren.2.me,Clren.2.cv)}
if (Disease==1){k12.n<-NormGen(n, k12.1.me,k12.1.cv)} else if (Disease==2)
{k12.n<-NormGen(n, k12.2.me,k12.2.cv)}
if (Disease==1){k21.n<-NormGen(n, k21.1.me,k21.1.cv)} else if (Disease==2)
{k21.n<-NormGen(n, k21.2.me,k21.2.cv)}
if (Disease==1){V0.n<-NormGen(n, V0.1.me,V0.1.cv)} else if (Disease==2)
{V0.n<-NormGen(n, V0.2.me,V0.2.cv)}
if (Disease==1){kga.n<-LogNormGen(n, kga.1.me,kga.1.cv)} else if (Disease==2)
{kga.n<-LogNormGen(n, kga.2.me,kga.2.cv)}
if (Disease==1){Foral.n<-NormGen(n,Foral.1.me,Foral.1.cv)/100} else if (Disease==2)
{Foral.n<-NormGen(n,Foral.2.me,Foral.2.cv)/100}

kpa.n<- LogNormGen(n, kpa.me,kpa.cv);kca.n<- LogNormGen(n, kca.me,kca.cv)
kpm.n<- LogNormGen(n, kpm.me,kpm.cv);kcm.n<- LogNormGen(n, kcm.me,kcm.cv)
if (kpd.me==0){kpd.n<-rep(0,n)} else if (kpd.me>0){kpd.n<-LogNormGen(n,
kpd.me,kpd.cv)}
if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n,
kcd.me,kcd.cv)}
if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n,
kpls1.me,kpls1.cv)}
if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n,
kpls2.me,kpls2.cv)}
if (kcls1.me==0){kcls1.n<-rep(0,n)} else if (kcls1.me>0){kcls1.n<-LogNormGen(n,
kcls1.me,kcls1.cv)}
if (kcls2.me==0){kcls2.n<-rep(0,n)} else if (kcls2.me>0){kcls2.n<-LogNormGen(n,
kcls2.me,kcls2.cv)}

TLVprop.n <- NormGen(n, TLVprop.me,TLVprop.cv)
if (kin.me==0){kin.n<-rep(0,n)} else if (kin.me>0){kin.n<-NormGen(n, kin.me,kin.cv)}

Fex.n<- NormGen(n, Fex.me,Fex.cv);Fpd.n<- NormGen(n, Fpd.me,Fpd.cv);Fcd.n<-NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n<-rep(0,n)} else if (Din.me>0){Din.n<-LogNormGen(n, Din.me,Din.cv)}

#########################################################################
#                         PBPK Model iterations                        #
#########################################################################
Cltot.n<-Clren.n+Clnonren.n
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n*0.8/1000
PLV.n <- TLV.n*0.2/1000
for (i in 1:n) {
  Cltot<-Cltot.n[i];k12<-k12.n[i];k21<-k21.n[i]; V0<-V0.n[i]; kga<-kga.n[i]
  Foral<-Foral.n[i];kpa<-kpa.n[i];kca<-kca.n[i];kpm<-kpm.n[i];kcm<-kcm.n[i];kpd<-kpd.n[i]
  kcd<-kcd.n[i];kpls1<- kpls1.n[i];kpls2<-kpls2.n[i];kcls1<-kcls1.n[i];kcls2<-kcls2.n[i];CLV<-CLV.n[i]
  PLV<-PLV.n[i];kin<-kin.n[i];Fex<-Fex.n[i];Fpd<-Fpd.n[i];Fcd<-Fcd.n[i];Din<-Din.n[i]
  LDose <- InhalerDose*Dtl; GIDose <-InhalerDose*DtlG
  CID<-TCBM(t,Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1,
            kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, LDose,GIDose, tinf)
  CLS.Data[i]<-CID[6];GI.Data[i] <-CID[7];CC.Data[i] <-CID[8];PC.Data[i] <-CID[9]

  LDose/GIDose*V0/CLV
  CS.Data[i]<-CID[4]/CLV;PL.Data[i]<-CID[3]/PLV
  Cmax.302B.n[i]<-CP.Data[10,i]*1000
  CpAUC.302B.n[i]<-AUC.Trap.Calc(t, CP.Data[,i])/60*1000
  }

png(width=10,height=7,units='in',res=1200, filename="C:/Users/Laptop/Desktop/Cip304B.png")
T304B <- c(5,15,45,60,90,120,150,180,240,360,480,720,960,1440)
C304B <-
c(0.055,0.115,0.249,0.259,0.249,0.190,0.190,0.151,0.107,0.070,0.044,0.022,0.011,0.007)
VPCP(CP.Data,'green',0.005,0.5,0,720,'CIP 304B Cp(t) -- Inhaler CF, Dose = 65mg','Plasma Conc (mg/l)', 'y')
points(T304B,C304B,col='red',pch=20,cex=2.5)
dev.off()
dev.off();set.seed(123091)
BW.me <- 64
Disease <- 2
InhalerDose<-32.5#mg
t<-seq(0,720,5)

# Generation of numbers

BW.n     <- NormGen(n, BW.me,BW.cv)
if (Disease==1){Clnonren.n<-NormGen(n, Clnonren.1.me,Clnonren.1.cv)} else if (Disease==2)
{Clnonren.n<-NormGen(n, Clnonren.2.me,Clnonren.2.cv)}
if (Disease==1){Clren.n<-NormGen(n, Clren.1.me,Clren.1.cv)} else if (Disease==2)
{Clren.n<-NormGen(n, Clren.2.me,Clren.2.cv)}
if (Disease==1){k12.n<-NormGen(n, k12.1.me,k12.1.cv)} else if (Disease==2)
{k12.n<-NormGen(n, k12.2.me,k12.2.cv)}
if (Disease==1){k21.n<-NormGen(n, k21.1.me,k21.1.cv)} else if (Disease==2)
{k21.n<-NormGen(n, k21.2.me,k21.2.cv)}
if (Disease==1){V0.n<-NormGen(n, V0.1.me,V0.1.cv)} else if (Disease==2)
{V0.n<-NormGen(n, V0.2.me,V0.2.cv)}
if (Disease==1){kga.n<-LogNormGen(n, kga.1.me,kga.1.cv)} else if (Disease==2)
{kga.n<-LogNormGen(n, kga.2.me,kga.2.cv)}
if (Disease==1){Foral.n<-NormGen(n, Foral.1.me,Foral.1.cv)/100} else if (Disease==2)
{Foral.n<-NormGen(n,Foral.2.me,Foral.2.cv)/100}
kpa.n<- LogNormGen(n, kpa.me,kpa.cv);kca.n<- LogNormGen(n, kca.me,kca.cv)
kpm.n<- LogNormGen(n, kpm.me,kpm.cv);kcm.n<- LogNormGen(n, kcm.me,kcm.cv)
if (kpd.me==0){kpd.n<-rep(0,n)} else if (kpd.me>0){kpd.n<-LogNormGen(n, kpd.me,kpd.cv)}
if (kcd.me==0){kcd.n<-rep(0,n)} else if (kcd.me>0){kcd.n<-LogNormGen(n, kcd.me,kcd.cv)}
if (kpls1.me==0){kpls1.n<-rep(0,n)} else if (kpls1.me>0){kpls1.n<-LogNormGen(n, kpls1.me,kpls1.cv)}
if (kpls2.me==0){kpls2.n<-rep(0,n)} else if (kpls2.me>0){kpls2.n<-LogNormGen(n, kpls2.me,kpls2.cv)}
if (kcls1.me==0) {kcls1.n <- rep(0, n)} else if (kcls1.me>0) {kcls1.n <- LogNormGen(n, kcls1.me, kcls1.cv)}
if (kcls2.me==0) {kcls2.n <- rep(0, n)} else if (kcls2.me>0) {kcls2.n <- LogNormGen(n, kcls2.me, kcls2.cv)}
TLVprop.n <- NormGen(n, TLVprop.me, TLVprop.cv)
if (kin.me==0) {kin.n <- rep(0, n)} else if (kin.me>0) {kin.n <- NormGen(n, kin.me, kin.cv)}
Fex.n <- NormGen(n, Fex.me, Fex.cv); Fpd.n <- NormGen(n, Fpd.me, Fpd.cv); Fcd.n <- NormGen(n, Fcd.me, Fcd.cv)

####################################################################
###############        PBPK Model iterations
################################
####################################################################

Cltot.n <- Clren.n + Clnonren.n
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n*0.8/1000
PLV.n <- TLV.n*0.2/1000
for (i in 1:n) {
  Cltot[i] <- Cltot.n[i]; k12[i] <- k12.n[i]; k21[i] <- k21.n[i]; V0[i] <- V0.n[i]; kga[i] <- kga.n[i]
  Foral[i] <- Foral.n[i]; kpa[i] <- kpa.n[i]; kca[i] <- kca.n[i]; kpm[i] <- kpm.n[i]; kcm[i] <- kcm.n[i]; kpd[i] <- kpd.n[i]
  kcd[i] <- kcd.n[i]; kpls1[i] <- kpls1.n[i]; kpls2[i] <- kpls2.n[i]; kcls1[i] <- kcls1.n[i]; kcls2[i] <- kcls2.n[i]; CLV[i] <- CLV.n[i]
  PLV[i] <- PLV.n[i]; kin[i] <- kin.n[i]; Fex[i] <- Fex.n[i]; Fpd[i] <- Fpd.n[i]; Fcd[i] <- Fcd.n[i]; Din[i] <- Din.n[i]
  LDose[i] <- InhalerDose*DtL; GI.Dose[i] <- InhalerDose*DtGI
  CID[i] <- TCBM(t, Cltot[i], k12[i], k21[i], V0[i], kpa[i], kca[i], kpm[i], kcm[i], kpd[i], kcd[i], kpls1[i],
                kpls2[i], kcls1[i], kcls2[i], kin[i], Fex[i], Fpd[i], Fcd[i], Din[i], LDose[i], GI.Dose[i], tinf[i])
  CLS.Data[i] <- C[6]; Gl.Data[i] <- C[7]; CC.Data[i] <- C[8]; PC.Data[i] <- C[9]
  CS.Data[i] <- C[3]/CLV; PL.Data[i] <- C[3]/PLV
  Cmax.302A[i] <- CP.Data[i][13, i]*1000
  CpAUC.302A[i] <- AUC.Trap.Calc(t, CP.Data[i])/60*1000
}

png(width=10, height=7, units='in', res=1200, filename="C:/Users/Laptop/Desktop/Cip304C.png")
T304C <- c(5, 15, 45, 60, 90, 120, 180, 240, 360, 480, 720)
C304C <- c(0.029, 0.032, 0.107, 0.124, 0.120, 0.115, 0.099, 0.070, 0.042, 0.028, 0.012)
VPCP(CP.Data, 'green', 0.005, 0.5, 0.720, 'CIP 304C Cp(t) -- CF, Inhaler Dose = 32.5mg', 'Plasma Conc (mg/l)', 'y')
points(T304C, C304C, col='red', pch=20, cex=2.5)
dev.off()
mean(CpAUC.301A.n)
mean(CpAUC.302A.n)
mean(CpAUC.302B.n)

sd(CpAUC.301A.n)
sd(CpAUC.302A.n)
sd(CpAUC.302B.n)

mean(Cmax.301A.n)
mean(Cmax.302A.n)
mean(Cmax.302B.n)

sd(Cmax.301A.n)
sd(Cmax.302A.n)
sd(Cmax.302B.n)

((mean(CpAUC.301A.n)-482)*100/482) +
((mean(CpAUC.302A.n)-520)*100/520) +
((mean(CpAUC.302B.n)-1237)*100/1237))/4

sd(c(((mean(CpAUC.301A.n)-482)*100/482),
((mean(CpAUC.302A.n)-520)*100/520),
((mean(CpAUC.302B.n)-1237)*100/1237)))

(((mean(Cmax.301A.n)-79)*100/79) +
((mean(Cmax.302A.n)-128)*100/128) +
((mean(Cmax.302B.n)-289)*100/289))/4
\[ \text{sd}(c((\text{mean}(C_{\text{max}.301A} \cdot n) - 79) \times 100/79),
((\text{mean}(C_{\text{max}.302A} \cdot n) - 127) \times 100/127),
((\text{mean}(C_{\text{max}.302B} \cdot n) - 289) \times 100/289))) \]
Code Utilized for Sensitivity Analysis After INH

```r
rm(list=ls()); cat("014"); dev.off(); set.seed(123091)

BW.me <- 70
BW.cv <- 0
n <- 3
Disease <- 1 # 1= Healthy 2= CF

InhalerDose <- 32.5 # mg
DtL <- 0.53
DtGI <- 0.43
t <- seq(0,720,5)

Clren.1.me <- 315 # Healthy
Clren.2.me <- 425 # CF
Clnonren.1.me <- 315 # Healthy
Clnonren.2.me <- 225 # CF
k12.1.me <- 0.025 # Healthy
k12.2.me <- 0.050 # CF
k21.1.me <- 0.015 # Healthy
k21.2.me <- 0.020 # CF
V0.1.me <- 75 # Healthy
V0.2.me <- 20 # CF

Clnonren.1.cv <- 0 # Healthy
Clnonren.2.cv <- 0 # CF
Clren.1.cv <- 0 # Healthy
Clren.2.cv <- 0 # CF
k12.1.cv <- 0 # Healthy
k12.2.cv <- 0 # CF
k21.1.cv <- 0 # Healthy
k21.2.cv <- 0 # CF
V0.1.cv <- 0 # Healthy
V0.2.cv <- 0 # CF

Foral.1.me <- 73
Foral.2.me <- 85
kga.1.me <- 0.020
kga.2.me <- 0.011
Foral.1.cv <- 0; Foral.2.cv <- 0; kga.1.cv <- 0; kga.2.cv <- 0

kpa.me <- 0.010
kca.me <- 0.020
kpm.me <- 0.020
```

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kcm.me <- 0.02
kpd.me <- 0.008
kcd.me <- 0.0002
kpls1.me <- 10000
kpls2.me <- 10000
kcls1.me <- 10000
kcls2.me <- 10000
TLV.me <- 30
kpa.cv <- 0;kca.cv <- 0;kcm.cv <- 0;kpd.cv <- 0;kcd.cv <- 0
kpls1.cv <- 0;kpls2.cv <- 0;kcls1.cv <- 0;kcls2.cv <- 0
TLVprop.me <- -1
TLVprop.cv <- -0
kin.me <- 0
Fex.me <- 0
Fpd.me <- 0.25
Fcd.me <- 0.75
Din.me <- 0
kin.cv <- 0; Fex.cv <- 0; Fpd.cv <- 0; Fcd.cv <- 0; Din.cv <- 0

### Normal Distribution Generator

```r
NormGen <- function(simulation.n, samp.m, samp.cv) {
  samp.sd <- samp.cv * samp.m / 100;
  out <- rnorm(simulation.n, samp.m, samp.sd);
  return(out)
}
```

```r
LogNormGen <- function(n, Mean, PCV) {
  CV <- PCV / 100; SD <- (CV^2); Variance <- SD^2;
  mean.in <- log(Mean^2 / sqrt(Variance + Mean^2));
  SD.in <- sqrt(log(Variance / Mean^2 + 1));
  x <- rlnorm(n, mean.in, SD.in);
  return(x)
}
```

```r
AUC.Trap.Calc <- function(Time, CP) {
  n <- length(Time);
  AUC.Values <- rep(0, n - 1);
  for (i in 1:(n - 1)) {
    if (i == 1) { AUC.Values[i] <- (Time[i + 1] - Time[i]) * (CP[i] + CP[i + 1]) / 2 }
    if (i > 1) { AUC.Values[i] <- (Time[i + 1] - Time[i]) * (CP[i] + CP[i + 1]) / 2 + AUC.Values[i - 1] }
  }
  return(AUC.Values[i])
}
```

### Generation of numbers

```r
BW.n <- NormGen(n, BW.me, BW.cv)
```
if (Disease==1){Clnonren.n<-NormGen(n, Clnonren.1.me,Clnonren.1.cv)} else if (Disease==2) {Clnonren.n<-NormGen(n, Clnonren.2.me,Clnonren.2.cv)}
if (Disease==1){Clren.n<-NormGen(n, Clren.1.me,Clren.1.cv)} else if (Disease==2) {Clren.n<-NormGen(n, Clren.2.me,Clren.2.cv)}
if (Disease==1){k12.n<-NormGen(n, k12.1.me,k12.1.cv)} else if (Disease==2) {k12.n<-NormGen(n, k12.2.me,k12.2.cv)}
if (Disease==1){k21.n<-NormGen(n, k21.1.me,k21.1.cv)} else if (Disease==2) {k21.n<-NormGen(n, k21.2.me,k21.2.cv)}
if (Disease==1){V0.n<-NormGen(n, V0.1.me,V0.1.cv)} else if (Disease==2) {V0.n<-NormGen(n, V0.2.me,V0.2.cv)}
if (Disease==1){kga.n<LogNormGen(n, kga.1.me,kga.1.cv)} else if (Disease==2) {kga.n<LogNormGen(n, kga.2.me,kga.2.cv)}
if (Disease==1){Foral.n<NormGen(n,Foral.1.me,Foral.1.cv)/100} else if (Disease==2) {Foral.n<NormGen(n,Foral.2.me,Foral.2.cv)/100} kpa.n< LogNormGen(n, kpa.me,kpa.cv);kca.n< LogNormGen(n, kca.me,kca.cv)
if (kpd.me==0){kpd.n<rep(0,n)} else if (kpd.me>0){kpd.n<LogNormGen(n, kpd.me,kpd.cv)}
if (kin.me==0){kin.n<rep(0,n)} else if (kin.me>0){kin.n<NormGen(n, kin.me,kin.cv)}
if (kcd.me==0){kcd.n<rep(0,n)} else if (kcd.me>0){kcd.n<LogNormGen(n, kcd.me,kcd.cv)}
if (kls1.me==0){kls1.n<rep(0,n)} else if (kls1.me>0){kls1.n<LogNormGen(n, kls1.me,kls1.cv)}
if (kls2.me==0){kls2.n<rep(0,n)} else if (kls2.me>0){kls2.n<LogNormGen(n, kls2.me,kls2.cv)}
if (kcs1.me==0){kcs1.n<rep(0,n)} else if (kcs1.me>0){kcs1.n<LogNormGen(n, kcs1.me,kcs1.cv)}
if (kcs2.me==0){kcs2.n<rep(0,n)} else if (kcs2.me>0){kcs2.n<LogNormGen(n, kcs2.me,kcs2.cv)}
TLVprop.n <- NormGen(n, TLVprop.me,TLVprop.cv)
if (kin.me==0){kin.n<rep(0,n)} else if (kin.me>0){kin.n<NormGen(n, kin.me,kin.cv)}
Fex.n< NormGen(n, Fex.me,Fex.cv);Fpd.n<- NormGen(n, Fpd.me,Fpd.cv);Fcd.n< NormGen(n, Fcd.me,Fcd.cv)
if (Din.me==0){Din.n<rep(0,n)} else if (Din.me>0){Din.n<LogNormGen(n, Din.me,Din.cv)}

Clren.n <- c(Clren.1.me/5,Clren.1.me,5*Clren.1.me)
#Clnonren.n <- c(Clnonren.1.me/5,Clnonren.1.me,5*Clnonren.1.me)
#k12.n <- c(k12.1.me/5,k12.1.me,5*k12.1.me)
#k21.n <- c(k21.1.me/5,k21.1.me,5*k21.1.me)
#V0.n <- c(V0.1.me/5,V0.1.me,5*V0.1.me)
#kga.n <- c(kga.1.me/5,kga.1.me,5*kga.1.me)
#Foral.n <- c(Foral.1.me/5,Foral.1.me,5*Foral.1.me)
#kpa.n <- c(kpa.me/10,kpa.me,10*kpa.me)
#kca.n <- c(kca.me/10,kca.me,10*kca.me)
#kpm.n <- c(kpm.me/10,kpm.me,10*kpm.me)
#kcm.n <- c(kpm.me/10,kpm.me,10*kpm.me)
#kpd.n <- c(kpd.me/10,kpd.me,10*kpd.me)
#kcd.n <- c(kcd.me/10,kcd.me,10*kcd.me)

#Clren.n <- c(Clren.2.me/5,Clren.2.me,5*Clren.2.me)
#Clnonren.n <- c(Clnonren.2.me/5,Clnonren.2.me,5*Clnonren.2.me)
#k12.n <- c(k12.2.me/5,k12.2.me,5*k12.2.me)
#k21.n <- c(k21.2.me/5,k21.2.me,5*k21.2.me)
#V0.n <- c(V0.2.me/5,V0.2.me,5*V0.2.me)
#kga.n <- c(kga.2.me/5,kga.2.me,5*kga.2.me)
#Foral.n <- c(Foral.2.me/5,Foral.2.me,5*Foral.2.me)
#kpa.n <- c(kpa.me/10,kpa.me,10*kpa.me)
#kca.n <- c(kca.me/10,kca.me,10*kca.me)
#kpm.n <- c(kpm.me/10,kpm.me,10*kpm.me)
#kcm.n <- c(kpm.me/10,kpm.me,10*kpm.me)
#kpd.n <- c(kpd.me/10,kpd.me,10*kpd.me)
#kcd.n <- c(kcd.me/10,kcd.me,10*kcd.me)

Hallo
 Parm <- c(Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1, kpls2, kcls1,
            kcls2, kin, Fex, Fpd, Fcd, Fgd)
 out <- ode(IC, t, Model.Function, Parm)
 return(out)

PBPK Model iterations

Cltot.n <- Clren.n + Clnonren.n
TLV.n <- TLV.me*(BW.n/mean(BW.n))^0.8*TLVprop.n
CLV.n <- TLV.n*0.8/1000
PLV.n <- TLV.n*0.2/1000

In.Data <- matrix(0, length(t), (n)); PLU.Data <- In.Data; Cmax.n <- rep(0, n); Smax.n <- Cmax.n
Foral <- Foral.n[i]; kga <- kga.n[i]

for (i in 1:n) {
  Cltot <- Cltot.n[i]; k12 <- k12.n[i]; k21 <- k21.n[i]; V0 <- V0.n[i]; kga <- kga.n[i]
  Foral <- Foral.n[i]; kpa <- kpa.n[i]; kca <- kca.n[i]; kpm <- kpm.n[i]; kcm <- kcm.n[i]; kpd <- kpd.n[i]
  kcd <- kcd.n[i]; kpls1 <- kpls1.n[i]; kpls2 <- kpls2.n[i]; kcls1 <- kcls1.n[i]; kcls2 <-
  kcls2.n[i]; CLV <- CLV.n[i]
  PLV <- PLV.n[i]; kin <- kin.n[i]; Fex <- Fex.n[i]; Fpd <- Fpd.n[i]; Fcd <- Fcd.n[i]; Din <- Din.n[i]
  LDose <- InhalerDose*DtL; GIDose <- InhalerDose*DGl
  CID <- TCBM(t, Cltot, k12, k21, V0, kpa, kca, kpm, kcm, kpd, kcd, kpls1,
              kpls2, kcls1, kcls2, kin, Fex, Fpd, Fcd, Din, LDose, GIDose, tinf)
  In.Data[i,] <- CID[2]; PLU.Data[i,] <- CID[3]; CLU.Data[i,] <- CID[4]; PLS.Data[i,] <- CID[5]
  CLS.Data[i,] <- CID[6]; Gl.Data[i,] <- CID[7]; CC.Data[i,] <- CID[8]; PC.Data[i,] <- CID[9]
  Ex.Data[i,] <- CID[10]; Fe.Data[i,] <- CID[11]; El.Data[i,] <- CID[12]; CP.Data[i,] <-
  CID[8]/V0
  CS.Data[i,] <- CID[4]/CLV; PL.Data[i,] <- CID[3]/PLV; Cmax.n[i,] <-
  max(CP.Data[i,]); Smax.n[i,] <- max(CS.Data[i,])
}

VPC Plotting Function for TS DATA

VPCP <- function(Data, Color, YMin, YMax, XMin, XMax, Title, Ytitle, LogAxis){
  Size.of.Data <- dim(Data); PlottingData <- matrix(0, Size.of.Data[1], 4);
  PlottingData[1,] <- CID[1];
  for (i in 1:length(t)) {
    PlottingData[i, 1] <- min(Data[i, 1]);
    PlottingData[i, 2] <- mean(Data[i, 1]);
    PlottingData[i, 3] <- max(Data[i, 1]);
    PlottingData[i, 4] <- Data[i, 1];
  }
}
Time<-PlottingData[,1]; Bottom<-PlottingData[,2]; Top<-PlottingData[,4]; Middle<-PlottingData[,3]
ColNumbers<-col2rgb(Color); par(TRUE); mycol4<-rgb(ColNumbers[1],ColNumbers[2],ColNumbers[3],max=255,alpha=90)

plot(Time,Bottom,type="n", col=Color, main=Title, ylim=c(YMin,YMax), log=LogAxis, xlim=c(XMin,XMax),

  ylab =Ytitle, xlab = 'Time (minutes)', font=2,font.lab =2)
polygon(c(Time, rev(Time)), c(Top, rev(Bottom)), col=mycol4,border=NA)
lines(Time, Middle, type= "l", col=Color, lwd=2.8);  return()

png(width=12,height=7,units='in',res=1200,filename="C:/Users/Laptop/Desktop/HV Clrenal.png");par(mfrow=c(1,2))
VPCP(CP.Data, 'green',0.001,10,0,720,"","Plasma Conc (mg/l)", 'y')
VPCP(CS.Data, 'cyan',0.1,1000,0,720,"","Sputum Conc (mg/l)", 'y')
mtext("Sensitivity of cp(t) (left plot) and cs(t) (right plot) to Clrenal after INH Dosing in HV", cex=1.2, side = 3, line = -2, outer=T, font=2); dev.off()

AUCLowerParmValue<-AUC.Trap.Calc(t,CP.Data[,1]); AUCHigherParmValue<-AUC.Trap.Calc(t,CP.Data[,3])
AUCS <-c(AUCLowerParmValue, AUCHigherParmValue); AUCMultiplier=1
if (AUCHigherParmValue<AUCLowerParmValue){AUCMultiplier=-1}
Cmaxlower <- Cmax.n[1]; Cmaxhigher<- Cmax.n[3]; Cmaxmultiplier<-1
if (Cmaxhigher<Cmaxlower){Cmaxmultiplier=-1}
SAUCLowerParmValue<-AUC.Trap.Calc(t,CS.Data[,1]); SAUCHigherParmValue<-AUC.Trap.Calc(t,CS.Data[,3])
SAUCS <-c(SAUCLowerParmValue,SAUCHigherParmValue); SAUCMultiplier=1
if (SAUCHigherParmValue<SAUCLowerParmValue){SAUCMultiplier=-1}
Smaxlower <- Smax.n[1]; Smaxhigher<- Smax.n[3]; Smaxmultiplier<-1
if (Smaxhigher<Smaxlower){Smaxmultiplier=-1}
Clrenal.1.AUC<-AUCMultiplier*max(AUCS)/min(AUCS)
Clrenal.1.cmax<-Cmaxmultiplier*max(Cmax.n)/min(Cmax.n)
Clrenal.1.SAUC<-SAUCMultiplier*max(SAUCS)/min(SAUCS)
Clrenal.1.smax <- Smaxmultiplier*max(Smax.n)/min(Smax.n)
VITA

Bishoy Hanna was born on September 29, 1990 in Cairo, Egypt. He came to the US when he was 8 years old and became a US citizen. He graduated from New Jersey Institute of Technology, Newark College of Engineering and Albert Dorman Honors College with a bachelor’s degree in Biomedical Engineering in May 2012. He graduated from New Jersey Institute of Technology with a master’s degree in Biomedical Engineering in December 2012. In 2013, he joined the master’s program at Department of Pharmaceutics, Virginia Commonwealth University (VCU), Richmond, VA, under supervision of Dr. Jürgen Venitz, M.D., Ph.D.. In 2014, Bishoy joined the Ph.D. program at Department of Pharmaceutics, Virginia Commonwealth University (VCU), Richmond, VA, under supervision of Dr. Jürgen Venitz, M.D., Ph.D..

Prior to pursing his Ph.D., Bishoy was an organics lab technician in June 2010 at Accutest Laboratories performing organic extractions. In May 2011, he was an imaging intern at the University of Medicine and Density of New Jersey developing Matlab Program to filter Function MRI data for imaging. In May 2012, Bishoy was a Clinical Biostatical R&D Intern at Johnson & Johnson, Janssen Pharmaceuticals analyzing protocol to understand the timeline of the study, procedure & the implications of the acquired data, establishing a clinical model for the interaction of Warfarin in patients based on various Phase III studies, and determining possible research routes as to how to safely transition patients to/from Warfarin to Xarelto.

While pursuing his Ph.D. Bishoy presented his research extramurally at American Society of Clinical Pharmacology and Therapeutics (ASCPT 2016, 2018) and American Association of Pharmaceutical Scientists (AAPS 2017), in addition to intramural presentations both within the department and School of Pharmacy. During the fall 2016 he successfully completed an internship in the Department of Quantitative Clinical Pharmacology at AstraZenca performing tumor modeling.

Bishoy received the Joseph P. Schwartz Graduate Student Travel award and Second Place Best Research Poster. He served as the American Association of Pharmaceutical Scientists Student Chapter Chair since August 2014. He has served on the Tompkins McCaw Graduate Advisory Committee since August 2014. He is the Graduate Pharmaceutics Student Association Treasurer/Secretary since August 2014. He also has been a Graduate teaching assistant for PCEU 615 for Pharm. D. students and PCEU 624 for graduate students in the School of Pharmacy teaching PK modeling.

He has accepted a Scientist position in Celgene in Summit, NJ, and will be working on Oncology drug development projects.