Physiologically Based Pharmacometric Models for Colistin and the Immune Response to Bacterial Infection

SALIM BOUCHENE
Antibiotic treatment failure might be due to bacterial resistance or suboptimal exposure at target site and there is a lack of knowledge on the interaction between antimicrobial pharmacodynamics (PD) and the immune response to bacterial infections. Therefore, it is crucial to develop tools to increase the understanding of drug disposition to better evaluate antibiotic candidates in drug development and to elucidate the role of the immune system in bacterial infections.

Colistin is used as salvage therapy against multidrug resistant Gram-negative infections. In this work, a whole-body physiologically based pharmacokinetic model (WBPBPK) was developed to characterize the pharmacokinetics (PK) of colistin and its prodrug colistin methanesulfonate (CMS) in animal and human. The scalability of the model from animal to human was assessed with satisfactory predictive performance for CMS and demonstrating the need for a mechanistic understanding of colistin elimination.

The WBPBPK model was applied to investigate the impact of pathophysiological changes commonly observed in critically ill patients on tissue distribution of colistin and to evaluate different dosing strategies.

Model predicted concentrations in tissue were used in combination with a semi-mechanistic PKPD model to predict bacterial killing in tissue for two strains of *Pseudomonas aeruginosa*.

Finally, a toxicokinetic (TK) model was constructed to describe the time course of *E. coli* endotoxin concentrations in plasma and the effect on pro-inflammatory cytokine release. The model adequately described the concentration-time profiles of endotoxin and its stimulation of IL-6 and TNF-α production using an indirect response model combined with a transit compartment chain with a tolerance component to endotoxemia.

The WBPBPK model developed in this work increased the knowledge on colistin tissue exposure under various conditions and could be used in drug development process to assess antibiotic efficacy or to test new drug combinations. The model describing endotoxin TK and its effect on cytokines is a new tool to be further applied in longitudinal studies to explore the immune response cascade induced by bacterial infections. The methodology applied in this thesis contributes to the development of an integrated modeling framework including physiology, drug distribution, bacterial growth and killing as well as the immune response to infection.

**Keywords:** PBPK model, endotoxin, colistin, WBPBPK-PD, CMS, inflammation, tissue distribution, Kp, predictions in tissue, interspecies scaling

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To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


III. **Bouchene S**, Friberg LE, Björkman S, Couet W, Karlsson MO (2015) Application of a Whole-Body Physiologically Based Pharmacokinetic Model to Describe the Plasma and Urine Disposition of Colistin and Colistin methanesulfonate in Healthy Volunteers. *In manuscript*

IV. **Bouchene S**, Friberg LE, Plachouras D, Björkman S, Karlsson MO (2016) A Whole-Body Physiologically Based Pharmacokinetic-Pharmacodynamic Model for Colistin in Critically Ill Patients. *In manuscript*

V. Thorsted A, **Bouchene S**, Tano E, Castegren M, Lipcsey M, Sjölin J, Karlsson MO, Friberg LE, Nielsen EI (2016) Toxicokinetics of Endotoxin and its Relation to Pro-Inflammatory Cytokines Tumor Necrosis Factor-α and Interleukin 6 in a Porcine Sepsis Model. *In manuscript*

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Abbreviations

CBA  Colistin Base Activity
C   Concentration
CDK  Chronic Kidney Disease
CFU  Colony forming unit
CL   Clearance
CMS  Colistin methanesulfonate
CrCl Creatinine Clearance
CSF  Cerebrospinal fluid
DDI  Drug-drug interactions
dOFV Delta OFV
EC50 Concentration that gives 50% on E\textsubscript{max}
ELF  Epithelial Lining Fluid
E\textsubscript{max} Maximum effect
EUCAST European Committee on Antimicrobial Susceptibility Testing
f\textsubscript{bl} fraction of residual blood in tissue homogenates
FOCE First Order Conditional Estimation
f\textsubscript{up} Unbound fraction in plasma
f\textsubscript{ut} Unbound fraction in tissue
GFR  Glomerular filtration rate
GIT  Gastrointestinal tract
h   Hours
Hb   Hemoglobin
HCT  Hematocrit
HPLC-MS/MS High-performance liquid chromatography coupled with tandem mass spectrometry
ICU  Intensive care unit
IIV  Interindividual variability
IL-6 Interleukin-6
i.m. Intramuscular
i.v. Intravenous
IVIVE  
*in vitro-in vivo* extrapolations

k  
Rate constant

$k_a$  
Rate of absorption

LC-MS/MS  
Liquid chromatography coupled with tandem mass spectrometry

LL  
Log-likelihood

LOQ  
Limit of quantification

MIC  
Minimum inhibitory concentration

MIU  
Million International Units

min  
Minutes

MU  
Million Units

NLME  
Nonlinear mixed effects

OFV  
Objective function value

PAMP  
Pathogen associated molecular patterns

PBPK  
Physiologically based pharmacokinetic

PBS  
Phosphate buffer saline

pcVPC  
Prediction corrected Visual Predictive Checks

PD  
Pharmacodynamics

PK  
Pharmacokinetics

Q  
Blood flow

R  
Resting state bacteria

SIR  
Sampling Importance Resampling

RUV  
Residual Unexplained Variability

S  
Susceptible (bacteria)

s.c.  
Subcutaneous

SD  
Standard deviation

Se  
Standard error

TNF-α  
Tumor Necrosis Factor α

UT  
Urinary tract

V  
Volume

$V_d$  
Volume of distribution

VPC  
Visual Predictive Checks

$V_{ss}$  
Volume of distribution at steady state

WBPBPK  
Whole-body Physiologically Based Pharmacokinetic

WT  
Total body weight

γ  
Gamma, Hill coefficient of the drug effect

θ  
Theta, population fixed effect

η  
Eta, individual random effect
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>κ</td>
<td>Kappa, occasion random effect</td>
</tr>
<tr>
<td>ε</td>
<td>Epsilon, residual</td>
</tr>
<tr>
<td>Ω</td>
<td>Omega, interindividual covariance matrix</td>
</tr>
<tr>
<td>Π</td>
<td>Pi, interoccasion covariance</td>
</tr>
<tr>
<td>Σ</td>
<td>Sigma, residual error covariance matrix</td>
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Introduction

The identification of penicillin by Alexander Fleming in 1928 has led to one of the most significant progress observed throughout the history of medicine. Beside their use to treat patients with bacterial infections reducing the associated morbidity and mortality, antibiotics contributed to major advances in organ transplantation, open surgery and in immunocompromised patients.

However, exposure to antibiotics is the driving force for bacterial resistance development. The intensive use of antibacterial drugs worldwide has triggered the development of antibiotic resistance which has become one of the greatest threats for healthcare systems (1, 2). Moreover, the decline in research and development of new antibiotics has worsened the situation with only two new antibiotic classes approved since 2000 (1, 3, 4). In view of the limited available therapeutic options, there is an urgent need to understand antibiotic distribution at the different target sites as well as the role of the immune system in bacterial killing in order to develop effective dosing strategies. Optimized dosing would increase therapeutic success in patients with severe infections and reduce the emergence of bacterial resistance. Furthermore, efficient and easy-to-use tools such as modeling and simulation would facilitate the identification of new antibiotic candidates by leveraging information across discovery and development phases (5, 6).

Colistin

Colistin is an antibiotic used in clinical practice that belongs to the polymyxin class (7). Colistin, or polymyxin E, is a cyclic decapeptide with a fatty acid tail. Five amino acid groups (Figure 1) present free amine moieties, which are cationic at physiological pH (7.4). More than 30 species of colistin have been identified even though the marketed drug is mainly composed of two species: colistin A and B or polymyxins E₁ and E₂, which account for almost 100% of the powder content. Colistin A and B differ from each other by their N-terminal fatty acyl group (8). Because of its chemical structure, colistin is amphipathic with a hydrophilic peptide structure and a hydrophobic fatty acid tail (Figure 1).

Colistin is mostly used as a prodrug, colistin methanesulfonate (CMS or colistimethate), and administered intravenously (i.v.) (9-11) or via nebuliza-
tion (12). CMS is an anionic molecule synthetized by adding a methanesulfonate groups (–CH₂SO₃⁻) to the free amines of colistin. CMS is less toxic than colistin but it is antimicrobiologically inactive (13). CMS is spontaneously hydrolyzed in aqueous and biological media by removal of the methanesulfonate groups from the amine groups to which they are linked. The resulting mixture is formed of various partially sulfomethylated derivatives of CMS, as well as of colistin (13).

Figure 1. Structures of colistin A and B (upper panel) and colistin methanesulfonate A and B (lower panel). Abbreviations: fatty acid: 6-methyloctanoic acid for colistin A and 6-methylheptanoic acid for colistin B; Thr, threonine; Dab, α,γ-diaminobutyric acid. α and γ indicate the respective -NH₂ involved in the peptide linkage (reprinted with permission from J. Antimicrob. Chemother. 2013 Oct 68(10) 2311-7).

In the 1970s, colistin was abandoned due to nephrotoxicity and neurotoxicity as well as to the availability of safer antibiotics (14, 15). Since the 2000s, colistin has reappeared in clinical practice because of the resurgence of multidrug resistant (MDR) Gram-negative bacterial infections, becoming the last resort therapeutic option for critically ill patients (16). As an old drug, colistin has not been subjected to the current drug development standards resulting in a lack of understanding of its pharmacokinetics (PK) and pharmacodynamics (PD). During the last decade, many studies have been performed to develop new analytical methods (17-20) leading to a better understanding of colistin PK (9, 10, 21) and PD (11, 22, 23).

The PK of CMS is well understood as the prodrug is mainly eliminated through renal excretion and spontaneous aqueous hydrolysis forming colistin
However, the determination of CMS disposition remains challenging because of the impossibility to quantify CMS itself or to determine its unbound fraction in plasma ($f_{up}$). The available analytical methods are only capable of measuring CMS concentrations in addition to the methanesulfonate intermediates at different stages of hydrolysis (17-20). Moreover, CMS is chemically too unstable to enable the quantification of its $f_{up}$. A study based on the structure–activity relationships (SAR) driving the binding of polymyxins to α-1-acid glycoprotein was not able to measure CMS binding (25). The disposition of colistin is not fully understood as its elimination mechanism remains unknown. Colistin that is formed in urine from renally excreted CMS undergoes extensive tubular reabsorption into the proximal tubular cells of the kidneys (24, 26) (Figure 2). As many antibiotics, CMS and colistin distribute into plasma and the interstitial fluid (24).

![Figure 2](image_url)  
**Figure 2.** Schematic representation of the disposition of colistin methanesulfonate (CMS) and formed colistin in the body following intravenous (i.v.) administration of CMS, adapted from Couet et al (24).

Colistin acts as a two-stage detergent-like antibiotic first targeting the lipopolysaccharide (LPS or endotoxins) layer on the bacteria outer membrane to impair membrane integrity and favor its penetration into the periplasmic space (27). After disrupting the plasma membrane, colistin increases the permeability of polar charged molecules leading to a failure in cell respiration and disruption of membrane integrity, which both ultimately lead to cell death and lysis (27). Gram-negative bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumanii* are susceptible to colistin. Even though the frequency of resistant bacterial strains to colistin has been low, the emergence of resistance has been observed because of the extensive use of colistin since the 1990s (28).

Most manufacturers express colistin doses in units related to the drug potency: in Europe, vial contents are expressed in International Units (IU or U), whereas in North America and Australia, they are labelled in Colistin Base Activity (CBA). The dosage recommendations from European manufacturers are 1-2 million IU (MIU or MU) q8h for patients weighting more than 60 kg, and 50000 IU/kg as daily dose, divided in three equal doses (q8h), for patients weighting less than 60 kg. After several clinical PK studies, such as in critically ill patients in Greece, new dosing strategies have
been suggested with an elevation of the dose to 3 MU q8h after a loading dose of 9 MU (9, 11).

**Bacteria**

*Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative bacterium with a high capacity to develop resistance against various classes of antibiotics (29). The mechanisms of resistance are either transient or adaptive. The resistance to colistin has been modeled as an adaptive mechanism (11, 22). *P. aeruginosa* is often involved in nosocomial infections within intensive care units (ICU).

*Escherichia coli*

*Escherichia coli* (*E. coli*) bacteria belong to the Enterobacteriaceae group which is part of the human commensal flora. *E. coli* are widely used in microbiology experiments because it is easy to cultivate and to handle with minimum harm. *E. coli* can become virulent and infect the gastrointestinal and urinary tracts.

**Innate immune response to bacterial infections**

The immune system constantly scans the body to detect infections. Bacteria can penetrate into the body through the skin or the mucosal epithelium lining the gastrointestinal, respiratory or urinary tracts.

Mechanistically, the innate immune response is strongly activated by endotoxins, which are located in the outer membrane of Gram-negative bacteria (30). When endotoxins reach the bloodstream, it is recognized through pathogen associated molecular patterns (PAMP) which activate the immune system of the infected host. The immune response is a complex network linking various causes and effects which are not fully elucidated. The interaction between the endotoxins and PAMPs activates the macrophages and neutrophils in tissues, which trigger the release of endogenous mediators such as pro-inflammatory cytokines inducing inflammation (31, 32). For example, interleukin-6 (IL-6) and the Tumor Necrosis Factor α (TNF-α) are released during the early phase the innate immune response. They stimulate inflammation and are implicated in many other processes such as the release of acute phase proteins or the activation of lymphocytes. TNF-α and IL-6 are also involved in the recruitment of the neutrophils to the site of infection (33).
Porcine models have been developed to study the immune response to the administration of *E.coli* endotoxins (34-36). The endotoxin effect is an important component of the immune response to bacterial infections with clinical consequences. It may cause severe conditions such as septic shock or sepsis (37, 38).

**Physiologically-Based Pharmacokinetic (PBPK) modeling**

Various PK models may be used to characterize the disposition of a molecule in human or in animal. Two main classes of compartmental PK models are often used: empirical (classical or mammillary) models and physiologically based models.

**Empirical models**

Empirical models (Figure 3) represent the body by relatively few compartments. Each compartment is designed as a space without any explicit physiological meaning where a homogenous distribution of the drug is assumed. A set of differential or analytical equations are used to describe mass transfer of the molecule across the compartments. The parameters derived from these models are the clearance(s) (CL) and volume(s) of distribution (V_d) which can be interpreted in terms of plasma protein and tissue binding or distribution into the extracellular space or deep tissues (e.g. adipose). The structures of the empirical PK models and the parameter estimates are data driven (top-down modeling strategy). These models are useful to adequately describe the concentration-time profile in plasma of a wide range of compounds. However they lack direct physiological meaning and are not suited for predicting explicitly tissue exposure of a drug.
Physiologically based pharmacokinetic models

Structure
Physiologically based pharmacokinetic (PBPK) models describe the body as a series of anatomical or physiological compartments representing specific organs or tissues. These models can be whole-body physiologically based pharmacokinetic (WBPBPK) models (39) representing the major tissues of interest in a body or minimal (or lumped) PBPK models (40, 41). In minimal PBPK models, only target organs or tissues are explicitly depicted whereas the remaining ones are lumped (tissues) or grouped (organs). The mass transfer of the studied compound is described by a set of differential equations (42, 43). Figure 4 displays the generic structure of a WBPBPK model.

The distribution of the drug into tissue compartments may be blood perfusion-limited or diffusion-limited. The distribution of drugs in PBPK models is often assumed to be perfusion-limited meaning that the equilibration between blood and tissue concentrations is instantaneous (39, 43). In more complex models, equilibration between blood and tissue concentrations can be slow where a diffusion-limited distribution applies more adequately. The choice of the PBPK model structure is chosen based on the intended use of it, the biological and physicochemical characteristics of the compound, and the target site.

System-specific parameters
Each compartment of a perfusion-limited PBPK model is defined by its physiological volume ($V_T$) and its regional blood flow ($Q_T$), specific to the species of interest. These physiological parameter values are usually collected from literature (43-45). Biological parameters such as the glomerular flow
rate (GFR), often approximated by the creatinine clearance (CrCL), or body weight (WT) can be used a priori in the model to scale the physiological parameters. The values of the biological parameters are either from literature (43-45) or from the data (e.g. CrCL or WT). Moreover, some databases exist to support PBPK modeling for elderly impaired patients (46), pediatrics (47), pregnancy (48), obesity (49), and environmental factors such as smoking (42).

**Drug-specific parameters**

The drug dependent physicochemical parameters such as the tissue-to-plasma partition coefficients (K<sub>p</sub>), hydrolysis or metabolic rate constants and drug transport affinity constants are usually included in PBPK models to either fit the concentration time course of a drug or to predict its exposure in plasma or tissue. The K<sub>p</sub> characterizes the degree of partitioning of a molecule to a specific tissue, defined as the ratio of the concentration in the given tissue over the plasma concentration at steady state.

![PBPK model diagram](image)

**Figure 4.** Example of a whole-body physiologically based pharmacokinetic model used in Papers I-IV. The compartments represent the main organs of a body, Q the regional blood flows of the tissues, K<sub>p</sub> the tissue-to-plasma partition coefficients, CL<sub>r</sub> the renal clearance, CL<sub>reab</sub> the reabsorption clearance and UFR the urinary flow rate. Carcass compartment represents tissues that were not included separately in the model structure.

**Utility of PBPK modeling**

During the last decade, PBPK models have gained in popularity with increasing application in drug development and regulatory science (50-53).
PBPK models have been used to facilitate in vitro-in vivo extrapolations (IVIVE), interspecies scaling and in drug-drug interaction (DDI) studies. PBPK models may also be used to predict the PK of drugs in specific subpopulations (e.g. pediatric patients). Originally, PBPK models have been used in simulation mode using animal and/or in vitro data to predict the human plasma PK in vivo. The adjustment of some parameters (e.g. clearance or \( K_p \)) was done a posteriori when observed data in vivo were available in order to calibrate the model and reduce the uncertainty associated with the predictions (54, 55).

During the last decade, several studies (56, 57) have demonstrated the possibility to combine the traditional PBPK modeling approach with parameter estimation techniques. The PBPK model is used to fit plasma in vivo data together with parameter estimation using in vitro, in silico data. In addition, when applying a population approach, PBPK models can be used to estimate inter and intraindividual variability inherent to PK (58, 59).

Semi-mechanistic PKPD model for colistin

The PKPD models are developed to summarize the relationship between the drug, dose, plasma concentration, drug effect and side effects. Knowledge on bacterial growth and bacterial killing can be obtained from various in vitro experiments (e.g. time-kill experiments). Bacterial growth and killing are usually studied for a range of static or dynamic drug concentrations. Semi-mechanistic PKPD models integrate prior mechanistic understanding about the system (i.e. bacteria and antibiotic) coupled to experimental data in order to strengthen model extrapolations (60). Nielsen et al have developed a semi-mechanistic model describing the bacterial count change over time for different antimicrobials (61). In this model, a growing drug-susceptible bacteria subpopulation (S) and a resting non-growing bacteria subpopulation (R) co-exist. The drug effect was implemented as a (sigmoid) maximum effect \( E_{\text{max}} \) killing rate of the susceptible bacteria. The non-susceptible resting bacteria subpopulation depicted both the biphasic kill observed experimentally, as well as the plateau of number of bacteria that is reached in the system. The bacterial growth and natural death of the bacteria are described by first order rate constants (\( k_{\text{growth}} \) and \( k_{\text{death}} \)). All bacteria were initially assumed to be susceptible with a transfer rate (\( k_{SR} \)) to the resting stage that increases with the total bacterial content in the system. Resting bacteria do not grow but share the same natural death kinetics (\( k_{\text{death}} \)) with the susceptible bacteria. The bacterial count reaches the stationary phase where the bacteria count is no longer increasing.

In this work, the semi-mechanistic PKPD model used to predict the killing of \( P. \ aeruginosa \) in tissue by colistin has been developed by Mohamed et al (22). The model structure consists of one compartment representing the
drug-susceptible growing bacteria (S) and one compartment representing the non-susceptible resting bacteria (R) (Figure 5). The PKPD parameter values were fixed to the published estimate values (22). Bacterial killing was driven by colistin unbound concentrations and an adaptive resistance function was included in the model. The resistance state (Re ON) was developing with a colistin concentration dependent rate constant (k_{on}) from a non-resistant state (Re OFF). The process was reversible with the rate constant to return to susceptibility state k_{off}. The effect of colistin concentration, k_{col}, was an E\textsubscript{max} model for both \textit{P. aeruginosa} strains, ATCC27853 (susceptible) and ARU552 (meropenem resistant), \((E_{\text{max}} \times C_{\text{col}})/(EC_{50} + C_{\text{col}})\) where \(C_{\text{col}}\) was colistin concentration. An increased fraction in Re ON resulted in a reduction of the maximum bacterial killing. In this work, this model was used to predict bacterial killing in tissues, therefore, CMS and colistin compartments were substituted with the given tissue compartments of the WBPBPK model. A start inoculum of 10\textsuperscript{8} CFU.mL\textsuperscript{-1} was used for all predictions.

**Figure 5.** Schematic illustration of the semi-mechanistic PKPD model for colistin developed by Mohamed et al. (22) (reprinted with permission from \textit{J. Antimicrob. Chemother. 2014;69:1350-1361}).

**PKPD models for immune response to bacterial infections**

Different models may be used to describe the immune response to bacterial infections. For instance, systems biology models are useful to study complex processes occurring during the immune response (62, 63) such as the initiation and the development inflammation. Empirical models have also been constructed to analyze the immune response to infections by bacterial pathogens. These models consist of simple mathematical equations able estimate
the clearance of bacteria by the neutrophils, (64-68), monocytes (69) or macrophages.

PBPK modeling has been applied to investigate the kinetics of endotoxins. These models intended to characterize the tissue distribution of endotoxin and to evaluate endotoxins as biomarkers for infections by Gram-negative bacteria (70, 71). A recent work by Gabrielsson et al (72) combined modeling and design of challenge tests to analyze inflammatory and metabolic biomarker studies. One of the case studies was to describe the release of cytokine (TNF-α) after *E. coli* endotoxin challenge in cynomolgus monkeys. Models describing the relationship between the systemic exposure of endotoxin and cytokine production are rare; one simple model depicting endotoxin effect on TNF-α production in rat has been published (73).

**Pharmacometrics**

Pharmacometrics has been defined as “the science of developing and applying mathematical and statistical methods to characterize, understand and predict a drugs pharmacokinetics, pharmacodynamics and biomarker outcomes behavior” (74). In other words, pharmacometric models can, by quantitatively describing the relationships between drug exposure or dose (PK) and drug effects (PD), summarize data into components of particular interest such as a drug’s half-life or its maximum effect on a biomarker, enable a more thorough understanding of the mechanisms involved in these processes as well as predict future scenarios such as new clinical trials, new dosing regimen or new patient populations. These models are a major asset to support drug development.

**Nonlinear mixed effects models**

The population approach to modeling makes use of nonlinear mixed effects (NLME) models, which are composed of a given number of parameters $\theta$ divided into fixed and random effects. The fixed effects represent the structural model, which describes the time course of a measured entity (e.g. drug or biomarker concentrations, or pharmacodynamic response). The structural model is often described by a set of algebraic (analytical) or differential equations.

The random effects relate to the stochastic (or statistical) model that describes the variability in the observed data. The random effects can be subdivided into three major components: interindividual variability (IIV), intraindividual or interoccasion variability (IOV) and residual unexplained variability (RUV). IIV relates to inherent differences between individuals of the same population, which will lead to a drug being eliminated faster in patient A than in patient B for example. A number of reasons can explain these dif-
ferences in biological processes, and often specific individual covariates (e.g. body weight or creatinine clearance) are used in the analysis to explain part of the IIV. IOV relates to differences in biological process within the same individual: the elimination of the drug might be faster for patient A on day and slower the next, depending on patient A’s food intake for example. RUV comprises all remaining unexplained variability, originating from different types of errors such as those related to dosing/treatment adherence, sampling, analytical errors or model misspecification.

In NLME models, each individual possesses a set of individual parameters which are functions of the typical population parameters adjusted by the individual random effects. One parameter value for a given individual \( P_{ik} \) can be described as:

\[
P_{ik} = \theta \cdot e^{\eta_i + \kappa_k}
\]

where \( \theta \) is the typical value of the parameter in the studied population, \( \eta_i \) is the random effect describing the difference between the typical parameter value and the parameter value in individual \( i \), and \( \kappa_k \) is the random effect describing the difference between individual parameter values from different occasions. \( \eta_i \) and \( \kappa_k \) are assumed to follow normal distributions with mean 0 and variances \( \omega_{IIV}^2 \) and \( \omega_{IOV}^2 \). Even though any kind of distribution can be assumed for the \( P_{ik} \), the hypothesis of a log-normal distribution (as displayed in equation 1) is often made for PK or PD parameters.

The general equation for the \( j \)th observation of the \( i \)th individual \( y_{ij} \) can then be expressed as:

\[
y_{ij} = f(x_{ij}, P_{ij}) + \varepsilon_{ij}
\]

where \( f \) symbolizes a nonlinear function, \( P_{ij} \) is a vector of individual model parameters and \( x_{ij} \) is a vector of independent variables which includes study design characteristics such as time, dose or covariates. \( \varepsilon_{ij} \) represents the residual error term describing the difference between the individual observation \( y_{ij} \) and the corresponding individual model prediction. The distribution of \( \varepsilon_{ij} \) is assumed to be normal with mean zero and variance \( \sigma^2 \). In equation 2, RUV is assumed to be additive. However, other assumptions are possible and proportional or combined (additive plus proportional) relationships are often used. Note that for simplicity reasons, the IOV index \( k \) was omitted for equation 2.

Maximum likelihood estimation

The NON-linear Mixed Effects Modeling (NONMEM) software (Icon development Solutions, Ellicot City, MD, USA) (75) was used to perform NLME modeling in the different projects presented in this work. Parameter
estimation using NONMEM was based on maximum likelihood theory. The likelihood of the data of an individual given the model is calculated as follows:

\[
L_i (y_i, \theta) = \int_{-\infty}^{+\infty} P(y_i | \theta, \eta, \Sigma) \cdot P(\eta | \Omega) \, d\eta
\]

where \( \Omega \) and \( \Sigma \) are the variance-covariances matrices of the random effects, \( P(y_i | \theta, \eta, \Sigma) \) is the probability density of the individual observations and \( P(\eta | \Omega) \) is the probability density of the individual parameters. The product refers to a joint density and the integral over the joint density gives the marginal likelihood. The population likelihood is given by the product of \( L_i \) over the number of subjects in the data \( (N) \):

\[
L (y, \theta) = \prod_{i=1}^{N} L_i (y_i, \theta)
\]

The negative of two times the log-likelihood (-2LL) is commonly used instead of the likelihood to facilitate computations. This quantity will be referred to as the objective function value (OFV). The best parameter description of the data depicted by the maximum likelihood estimate is obtained by minimizing -2LL over the parameter space.

No analytical solution exists for the likelihood, and approximations need to be used. This can be performed by Monte-Carlo integration or by linearization. In the current work, a linearization method was used: the First Order Conditional Estimation (FOCE) with and without interaction. With this method, NONMEM approximates the likelihood by first-order Taylor series linearization around the current conditional mean estimate of the individual random effects. Interaction is present whenever residual errors and individual random effects are not independent, i.e. when the variance of the RUV depends on model predictions (e.g. with proportional or combined RUV).

Implementing frequentist priors

WBPBPK models provide a mechanistic description of the disposition of a drug in the body. However, these models may be limited because of the vast amount of data needed on physiological and drug specific parameters, as well as because of the high computational burden due to their high dimensionality and complex implementation. Attempts to overcome these hurdles have included fixing a large number of model parameters, or reducing the complexity of the models (e.g. limiting the number of tissue compartments) \((57, 76)\). However these approaches underuse information contained in the data, and potentially limit the physiological interpretability of modeling outcomes.

A more interesting approach, which enables the estimation of WBPBPK model parameters within a frequentist framework while still incorporating
prior information, has been developed (76). It has the advantage of being much faster than Bayesian estimation methods (57). The incorporation of the prior information with this approach can be seen as a penalty function, which is added to the -2LL to minimize and which increases when parameters move away from their prior value. The rationale behind this approach rests on the following: the simultaneous analysis of two independent datasets with the same structural model would result in an OFV equal to the sum of the two OFVs obtained when fitting the datasets separately. If one of the datasets (i.e. the data leading to the prior information) is not available for the simultaneous analysis, a representation of its OFV can be used, which is a function of the parameters of the model. Here the representation of the OFV is set to minus two times the likelihood of the estimated parameters given their prior distributions. This term is then added to the OFV of the observed data, and the sum of both terms is then minimized with respect to the parameters to estimate.

Model comparison

The OFV is used in the likelihood ratio test to determine which of two models describes best description the data. Indeed, when two models are hierarchical or “nested” (i.e. fixing one or more parameter values to specific values in one model comes back to the other model), the difference in OFV (dOFV) between them is expected to follow a $\chi^2$ distribution with $n$ degrees of freedom, $n$ being the difference in the number of parameters between the nested models. A model is thus significantly better if the OFV decreases by a value larger than the predicted theoretical value determined by the $\chi^2$ distribution. An often used cut-off is a drop in OFV of 3.84 (dOFV = -3.84), which corresponds to a $p$-value of 0.05 for one degree of freedom (i.e. one extra parameter, $n=1$).
Aims

General aims
The general aims of this thesis were to develop translational whole-body physiologically based pharmacokinetic (WBPBPK) models to characterize the disposition of colistin across animals and humans, and to develop pharmacokinetic-pharmacodynamic (PKPD) models describing the initiation of the immune response to Gram-negative bacterial infections mediated by endotoxins. The developed models allow to better understand the processes driving bacterial killing at target site, providing support for the optimization of currently available antibacterial treatments and for the development of new antibiotics.

Specific aims
1. To develop WBPBPK models that describe the disposition of colistin and colistin methanesulfonate (CMS) from a mechanistic standpoint in animal and human, incorporating various sources of prior data.
2. To use the WBPBPK models to extrapolate colistin and CMS PK from animal to human.
3. To predict tissue distribution of colistin and CMS under various pathophysiological conditions and diverse dosing strategies.
4. To simulate bacterial killing at sites of infection by combining the WBPBPK model predicted tissue concentration-time profiles with a semi-mechanistic PKPD model.
5. To develop a model to explore the effect of *E. coli* endotoxins on the release of pro-inflammatory cytokines.
Methods

Data
Tissue-to-plasma partition coefficients ($K_p$) are crucial drug-specific parameters included in PBPK models to measure tissue distribution. They are different depending on tissue composition and the nature of the compound.

Determination of prior $K_p$ values

**CMS and colistin $K_p$ from rat tissue homogenates (Paper I)**
A tissue distribution study was performed using rat tissue homogenates to obtain the experimental $K_p$ of MS and colistin to be implemented in the WBPBPK model. $K_p$ were calculated, for both CMS and colistin, as the ratio of the concentration in the tissue homogenate over the plasma concentration at steady state (77).

**Colistin and CMS administration and blood sampling**
Six rats received a 3-h constant i.v. infusion of 8.5 mg.h$^{-1}$.kg CMS (Colymicine 1 MU; Sanofi Aventis, Paris, France). Blood was sampled 2 h and 3 h after the start of the infusion (i.e. end of the constant infusion) to ensure that steady state was attained. The same procedure was performed for colistin (Sigma, Saint Quentin-Fallavier, France), with six rats receiving a 4-h constant i.v. infusion of colistin sulfate (0.35 mg.h$^{-1}$.kg$^{-1}$). Blood samples were collected 3 h and 4 h after the start of the infusion (i.e. at the end of the constant infusion). Plasma was separated from other components of blood by centrifugation.

**Organ sampling**
The rats were anesthetized with isoflurane 2.5 to 3% inhalation at the end of the infusion and sacrificed by intracardiac exsanguination. The entire brain, heart, lungs and kidneys were collected for each rat. Samples of thigh muscle, neck fat, liver and duodenum were extracted from each rat.

**Tissue homogenates preparation**
After cleaning and flushing with saline, organ samples were weighted and freshly prepared as 20% tissue homogenates in PBS (i.e. diluted 6 times) using a T-18 Ultra-Turrax homogenizer (KA®-Werke GmbH & Co. KG,
Germany). The experimental procedure was completed on dried ice to maintain a low temperature and minimize the conversion of CMS into colistin. Tissue homogenates were centrifuged and CMS and colistin concentrations were assayed in the supernatant. Blood contamination was quantified in the tissue homogenates of lungs, heart, liver and kidneys. The correction was based on the comparison of the hemoglobin (Hb) content in the supernatant of the tissue homogenates and the Hb content in whole blood (78). The fraction of residual blood ($f_{bl}$) in each tissue homogenate was calculated as (Eq.5):

$$
f_{bl} = \frac{Hb_{tissue}}{Hb_{bl}}
$$

where $Hb_{tissue}$ and $Hb_{bl}$ are the concentrations of Hb in the tissue homogenates and in blood, respectively.

The corrected concentrations of CMS and colistin in the tissue homogenates ($C_{corr}$) were calculated from the measured total concentrations of CMS and colistin in tissue homogenates ($C_{tot}$) and blood ($C_{bl}$) (Eq.6):

$$
C_{corr} = C_{tot} - f_{bl} \cdot C_{bl}
$$

CMS and colistin do not distribute into red blood cells, so blood concentrations ($C_{bl}$) were calculated as (Eq.7):

$$
C_{bl} = (1 - HCT) \cdot C_p
$$

where HCT was the hematocrit of a typical rat obtained from literature data (79) and $C_p$ the measured plasma concentrations.

**In silico determination of CMS and colistin $K_p$ (Paper I)**

The $K_p$ were predicted using an *in silico* model (54) based on the physiological description of tissues and the binding properties of the compound (Eq.8):

$$
K_{p-prior-tissue} = PV_f + IV_f \cdot f_{up} + IV_f \cdot r_a \cdot (1 - f_{up})
$$

where $PV_f$ is the plasma volume fraction, $IV_f$ the interstitial volume fraction, $r_a$ the interstitium:plasma albumin ratio and $f_{up}$ the unbound fraction in plasma.

CMS and colistin do not distribute into the cells (19), so the terms related to the distribution into the erythrocytes and tissue cells were ignored.
Animal data

**In vivo studies (Paper II)**

Plasma concentration-time profiles for CMS and colistin in mouse, rabbit and pig were used to develop the interspecies WBPBPK model (Paper II). The animals were dosed with CMS (Colymicine, 1 MU; Sanofi-Aventis, Paris, France). Male Swiss mice (n=40, 4 per time point) received a subcutaneous (s.c.) administration of CMS single dose of 15 mg.kg⁻¹. Venous blood samples were collected at 0-4 h post-dose. Male New Zealand White rabbits (n=3) were dosed with an i.v. bolus of CMS at a single dose of 15 mg.kg⁻¹ and arterial blood was sampled at 0-7 h post-dose. Finally, two Large White male pigs received a single dose of 150 mg CMS though a 1-h i.v. infusion and venous blood was collected at 0-18 h post-dose.

**Literature**

*CMS and colistin plasma concentrations (Papers I and II)*

Plasma concentrations for CMS and colistin in rat (n=6) and in baboon (n=3) were obtained from previously published studies (80, 81).

*Plasma unbound fractions of CMS and colistin (f_{up}) (Papers I and II)*

The plasma unbound fractions (f_{up}) of colistin in rat mouse were gathered from literature (23, 82) whereas no data was available in rabbit, baboon and pig. Therefore, colistin f_{up} for mouse and rabbit was assumed be equal to the rat value (f_{up}=0.44) whereas f_{up} of colistin in baboons and pigs were set to human value (f_{up}=0.34) (11). These assumptions were supported by the relatively close serum albumin and α-acid glycoprotein levels in the species sharing the same f_{up} (79). No data was available in the literature on CMS f_{up}. The high instability of CMS makes experimental determination of CMS f_{up} very difficult. Therefore, CMS f_{up} was predicted using the online Simcyp prediction tools (83) accounting for the physicochemical properties of CMS and resulting in a value of 0.75 set for all species (84).

*Endotoxin and cytokines plasma concentrations (Paper V)*

*E. coli* endotoxin (ETX), IL-6 and TNF-concentrations plasma of piglets were obtained from two published studies (37, 85). In study A, 20 piglets were allocated to six dose groups and one control group. The dose groups received a 6-h continuous i.v. infusion at doses of 0.063 (n=3), 0.25 (n=3), 1.0 (n=3), 4.0 (n=3), 8.0 (n=3) and 16 (n=2) μg⁻¹kg⁻¹h⁻¹ ETX. The animals in the control group (n=3) received saline (NaCl) following the same procedure. ETX plasma concentrations were measured at baseline and every 2 h while plasma samples of TNF-α and IL-6 were taken at baseline and at every following hour.

In study B, 26 piglets were initially allocated randomly to a continuous i.v. infusion of either 0.063 (n=12) or 4.0 (n=12) μg⁻¹kg⁻¹h⁻¹ ETX, plus a control
group (n=2). After initiation of ETX infusion, treated piglets were random-
ized a second time to varying durations of infusion: 1 h (n=6), 2 h (n=6) or 6 h (n=12). Blood samples for measurement of ETX, TNF-α and IL-6 were col-
clected at baseline and at every following hour.

Human data
CMS and colistin PK data for model development in human were obtained
from previously published studies (9, 11, 21).

Healthy volunteer data (Paper III)
Twelve male healthy subjects received each a single dose of 1 MU (equiv-
alent to 34 mg CBA) of CMS sodium (Colymicine, Sanofi Aventis, Paris,
France) administered as a 1-h i.v. infusion. Venous blood was sampled be-
tween 0 and 18 h after the start of the infusion. In addition, fractionated urine
samples were collected for all subjects at the following time intervals: 0-2, 2-
4, 4-8, 8-12 and 12-24 h after the start of the infusion.

Critically ill patient data (Paper IV)
A total of 27 patients (10 women, 17 men) received colistin as part of their
standard of care to treat an infection by a multidrug Gram-negative (MDR
GNB). Physiological and demographic data were recorded on the first day of
treatment for each patient. The patients were dosed with different initial dos-
es of CMS sodium (Colistin; Norma, Greece): 2, 3 or a loading dose of 6
MU (equivalent to 60 mg, 90 mg and 180 mg CBA, respectively). The
maintenance doses of CMS were of 1, 2 or 3 MU. CMS was injected to pa-
tients every 8 h through a 15-min i.v. infusion. Venous blood was collected
typically after the 1st dose for all patients and then after the 4th, 6th, 7th or 8th
dose. Samples were taken between 0 and 480 min after the start of the infu-
sion.

Physiological data
Tissue volumes (V_{tissue}), tissue blood flow rates (Q_{tissue}), hematocrit, urinary
flow rates (UFR) the glomerular filtration rates (GFR) for the different spe-
cies used in this thesis work were from literature (21, 43, 45, 79, 86-88) (89)
(Table 1). Physiological data in baboons were scarce in the literature, so
rhesus monkey data were extrapolated and scaled with respect to the body
weight of the baboons. All physiological parameters were fixed in the model.
Table 1. Physiological parameters for mice, rats, rabbits, baboons, pigs and human.

<table>
<thead>
<tr>
<th></th>
<th>Blood flow, % of cardiac output</th>
<th>Tissue volume, % of total body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse (86) Rat (86) Rabbit (88) Baboon (87) Pig (87) Human (43)</td>
<td>Mouse (45) Rat (43) Rabbit (86) Baboon (43) Pig (87) Human (43)</td>
</tr>
<tr>
<td><strong>Arteries</strong></td>
<td>100 (86) 100 (86) 100 (86) 100 (88) 100 (88) 100 (88) 100 (88) 100</td>
<td>1.63 (45) 2.47 2.20 (86) 2.24 (43) 2.67 2.57</td>
</tr>
<tr>
<td><strong>Veins</strong></td>
<td>100 (86) 100 (86) 100 (86) 100 (88) 100 (88) 100 (88) 100 (88) 100</td>
<td>3.27 (45) 4.93 4.40 (86) 4.52 (43) 5.35 5.14</td>
</tr>
<tr>
<td><strong>Lungs</strong></td>
<td>3.3 (86) 2 (43) 1.8 (86) 10.54 (43) 12.67 12.58</td>
<td>0.70 (45) 0.50 0.68 (86) 0.77 (45) 0.78 1.67</td>
</tr>
<tr>
<td><strong>Brain</strong></td>
<td>6.6 (86) 4.9 (43) 3.02 (86) 8.78 (43) 15.84 2.65</td>
<td>1.70 (45) 0.60 0.54 (86) 1.90 (45) 0.31 2.07</td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td>5.8 (86) 5.8 (43) 9 (89) 7.9 (43) 2.68 5.32</td>
<td>0.50 (45) 0.30 0.24 (86) 0.34 (45) 0.29 0.38</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td>15.9 (86) 27.8 (43) 29.25 (86) 13.17 (43) 3.81 13.24</td>
<td>16.50 (45) 19.00 4.40 (86) 10.00 (43) 4.46 11.10</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td>7.02 (86) 7.43 (43) 6.04 (86) 2.93 (43) 3.92 4.58</td>
<td>38.40 (45) 40.40 54.00 (86) 50.00 (43) 31.37 42.90</td>
</tr>
<tr>
<td><strong>Adipose</strong></td>
<td>1.13 (86) 0.85 (43) 1.7 (86) 3.07 (43) 2.27 1.36</td>
<td>7.00 (45) 7.00 4.80 (86) 13.04 (43) 23.53 14.30</td>
</tr>
<tr>
<td><strong>Gastrointestinal Tract</strong></td>
<td>18.75 (86) 10.14 (43) 20.94 (86) 19.75 (43) 4.53 19.13</td>
<td>0.50 (86) 0.30 0.04 (86) 0.17 (45) 0.16 0.27</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td>24.25 (86) 13.39 (43) 29.62 (86) 27.52 (43) 22.76 25.99</td>
<td>4.20 (45) 2.70 4.80 (86) 5.04 (45) 3.92 2.70</td>
</tr>
<tr>
<td><strong>Kidneys</strong></td>
<td>9.1 (86) 14.1 (43) 15.09 (86) 20.19 (43) 4.92 19.43</td>
<td>5.50 (45) 3.40 4.00 (86) 2.70 (45) 2.31 2.41</td>
</tr>
<tr>
<td><strong>Hematocrit (79)</strong></td>
<td>0.45 0.46 0.36 0.41 0.45</td>
<td>0.45 0.45</td>
</tr>
<tr>
<td><strong>GFR (mL.min⁻¹)</strong></td>
<td>0.66 (45) 3.78 (45) 14.14 (45) 40.71 (45) 115.11 (45) 127.1 (21)</td>
<td>0.66 (45) 3.78 (45) 14.14 (45) 40.71 (45) 115.11 (45) 127.1 (21)</td>
</tr>
<tr>
<td><strong>UFR (mL.day⁻¹.kg⁻¹)(86)</strong></td>
<td>50 200 60 75 27 20</td>
<td>50 200 60 75 27 20</td>
</tr>
</tbody>
</table>

Data are from (21, 43, 45, 79, 86-89)
Analytical method
CMS and colistin (Papers I-IV)
CMS and colistin concentrations in plasma, urine and tissue homogenates were determined using published high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) methods (19, 20).

ETX and cytokines (Paper V)
ETX concentrations were measured in duplicates using the chromogenic limulus amoebocyte lysate assay (Endochrome-KTM, Charles River Endosafe, Charleston, SC). For study A, commercial sandwich ELISA assays were used for TNF-α (KSC3012, BioSource International, Nivelles, Belgium) and IL-6 (Quantikine™ porcine IL-6, P6000, R&D Systems, Minneapolis, MN), with a lower limit of quantification (LOQ) of 10 ng L⁻¹. In study B, plasma TNF-α and IL-6 was quantified with two commercial ELISA assays (DY686 and DY690, R&D Systems, Minneapolis, MN, US) with a LOQ of 60 ng L⁻¹ for both cytokines.

Model development
WBPBPK model for CMS and colistin (Papers I-IV)
Generic model structure (Papers I-IV)
The structure of the WBPBPK model included 11 tissue compartments (Figure 4): lungs, brain, heart, skin, adipose, muscle, spleen, gastrointestinal tract (GIT), liver, kidneys and carcass. The remaining tissues were lumped into a carcass compartment. Tissue compartments were linked together via the arterial and venous blood compartments in a closed-loop format. Mass transfer of CMS and colistin was assumed perfusion-limited with a well-stirred distribution in each tissue compartment. A specific blood flow (Q_tissue) and a physiological volume (V_tissue) was allocated to each tissue compartment (43, 90). The affinity of CMS and colistin for each tissue was defined by a specific tissue-to-plasma partition coefficient (K_{p,tissue}). The mass transfer and elimination processes for CMS and colistin in a “regular” tissue are displayed in Figure 6-i and detailed in equations Eq.9 and 10.
\[
\frac{dC_{tissue}}{dt} = Q_{tissue} \cdot C_{art-CMS} - \frac{C_{tissue-CMS} \cdot CL_{hyd-CMS} \cdot R_{bp-CMS}}{K_p-tissue-CMS} - \frac{Q_{tissue} \cdot C_{tissue-CMS} \cdot R_{bp-CMS}}{K_p-tissue-CMS}
\]

\[
\frac{dcol}_{tissue} = Q_{tissue} \cdot C_{art-coli} + \frac{C_{tissue-Ccoli} \cdot CL_{hyd-Ccoli} \cdot R_{bp-Ccoli}}{K_p-tissue-coli} - \frac{Q_{tissue} \cdot C_{tissue-ccoli} \cdot R_{bp-ccoli}}{K_p-tissue-ccoli}
\]

where \(Q_{tissue}\) is the blood flow for a given tissue, \(C_{art-CMS}\) and \(C_{art-coli}\) the arterial blood concentrations of CMS and colistin, respectively. \(CL_{hyd-CMS}\) is the intrinsic hydrolysis clearance of CMS in the tissue and \(CL_{nr-coli}\) the intrinsic non-renal clearance of colistin in the tissue. \(R_{bp-CMS}\) and \(R_{bp-coli}\) are the blood/plasma ratios of CMS and colistin, respectively. \(C_{tissue-CMS}\) and \(C_{tissue-ccoli}\) are the tissue concentrations of CMS and colistin. \(K_p-tissue-CMS\) and \(K_p-tissue-ccoli\) are the tissue-to-plasma partition coefficients of CMS and colistin for the given tissue.
Implementation of the $K_p$ prior values (I-IV)

The $K_p$ prior values ($K_p$-tissue-prior) of CMS and colistin were determined for each tissue compartment of each species following the two methods described earlier: either from rat tissue homogenates or from a published *in silico* model (54). Experimental $K_p$-tissue-prior of CMS and colistin for the skin and the spleen were not available, therefore the *in silico* values were used instead. CMS and colistin $K_p$-tissue-prior for carcass were calculated as the mean of the $K_p$-tissue-prior of all the tissues in the model. The precision (standard error, SE) on $K_p$-tissue-prior determined from the rat tissue homogenates was derived from the experimental standard deviation (SD) and the number of samples ($n=6$). For the *in silico* $K_p$-tissue-priors different precisions were tested.

The equilibration rate constant of each tissue ($K_{T\text{-tissue}}$) was calculated to determine those with similar distribution kinetics (Eq.11):

$$K_{T\text{-tissue}} = \frac{Q_{tissue}}{k_{p\text{-tissue-prior}} V_{tissue}} \quad \text{Eq.11}$$

where $Q_{tissue}$ is the specific blood flow for a given tissue, $K_{p\text{-tissue-prior}}$ the prior value of the tissue-to-plasma partition coefficient and $V_{tissue}$ the specific physiological volume of the tissue.

Tissues with the same distribution kinetics were grouped into pools, as it would not be possible to distinguish those independently using plasma data alone. A proportionality factor ($F_{\text{pool}}$) was estimated for each pool of tissues with a prior value of $F_{\text{pool}}$ set to 1 (Table 2). The SE of each $F_{\text{pool}}$ prior was calculated as the mean of the SE of the $K_{p\text{-tissue-prior}}$ of the tissues in the pool (Table 2). Each $F_{\text{pool}}$ was multiplied to $K_{p\text{-tissue-prior}}$ of each tissue in the pool, corrected for the intrinsic clearances in the given tissue (Table 2) and $Q_{tissue}$ in order to derive $K_p$ values of CMS and colistin for each tissue in the pool.
Table 2. Derived prior values of $F_{pool}$.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CMS $F_{pool}$ Experimental Prior (SE)</th>
<th>CMS $F_{pool}$ In silico Prior (SE)</th>
<th>Colistin $F_{pool}$ Experimental Prior (SE)</th>
<th>Colistin $F_{pool}$ In silico Prior (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidneys</td>
<td>1 (0.15)</td>
<td>1 (0.50)</td>
<td>1 (0.13)</td>
<td>1 (0.50)</td>
</tr>
<tr>
<td>Lungs</td>
<td>1 (0.19)</td>
<td>1 (0.50)</td>
<td>1 (0.17)</td>
<td>1 (0.50)</td>
</tr>
<tr>
<td>Muscle, adipose, skin and carcass</td>
<td>1 (0.11)</td>
<td>1 (0.25)</td>
<td>1 (0.16)</td>
<td>1 (0.25)</td>
</tr>
<tr>
<td>Brain, heart, spleen, GIT, liver</td>
<td>1 (0.17)</td>
<td>1 (0.25)</td>
<td>1 (0.06)</td>
<td>1 (0.25)</td>
</tr>
</tbody>
</table>

The volumes of distribution at steady state of CMS and colistin ($V_{ss-CMS}$ and $V_{ss-coli}$) were calculated from the derived $K_p$ values (Table 3).

**Three different approaches to estimate $K_p$ in rat (Paper I)**

Three scenarios were evaluated to estimate CMS and colistin $K_p$ with the WBPBPK model. In scenario I, $K_p$ were estimated using the in silico priors (54). In scenario II, $K_p$ were estimated using experimental priors from rat tissue homogenates. In scenario III, $K_p$ were fixed to their experimental values without being re-estimated.

**The urinary tract submodel (Paper III)**

The disposition of CMS and colistin in urine was described by constructing a specific urinary tract (UT) submodel (Figure 6-ii) linked to the generic WBPBPK model structure. Colistin present in urine was assumed to be exclusively formed from the hydrolysis of CMS that was renally excreted (24). Based on the literature, colistin was assumed to be extensively reabsorbed (26). Different UT submodel structures were evaluated, starting from a simple urine output compartment towards more complex physiological structures. Linear and non-linear processes were tested in order to describe the reabsorption clearance of colistin ($CL_{rea-coli}$). The differential equations Eq.12-16 describe the mass transfer of CMS to the arterial blood, kidney tissue, kidney tubules, collecting system and bladder.
\[ \frac{d\text{CMS}_{\text{arterial}}}{dt} = \frac{\text{CO} \cdot C_{\text{lung-CMS}} \cdot R_{\text{bp-CMS}}}{K_{p-\text{lung-CMS}}} - C_{\text{art-CMS}} \cdot CL_{\text{hyd-CMS-arterial}} - \frac{\text{CO} \cdot C_{\text{art-CMS}} - CL_{\text{r-CMS}} \cdot C_{\text{art-CMS}}}{K_{p-\text{lung-CMS}}} \]  
Eq. 12

\[ \frac{d\text{CMS}_{\text{kidney}}}{dt} = Q_{\text{kidney}} \cdot C_{\text{art-CMS}} - \frac{C_{\text{kidney-CMS}} \cdot CL_{\text{hyd-CMS-kidney}} \cdot R_{\text{bp-CMS}}}{K_{p-\text{kidney-CMS}}} - \frac{Q_{\text{kidney}} \cdot C_{\text{kidney-CMS}} \cdot R_{\text{bp-CMS}}}{K_{p-\text{kidney-CMS}}} \]  
Eq. 13

\[ \frac{d\text{CMS}_{\text{tubule}}}{dt} = C_{\text{art-CMS}} \cdot CL_{\text{r-CMS}} - C_{\text{tubule-CMS}} \cdot CL_{\text{hyd-CMS-urine}} - C_{\text{tubule-CMS}} \cdot UFR \]  
Eq. 14

\[ \frac{d\text{CMS}_{\text{collect}}}{dt} = C_{\text{tubule-CMS}} \cdot UFR - C_{\text{collect-CMS}} \cdot CL_{\text{hyd-CMS-urine}} - C_{\text{collect-CMS}} \cdot UFR \]  
Eq. 15

\[ \frac{d\text{CMS}_{\text{bladder}}}{dt} = C_{\text{collect-CMS}} \cdot UFR - C_{\text{bladder-CMS}} \cdot CL_{\text{hyd-CMS-urine}} - C_{\text{bladder-CMS}} \cdot UFR \]  
Eq. 16

where CO is the cardiac output, \( Q_{\text{kidney}} \) the kidney blood flow, \( C_{\text{art-CMS}}, C_{\text{lung-CMS}}, C_{\text{kidney-CMS}}, C_{\text{tubule-CMS}}, C_{\text{collect-CMS}} \) and \( C_{\text{bladder-CMS}} \) CMS concentrations of CMS in the arterial blood, lungs, kidney tissue, kidney tubules, collecting system and bladder, respectively. \( CL_{\text{hyd-CMS-art}}, CL_{\text{hyd-CMS-kidney}} \) and \( CL_{\text{hyd-CMS-urine}} \) are the intrinsic hydrolysis clearances of CMS in the arterial blood, kidney tissue and urine. \( R_{\text{bp-CMS}} \) is the blood/plasma ratio for CMS. \( K_{p-\text{lung-CMS}} \) and \( K_{p-\text{kidney-CMS}} \) are the tissue-to-plasma partition coefficients of CMS for lungs and kidneys, respectively.
The differential equations Eq.17-21 describe the mass transfer of colistin to arterial blood, kidney tissue, kidney tubules, collecting system and bladder, respectively.

\[ \frac{d\text{coli}_{\text{arterial}}}{dt} = \frac{CO \cdot C_{\text{tung-coli}} \cdot R_{bp-coli}}{K_{p-\text{tung-coli}}} + C_{\text{art-CMS}} \cdot CL_{\text{hyd-CMS-art}} - CO \cdot C_{\text{art-coli}} - C_{\text{art-coli}} \cdot CL_{\text{nr-coli-art}} \]  
Eq.17

\[ \frac{d\text{coli}_{\text{kidney}}}{dt} = \frac{Q_{\text{kidney}} \cdot C_{\text{art-coli}} + C_{\text{kidney-CMS}} \cdot CL_{\text{hyd-CMS-kidney}} \cdot R_{bp-CMS}}{K_{p-\text{kidney-CMS}}} - Q_{\text{kidney}} \cdot C_{\text{kidney-coli}} \cdot R_{bp-coli} - \frac{C_{\text{kidney-coli}} \cdot CL_{\text{nr-coli-kidney-Rbp-coli}}}{K_{p-\text{kidney-coli}}} + \frac{C_{\text{kidney-coli}} \cdot CL_{\text{nr-coli-kidney-Rbp-coli}}}{K_{p-\text{kidney-coli}}} \]  
Eq.18

\[ \frac{d\text{coli}_{\text{tubule}}}{dt} = C_{\text{tubule-CMS}} \cdot CL_{\text{hyd-CMS-urine}} - C_{\text{tubule-coli}} \cdot UFR - C_{\text{tubule-coli}} \cdot CL_{\text{rea-coli}} - A_{\text{tubule-coli}} \cdot k_{\text{ON}} + A_{\text{tubule-coli}} \cdot k_{\text{OFF}} \]  
Eq.19

\[ \frac{d\text{coli}_{\text{collect}}}{dt} = C_{\text{collect-CMS}} \cdot CL_{\text{hyd-CMS-urine}} + C_{\text{tubule-coli}} \cdot UFR - C_{\text{collect-coli}} \cdot UFR - A_{\text{collect-coli}} \cdot k_{\text{ON}} + A_{\text{collect-coli}} \cdot k_{\text{OFF}} \]  
Eq.20
\[
\frac{d\text{col} \text{bladder}}{dt} = 
C_{\text{bladder-CMS}} \cdot CL_{\text{hyd-CMS-urine}} + C_{\text{collect-coli}} \cdot UFR - C_{\text{bladder-coli}} \cdot UFR - A_{\text{bladder-coli}} \cdot k_{ON} + A_{\text{bladder-coli}} \cdot k_{OFF} \quad \text{Eq. 21}
\]

where CO is the cardiac output, \( Q_{\text{kidney}} \) the kidney blood flow, \( C_{\text{art-CMS}}, C_{\text{kidney-CMS}}, C_{\text{tubule-CMS}}, C_{\text{collect-CMS}} \) and \( C_{\text{bladder-CMS}} \), the concentrations of CMS in the arterial blood, kidney tissue, kidney tubules, collecting system and bladder, respectively. \( C_{\text{art-coli}}, C_{\text{lung-coli}}, C_{\text{kidney-coli}}, C_{\text{tubule-coli}}, C_{\text{collect-coli}} \) and \( C_{\text{bladder-coli}} \) are the concentrations of colistin in the arterial blood, lungs, kidney tissue, kidney tubules, collecting system and bladder, respectively. \( A_{\text{tubule-coli}}, A_{\text{collect-coli}} \) and \( A_{\text{bladder-coli}} \) are the amounts of colistin in the kidney tubules, collecting system and bladder, respectively. \( K_{ON} \) and \( K_{OFF} \) are the non-specific binding rate constants to the urinary tract epithelium for colistin. \( CL_{\text{hyd-CMS-art}}, CL_{\text{hyd-CMS-kidney}} \) and \( CL_{\text{hyd-CMS-urine}} \) are the intrinsic hydrolysis clearances of CMS in the arterial blood, kidney tissue and urine. \( CL_{\text{nr-coli-art}} \) and \( CL_{\text{nr-coli-kidney}} \) are the intrinsic non-renal clearances of colistin in the arterial blood and kidney tissue. \( R_{\text{bp-CMS}} \) and \( R_{\text{bp-coli}} \) are the blood/plasma ratios of CMS and colistin, respectively. \( K_{p\text{-lung-coli}} \) and \( K_{p\text{-kidney-coli}} \) are the tissue-to-plasma partition coefficients of colistin for lungs and kidneys, and \( K_{p\text{-kidney-CMS}} \) the tissue-to-plasma partition coefficient of CMS for kidneys.
Figure 6. Mass balance and clearance processes in regular tissue compartments (i) and in the urinary tract submodel (ii) forming the full WBPBPK model. The urinary tract submodel includes the kidney tissue, kidney tubules lumen, the collecting system lumen and the bladder lumen.

**Interspecies scaling (Paper II)**

The drug specific parameters in the model are the tissue-to-plasma partition coefficients ($K_p$) and the clearance parameters ($CL_{r-CMS}$, $CL_{hyd-CMS}$ and $CL_{nr-coli}$). The $K_p$ of CMS and colistin were derived for each tissue of each species by estimating species-independent $F_{pool}$ times $K_p$-tissue-prior with correction by the species-specific $Q_{tissue}$ and the intrinsic clearances occurring in the tissue ($CL_{hyd-CMS-int}$ and $CL_{nr-coli-int}$). The grouping of tissues was identical across species and no experimental $K_p$ prior existed for any species except rats, so rat values were used as priors in all species.

The renal clearance of CMS ($CL_{r-CMS}$) was a linear function of the species-specific GFR, and a proportional factor ($Slope_{r-CMS}$) was estimated as shown in Table 3. The hydrolysis of CMS in each compartment ($CL_{hyd-CMS-int}$) was calculated by estimating a parameter ($Slope_{hyd-CMS}$) allometrically scaled by a power ($EXP_{hyd-CMS}$) of the species-specific tissue volume ($V_{tissue}$). $Slope_{r-CMS}$, $Slope_{hyd-CMS}$ and $EXP_{hyd-CMS}$ were set to be identical across species (Table 3).
Different scaling strategies were tested for the intrinsic non-renal clearance of colistin (CL\textsubscript{nr-coli-int}), as displayed in Table 3:

- **Model A:** CL\textsubscript{nr-coli-int} was calculated for tissues, plasma and urine, by estimating a common proportional factor for all tissues (Slope\textsubscript{nr-coli}) allometrically scaled to a power (EXP\textsubscript{nr-coli}) of the species-specific V\textsubscript{tissue}. EXP\textsubscript{nr-coli} in all tissues, plasma and urine (Table 3) was the same across species.

- **Model B:** model A was further developed by scaling to the maximum lifespan potential (MLP). The species-specific MLP was calculated using a published equation (91).

- **Model C:** CL\textsubscript{nr-coli-int} in tissue and plasma were calculated from an estimated species-specific proportional factor (Slope\textsubscript{nr-coli-species}) allometrically scaled to the species-specific physiological volumes (V\textsubscript{tissue}). Here the exponent EXP\textsubscript{nr-coli} was fixed to 1.

**Parameter estimation and model evaluation (Papers I-IV)**

A sequential modeling approach was applied to fit first plasma (and urine in healthy volunteers) CMS concentrations. When a satisfactory WBPBPK model was obtained for CMS, the population parameter estimates of CMS were fixed and then colistin PK parameters were estimated. The final step was to estimate all CMS and colistin parameters simultaneously. Model selection and evaluation were based on physiological plausibility and the maximum likelihood statistic based on the objective function value (OFV). The more complex model was selected if the reduction in OFV (dOFV) was at least 3.84, corresponding to a p-value < 0.05 for 1 degree of freedom. Visual predictive checks (VPC) (92) were employed for model evaluation and standard errors of the estimated parameters were obtained using Sampling Importance Resampling (SIR) (93).
Table 3. Implementation of the pharmacokinetic parameters for CMS and colistin in the interspecies WBPBPK model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Implementation in the WBPBPK model</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CMS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( CL_{r-CMS} )</td>
<td>Renal clearance of CMS</td>
<td>( CL_{r-CMS} = \text{Slope}_{CMS} \cdot GFR )</td>
<td>Linear relationship to GFR; A common ( \text{Slope}_{CMS} ) was estimated across species</td>
</tr>
<tr>
<td>( CL_{hyd-CMS-int} )</td>
<td>Tissue-specific intrinsic hydrolysis clearance of CMS</td>
<td>( CL_{hyd-CMS-int} = \text{Slope}<em>{hyd-CMS-int} \cdot V</em>{tissue}^{\text{EXP}_{hyd-CMS}} )</td>
<td>Power relationship to ( V_{tissue} ); Common ( \text{Slope}<em>{hyd-CMS} ) and ( \text{EXP}</em>{hyd-CMS} ) were estimated across species</td>
</tr>
<tr>
<td>( CL_{hyd-CMS} )</td>
<td>Total hydrolysis clearance of CMS</td>
<td>( CL_{hyd-CMS} = \sum(\text{CL}_{hyd-CMS-int}) )</td>
<td>Derived parameters</td>
</tr>
<tr>
<td>( K_{p-CMS} )</td>
<td>Tissue-to-plasma partition coefficient</td>
<td>( K_{p-CMS} = F_{pool} \cdot K_{p-CMS-prior} \cdot \frac{Q_{tissue} + CL_{hyd-CMS-int}}{V_{tissue}} )</td>
<td>A common ( F_{pool} ) was estimated across species</td>
</tr>
<tr>
<td>( V_{ss-CMS} )</td>
<td>Steady-state volume of distribution of CMS</td>
<td>( V_{ss-CMS} = \sum(V_{tissue} \cdot K_{p-CMS}) )</td>
<td>Derived parameters</td>
</tr>
</tbody>
</table>

| **Colistin** | | | |
| \( CL_{nr-coli-int} \) | Tissue-specific non-renal intrinsic clearance of colistin | | |
| \( CL_{nr-coli} \) | Total non-renal clearance of colistin | \( CL_{nr-coli} = \sum(\text{CL}_{nr-coli-int}) \) | Derived parameter |
| \( CL_{rea-coli} \) | Reabsorption clearance of colistin in the kidney tubules | \( CL_{rea-coli} = 9 \cdot \text{Qureine} \) | \( CL_{rea-coli} \) was fixed (26) |
| \( K_{p-coli} \) | Tissue-to-plasma partition coefficient | \( K_{p-coli} = F_{pool} \cdot K_{p-coli-prior} \cdot \frac{Q_{tissue} + CL_{nr-coli-int}}{Q_{tissue}} \) | A common \( F_{pool} \) was estimated across species |
| \( V_{ss-coli} \) | Steady-state volume of distribution of colistin | \( V_{ss-coli} = \sum(V_{tissue} \cdot K_{p-coli}) \) | Derived parameter |

* the parameters in bold in the equations are estimated
Modeling of the immune response to endotoxin exposure

**Toxicokinetic model for ETX (Paper V)**

Different models were tested to describe the plasma concentration-time profile of ETX in piglet with one or two-compartments, linear, non-linear or combined elimination mechanisms. Physiologically, the clearance of ETX is partly due to the reticuloendothelial system (e.g. Kupffer cells in liver and spleen), therefore a saturable elimination could be expected (94). Different approaches were also evaluated to depict the assay baseline level of ETX informed by the observations from the control groups, as well as a general tendency of higher ETX measurements at baseline measurements observed in all groups.

**Exposure response model for the cytokines: IL-6 and TNFα (Paper V)**

The infusion of ETX induces an exposure-dependent increase of the plasma levels of the pro-inflammatory cytokines, IL-6 and TNF-α. This increase resembles a surge from below quantifiable levels to a peak and finally a return towards baseline levels. A chain of transit compartments was used to depict the time delay and the shape of IL-6 and TNF-α profiles. Cytokines production was described by a zero order constant \( k_{in} \) into the first compartment of the transit chain. \( k_{in} \) was stimulated by ETX concentration \( C_{ETX} \) and in the last compartment of the transit chain, plasma levels of IL-6 and TNF-α were measured. The optimal number of transit compartments was obtained manually, and both a normal and sigmoidal \( E_{max} \) exposure-response relationship between ETX and each of the cytokines were tested. The chain could be described by the equations Eq. 22-25, with the \( E_{max} \) exposure-response relationship added on \( k_{in} \):

\[
\frac{dA}{dt}_1 = k_{in} \times \left( 1 + \frac{E_{max} \cdot C_{ETX}}{EC_{50} + C_{ETX}} \right) - k_{tr} \times A_1 \quad \text{Eq. 22}
\]

\[
\frac{dA}{dt}_n = k_{tr} \times A_{n-1} - k_{tr} \times A_n \quad \text{Eq. 23}
\]

\[
\frac{dA}{dt}_{obs} = k_{tr} \times A_{obs-1} - k_{out} \times A_{obs} \quad \text{Eq. 24}
\]

where \( C_{ETX} \) is the plasma concentration of endotoxin, \( k_{tr} \) the first-order rate constant describing transit between the compartments of the chain and defined as (Eq.25):

\[
k_{tr} = \frac{n+1}{MTT} \quad \text{Eq. 25}
\]
where \( n \) is the number of transit compartments and \( MTT \) the estimated mean transit time.

\( k_{\text{out}} \) was set equal to \( k_{\alpha} \), and \( k_{\text{in}} \) was set equal to \( k_{\text{out}} \times S_0 \), where \( S_0 \) represents the baseline cytokine level to which all compartments are initialized. For the exposure-response relationship, \( E_{\text{max}} \) is the maximum effect (proportional change relative to \( S_0 \)) and \( EC_{50} \) is the potency defined as the ETX concentration necessary to reach 50% of \( E_{\text{max}} \).

**Tolerance model (Paper V)**

The tolerance to ETX observed in the cytokine data was investigated with different mathematical models previously used in the literature (95). In addition, an empirical model describing the tolerance development as a time-dependent increase in \( EC_{50} \) was tested (Eq. 26):

\[
EC_{50,t} = EC_{50_t=0} \times e^{k_{tol} \times t} \quad \text{Eq. 26}
\]

where \( EC_{50_t=0} \) is the parameter describing the potency at baseline, \( EC_{50,t} \) describes the potency at time \( t \), and \( k_{tol} \) is an estimated first-order rate constant describing the time-dependent increase of \( EC_{50} \).

**Parameter estimation and model evaluation (Paper V)**

The model was developed using a sequential approach, initially estimating the parameters of ETX. When the best model for ETX TK was obtained, ETX parameters were fixed and the parameters of the response model for TNF-\( \alpha \) and IL-6 were estimated (96). Eventually all the parameters were estimated simultaneously.

The model evaluation was based on the objective function value (OFV), the precision of the parameter estimates (SE), goodness-of-fit plots and prediction-corrected visual predictive checks (pcVPC) (92). A reduction in OFV greater than 3.84 (\( p \leq 0.05 \) for 1 degree of freedom) was required for inclusion of one additional parameter in the model.

**Simulations**

**Design and strategy**

In the rat and interspecies studies (Papers I and II), model predictions were performed following the same study design and dosing regimens as in the original dataset. In the healthy volunteer study (Paper III) the full WBPBPK model was employed to investigate the impact of pathophysiological changes (9, 11) on CMS and colistin tissue distribution: (i) typical
individuals at the 5 stages of renal dysfunction (Chronic Kidney Disease, CDK, www.renal.org) and (ii) typical individuals with different interstitial fraction volumes \( (V_i) \) mimicking edema. In the same analysis, model predictions were made to examine colistin tissue distribution following different dosing regimens already tested by Mohamed et al with a classical compartmental model predicting colistin plasma concentration-time course (11).

Determination of the unbound fraction in tissue \( (f_{ut}) \) for colistin and colistin

Colistin and CMS unbound fractions in tissue \( (f_{ut}) \) were predicted from an *in silico* model (Eq.27) previously described in literature (97):

\[
f_{ut} = \frac{1}{1 + r_{a \cdot f_{up}^{-1}}} \quad \text{Eq.27}
\]

As CMS and colistin were assumed not to distribute in tissue cells, the predicted concentrations in tissue were corrected for a distribution only into the interstitial space (43).

Prediction of tissue distribution in typical individuals (Papers I, II, III and IV)

The WBPBPK model developed to characterize the disposition of CMS and colistin was employed to predict CMS and colistin total and unbound concentration-time courses in tissues typical individuals (animals and humans).

Predictions of tissue distribution including variability and parameter uncertainty (Papers I, II and III)

The WBPBPK models were used to perform simulations \( (n=1000) \) accounting for IIV, RUV and the uncertainty on parameter estimates to predict the unbound concentration-time profiles of CMS and colistin in tissue (animals and humans).

Prediction of bacterial killing at target site (Paper IV)

The predicted unbound colistin concentration-time profiles from the WBPBPK model were used to drive the bacterial killing in a semi-
mechanistic PKPD model (22) developed from in vitro time-kill experiments on *Pseudomonas aeruginosa*. The published PKPD parameter estimates for the wild-type (ATCC 27853) and the clinical meropenem strain (ARU 552) were used. Bacterial killing was predicted following the original dosing schedule and with the first dose of original dosing schedule replaced by a loading dose of 9 MU. A starting inoculum of $10^8$ CFU.mL$^{-1}$ was assumed for all predictions.

Scale-up of colistin and CMS PK from animal to human (Paper II)

The interspecies WBPBPK models developed in this thesis were extrapolated to predict the disposition of CMS and colistin in human. Human physiological parameters ($V_{\text{tissue}}$, $Q_{\text{tissue}}$, GFR and UFR) were obtained either from the original dataset or from literature (Table I). For comparison, the dose and study design used for the predictions were identical to those in the original publication (7). The $K_p$ priors were kept identical to those used in animal for a given tissue. $CL_{\text{nr-coli-int}}$ was derived following the same scaling strategy as for the animal species. For models A and B, the final interspecies estimates for $\text{Slope}_{\text{nr-coli}}$ and $\text{EXP}_{\text{nr-coli}}$ were utilized. In the case of model C, $CL_{\text{nr-coli}}$ in human was derived using the value of $\text{Slope}_{\text{nr-coli}}$ estimated in baboons scaled to the ratio of the body weights to the -0.25 power, as it is commonly done for allometric scaling of rates. CMS and colistin plasma PK profiles in human were simulated (n=200 replicates) including IIV, RUV and uncertainty on the estimated.

Software

In all studies, the software used for the modelling was NONMEM 7.3 (75). The evaluation of goodness-of-fit plots was also considered for model selection. Model runs were managed using the software Pirana (98). Model predictions and calculations to produce the Visual predictive checks (VPC) were performed using PsN (99) and visualized using Xpose version 4.4.2.2 (100) and R 3.1.2 (www.R-project.org).
Results

Experimental $K_p$ priors for CMS and colistin (Paper I)

Plasma concentrations of CMS at 2 h and 3 h after the start of the constant infusion of CMS were $15.1 \pm 4.3 \mu g.mL^{-1}$ and $14.2 \pm 3.7 \mu g.mL^{-1}$, respectively. Colistin plasma concentrations at 3 h and 4 h after the start of the constant infusion of colistin sulfate were $0.8 \mu g \pm 0.1 \mu g.mL^{-1}$ and $0.9 \pm 0.1 \mu g.mL^{-1}$, respectively. These results confirm that steady-state was reached at 3 h for CMS and at 4 h for colistin. Each experimental $K_{p,tissue-prior}$ of CMS was calculated as the ratio of the concentration in the tissue homogenate over the concentration in plasma at 3 h after the start of the infusion of CMS. Similarly, each experimental $K_{p,tissue-prior}$ of colistin was calculated at 4 h after the start of the infusion of colistin sulfate. Experimental and *in silico* $K_p$ of CMS and colistin are presented in Table 4. The experimental values of both compounds were higher for kidney than for all the other tissues indicating a potential accumulation in renal tissue. The calculated fractions of residual blood ($f_{bl}$) in lungs, heart, liver and kidneys are reported in Table 4.

Development of WBPBPK models for CMS and colistin

Different approaches to estimate $K_p$ of CMS and colistin in rat (Paper I)

The developed WBPBPK model well described CMS plasma concentration-time profiles in all scenarios (Figure 7). Colistin concentrations were underpredicted after 5 min post-dose in scenario I while they were adequately described in scenarios II and III. A common hydrolysis rate constant ($k_{hyd-CMS}$) in plasma, tissues and urine was estimated. CMS hydrolysis was quicker in scenario I and II compared to scenario III (Table 5).
Table 4. CMS and colistin $K_{p\text{-tissue-prior}}$ calculated from rat tissue homogenates (experimental) and from the *in silico* model (54) as well as the calculated residual blood fractions in lung, heart, liver and kidney tissue homogenates (Paper I).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CMS (SD)</th>
<th>Colistin (SD)</th>
<th>CMS (SD)</th>
<th>Colistin (SD)</th>
<th>$f_{bl}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>0.515 (0.069)</td>
<td>0.485 (0.078)</td>
<td>0.151</td>
<td>0.698 (0.078)</td>
<td>0.17</td>
</tr>
<tr>
<td>Brain</td>
<td>0.107 (0.040)</td>
<td>0.0925 (0.12)</td>
<td>0.0685</td>
<td>0.306 (0.12)</td>
<td>0.16</td>
</tr>
<tr>
<td>Heart</td>
<td>0.275 (0.026)</td>
<td>0.257 (0.038)</td>
<td>0.152</td>
<td>0.414 (0.038)</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>0.312 (0.026)</td>
<td>0.312 (0.038)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.125 (0.032)</td>
<td>0.111 (0.19)</td>
<td>0.0722</td>
<td>0.393 (0.19)</td>
<td></td>
</tr>
<tr>
<td>Adipose</td>
<td>0.0995 (0.050)</td>
<td>0.0845 (0.24)</td>
<td>0.244</td>
<td>0.709 (0.24)</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.197 (0.013)</td>
<td>0.182 (0.064)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIT</td>
<td>0.119 (0.13)</td>
<td>0.116 (0.64)</td>
<td>0.277</td>
<td>1.36 (0.64)</td>
<td>0.075</td>
</tr>
<tr>
<td>Liver</td>
<td>0.329 (0.061)</td>
<td>0.303 (0.12)</td>
<td>0.118</td>
<td>0.795 (0.12)</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.260 (2.085)</td>
<td>0.230 (3)</td>
<td>5.67</td>
<td>20.6 (3)</td>
<td>0.083</td>
</tr>
<tr>
<td>Carcass</td>
<td>0.224 (0.085)</td>
<td>0.208 (0.03)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GIT: Gastrointestinal tract
Figure 7. Visual Predictive Checks (VPC) of plasma concentration-time profiles for CMS and colistin in rats estimating $K_p$ using *in silico* $K_p$ priors (scenario I), estimating $K_p$ using experimental $K_p$ priors (scenario II) and fixing $K_p$ to the experimental $K_p$ values (scenario III). The circles represent CMS and colistin observed plasma concentrations, the solid red line represents the median of the observed data, and the grey shaded area the 95% confidence interval of the median of the model predictions.

The pooling of the tissues based on their $K_T$ values in order to estimate $K_p$ stabilized the model and reduced run times. Kidneys and lungs represented a pool each; muscle, adipose, skin and carcass formed the 3rd pool and the rest of the tissues the 4th pool. Therefore, four proportionality factors ($F_{pool}$) were estimated for CMS and four for colistin (i.e. 8 factors in total). In scenario I, uncertainty (relative standard error; RSE%) on $K_p$-tissue-prior was set to 50% for small tissues (lungs, brain, heart, spleen, GIT, liver and kidneys) while it was set to 25% for large tissues (adipose, muscle, skin and carcass). The choice for a lower uncertainty for the large tissues than for the small tissues was to decrease their influence on tissue distribution of CMS and colistin. In scenario I, colistin accumulation in kidney was not predicted (26, 101-103) which might have caused the inadequate depiction of colistin plasma PK. Interestingly the IIIV on colistin $K_p$ estimates is high (i.e. CV=874%) The $K_p$ model estimates from scenarios I and II are presented in Table 5. The volumes of distribution at steady state ($V_{ss}$) were higher for colistin (340 mL.kg$^{-1}$ in scenario I, 1580 mL.kg$^{-1}$ in scenario II and 1322 mL.kg$^{-1}$ in scenario III) than for CMS (278 mL.kg$^{-1}$ in scenario I, 330 mL.kg$^{-1}$ in scenario II and 380 mL.kg$^{-1}$ in scenario III) in all cases (Table 7). High concentrations of colistin were observed at the first time point *in vivo*, therefore a fraction of colistin ($F_0$) preformed from the administered CMS was estimated. The estimate of $F_0$ was high in all scenarios (30-40%). The precision of the model parameter estimates was reasonable (Table 5 and 6).
Table 5. $K_p$ estimates for CMS and colistin in scenarios I and II (Paper I).

<table>
<thead>
<tr>
<th></th>
<th>$K_p$ estimates from scenario I$^a$</th>
<th></th>
<th>$K_p$ estimates from scenario II$^b$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMS</td>
<td>Colistin</td>
<td>CMS</td>
<td>Colistin</td>
</tr>
<tr>
<td></td>
<td>Typical value (RSE%)</td>
<td>Typical value (RSE%)</td>
<td>Typical value (RSE%)</td>
<td>Typical value (RSE%)</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.511 (66)</td>
<td>0.516 (56)</td>
<td>0.151 (19)</td>
<td>0.630 (4)</td>
</tr>
<tr>
<td>Brain</td>
<td>0.102 (55)</td>
<td>0.0396 (63)</td>
<td>0.067 (18)</td>
<td>0.329 (15)</td>
</tr>
<tr>
<td>Heart</td>
<td>0.260 (55)</td>
<td>0.103 (63)</td>
<td>0.149 (18)</td>
<td>0.348 (15)</td>
</tr>
<tr>
<td>Skin</td>
<td>0.408 (13)</td>
<td>0.719 (22)</td>
<td>0.273 (12)</td>
<td>0.785 (9)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.155 (13)</td>
<td>0.185 (22)</td>
<td>0.059 (12)</td>
<td>0.711 (9)</td>
</tr>
<tr>
<td>Adipose</td>
<td>0.122 (13)</td>
<td>0.127 (22)</td>
<td>0.199 (12)</td>
<td>1.16 (9)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.188 (55)</td>
<td>0.079 (63)</td>
<td>0.194 (18)</td>
<td>0.199 (15)</td>
</tr>
<tr>
<td>GIT$^d$</td>
<td>0.114 (55)</td>
<td>0.049 (63)</td>
<td>0.272 (18)</td>
<td>1.45 (15)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.312 (55)</td>
<td>0.127 (63)</td>
<td>0.116 (15)</td>
<td>0.815 (15)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.260 (53)</td>
<td>0.270 (42)</td>
<td>5.23 (17)</td>
<td>20.8 (7)</td>
</tr>
<tr>
<td>Carcass</td>
<td>0.271 (13)</td>
<td>0.291 (22)</td>
<td>0.682 (12)</td>
<td>4.77 (9)</td>
</tr>
<tr>
<td>IIV$^c$ (RSE%)</td>
<td>874 (32)</td>
<td>17 (33)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ In silico $K_p$ priors of CMS and colistin were implemented in the WBPBPK model with an RSE of 50% for lungs, brain, heart, spleen, GIT and liver. RSE of 25% were implemented for adipose, muscle, skin and carcass.

$^b$ The experimental $K_p$ priors for CMS and colistin were implemented in the WBPBPK model with a RSE calculated from the experimental data $SE = \frac{SD}{\sqrt{n}}$, where SD is the experimental standard deviation for each tissue and n the number of replicates (n=6 rats).

$^c$ A unique IIV was estimated for all $K_p$ of colistin and expressed as a coefficient of variation (CV%).

$^d$ GIT: gastrointestinal tract.
Table 6. CMS and colistin PK parameter estimates together with the IIV and RUV for the three investigated scenarios (Paper I).

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>CMS</th>
<th>Colistin</th>
<th>CMS</th>
<th>Colistin</th>
<th>CMS</th>
<th>Colistin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Typical value (RSE%)</td>
<td>IIV (RSE%)</td>
<td>Typical value (RSE%)</td>
<td>IIV (RSE%)</td>
<td>Typical value (RSE%)</td>
<td>IIV (RSE%)</td>
</tr>
<tr>
<td>Slope(_{CMS}) (no units)</td>
<td>1.12 (24)</td>
<td>37 (39)</td>
<td>1.27 (17)</td>
<td>36 (27)</td>
<td>1.29 (16)</td>
<td>44 (18)</td>
</tr>
<tr>
<td>(k_{hyd-CMS}) (h(^{-1}))</td>
<td>0.589 (20)</td>
<td>5.82 (11)</td>
<td>8.3 (53)</td>
<td>0.637 (18)</td>
<td>5.94 (10)</td>
<td>10 (30)</td>
</tr>
<tr>
<td>(k_{nr-coli}) (h(^{-1}))</td>
<td>40 (11)</td>
<td>39 (8.4)</td>
<td>39 (8.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(_0) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL(_{r-CMS}) (mL.min(^{-1}).kg(^{-1}))(^a)</td>
<td>6.71</td>
<td>7.63</td>
<td>7.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL(_{hyd-CMS}) (mL.min(^{-1}).kg(^{-1}))(^a)</td>
<td>9.36</td>
<td>10.1</td>
<td>13.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL(_{nr-coli}) (mL.min(^{-1}).kg(^{-1}))(^a)</td>
<td>92.5</td>
<td>94.5</td>
<td>93.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V(_w-CMS) (mL.kg(^{-1}))(^a)</td>
<td>278.0</td>
<td>330.0</td>
<td>380.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V(_ss-coli) (mL.kg(^{-1}))(^a)</td>
<td>340.0</td>
<td>1580</td>
<td>1322</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportional RUV (CV%)</td>
<td>57 (13)</td>
<td>38 (32)</td>
<td>56 (14)</td>
<td>29 (28)</td>
<td>56 (16)</td>
<td>30 (45)</td>
</tr>
<tr>
<td>Common RUV (mg.L(^{-1}))</td>
<td>0.161 (65)</td>
<td>0.115 (65)</td>
<td>0.295 (16)</td>
<td>0.211 (16)</td>
<td>0.350 (17)</td>
<td>0.251 (17)</td>
</tr>
</tbody>
</table>

\(^a\) Derived parameters from \(K_p\), \(k_{hyd-CMS}\), \(k_{nr-coli}\) and the physiological parameters (GFR, \(V_{tissue}\) and \(Q_{tissue}\)).

\(^b\) IIV was expressed as a coefficient of variation (CV%).
Interspecies scaling approach for CMS and colistin (Paper II)

The WBPK model developed in rats (84) was applied to simultaneously fit the time course of plasma concentrations of CMS and colistin in mice, rats, rabbits, baboons and pigs. The VPC plots displayed in Figure 8 illustrate the match between the three scaling model predictions and the animal plasma data. Model A fairly well described plasma PK profiles for both compounds in all species except colistin in mice. A minor underprediction was observed in rabbits for which the peak of colistin was predicted too early. Model B was able to capture the CMS plasma PK profiles across species well. For mice, colistin concentrations were included within the model prediction interval even though the variability was very high. In models A and B, the median of the observed data was at the upper and lower limit of the 95% confidence interval (CI) for rabbits and rats, respectively. Finally, model C fairly well described the concentration-time courses of CMS and colistin for all species. The $K_p$ of colistin for kidney was consistently higher than 1 (by approximately 10-fold) across all species, indicating a clear accumulation of colistin in kidney tissue. The derived $CL_{r-CMS}$, $CL_{hyd-CMS}$ and $CL_{nr-coli}$ from models A, B and C for each animal species are displayed in Tables 7, 8 and 9. In agreement with allometric scaling, the estimated $CL_{hyd-CMS}$ decreased for larger animals in all tested models. In most cases, the estimated $CL_{r-CMS}$ and $CL_{hyd-CMS}$ were fairly close for a given species across the different models. The IIV estimate of $CL_{r-CMS}$ was high across all models (CV=98-130%). IIV was also high for $CL_{hyd-CMS}$ in model C. The precision of clearance related parameters were reasonable in all tested models (Table 8).
Figure 8. Visual Predictive Checks (VPC) of plasma concentration-time profiles of CMS and colistin in mice, rats, rabbits, baboons and pigs for the three tested models (A, B and C). The circles represent CMS and colistin observed plasma concentrations, the black solid line represents the median of the observed data, and the grey shaded area the 95% confidence intervals of the medians of the model predictions.
Table 7. CMS and colistin PK parameter estimates together with their IIV and the RUV for model A (Paper II).

<table>
<thead>
<tr>
<th>Objective function value</th>
<th>model A</th>
<th>Mouse</th>
<th>Rat</th>
<th>Rabbit</th>
<th>Baboon</th>
<th>Pig</th>
<th>RSE%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-178.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Slope_{CMS} (no unit)</td>
<td></td>
<td>1.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Slope_{hyd-CMS} (h⁻¹)</td>
<td></td>
<td>0.153</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>EXP_{hyd-CMS} (no units)</td>
<td></td>
<td>0.835</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Slope_nr-coli (h⁻¹)</td>
<td></td>
<td>0.276</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>EXP_nr-coli (no unit)</td>
<td></td>
<td>0.359</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>ka (h⁻¹)</td>
<td>8.11</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>F₀ (%)</td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>IIV Slope_{CMS} (CV%)</td>
<td></td>
<td>113</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
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<tr>
<td>IIV Slope_{hyd-CMS} (CV%)</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIV EXP_{hyd-CMS} (CV%)</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIV Slope_nr-coli (CV%)</td>
<td></td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>IIV EXP_nr-coli (CV%)</td>
<td></td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>IIV Coli RUV (CV%)</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>CL_{r-CMS} (mL.min⁻¹.kg⁻¹)</td>
<td></td>
<td>18.4</td>
<td>19.3</td>
<td>0.9</td>
<td>1.7</td>
<td>2.3</td>
<td>-</td>
</tr>
<tr>
<td>CL_{hyd-CMS} (mL.min⁻¹.kg⁻¹)</td>
<td></td>
<td>6.5</td>
<td>4.3</td>
<td>3.1</td>
<td>2.4</td>
<td>1.8</td>
<td>-</td>
</tr>
<tr>
<td>CL_{nr-coli} (mL.min⁻¹.kg⁻¹)</td>
<td></td>
<td>243.9</td>
<td>470.7</td>
<td>11.2</td>
<td>3.9</td>
<td>6.1</td>
<td>-</td>
</tr>
<tr>
<td>V_{ss-CMS} (mL.kg⁻¹)</td>
<td></td>
<td>779.9</td>
<td>365.7</td>
<td>181.7</td>
<td>142.9</td>
<td>187.3</td>
<td>-</td>
</tr>
<tr>
<td>V_{ss-coli} (mL.kg⁻¹)</td>
<td></td>
<td>780.1</td>
<td>682.0</td>
<td>758.1</td>
<td>471.4</td>
<td>763.3</td>
<td>-</td>
</tr>
<tr>
<td>CMS prop RUV (CV%)</td>
<td></td>
<td>50.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Coli prop RUV (CV%)</td>
<td></td>
<td>41.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Coli add RUV (mg/L)</td>
<td></td>
<td>0.058</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>CMS Common RUV (mg.L⁻¹)</td>
<td></td>
<td>0.241</td>
<td></td>
<td></td>
<td></td>
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<td>19</td>
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<tr>
<td>Coli Common RUV (mg.L⁻¹)</td>
<td></td>
<td>0.173</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

\( ^a \) Derived parameters from the \( K_p \) values and the physiological tissue volumes.

\( ^b \) IIV was shared between the slopes and exponents estimates both for CMS hydrolysis clearance and colistin non-renal clearance.

\( ^c \) A separate slope was estimated for each species therefore the RSE% is reported between parentheses for each estimate.
Table 8. CMS and colistin PK parameter estimates together with their IIV and the RUV for model B (Paper II).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mouse</th>
<th>Rat</th>
<th>Rabbit</th>
<th>Baboon</th>
<th>Pig</th>
<th>RSE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective function value</td>
<td></td>
<td>-197.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope\textsubscript{CMS} (no unit)</td>
<td></td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Slope\textsubscript{hyd-CMS} (h\textsuperscript{-1})</td>
<td></td>
<td>0.134</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>EXP\textsubscript{hyd-CMS} (no units)</td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Slope\textsubscript{nr-coli} (h\textsuperscript{-1})</td>
<td></td>
<td>5.09</td>
<td></td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>EXP\textsubscript{nr-coli} (no unit)</td>
<td></td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>ka (h\textsuperscript{-1})</td>
<td>1.33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>(F_0) (%)</td>
<td>11</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV Slope\textsubscript{CMS} (CV%)</td>
<td></td>
<td>98</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>IIV Slope\textsubscript{hyd-CMS} (CV%)\textsuperscript{b}</td>
<td></td>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>IIV EXP\textsubscript{hyd-CMS} (CV%)\textsuperscript{b}</td>
<td></td>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>IIV Slope\textsubscript{nr-coli} (CV%)\textsuperscript{b}</td>
<td></td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>IIV EXP\textsubscript{nr-coli} (CV%)\textsuperscript{b}</td>
<td></td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>IIV Coli RUV (CV%)</td>
<td></td>
<td>84</td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>(CL\textsubscript{CMS} (mL.min\textsuperscript{-1}.kg\textsuperscript{-1})\textsuperscript{a}</td>
<td>13.3</td>
<td>7.6</td>
<td>5.1</td>
<td>3.4</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>(CL\textsubscript{hyd-CMS} (mL.min\textsuperscript{-1}.kg\textsuperscript{-1})\textsuperscript{a}</td>
<td>6.9</td>
<td>1.1</td>
<td>2.4</td>
<td>1.7</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>(CL\textsubscript{nr-coli} (mL.min\textsuperscript{-1}.kg\textsuperscript{-1})\textsuperscript{a}</td>
<td>48.7</td>
<td>20.5</td>
<td>9.4</td>
<td>2.9</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>(V\textsubscript{ss-CMS} (mL.kg\textsuperscript{-1})\textsuperscript{a}</td>
<td>97.7</td>
<td>341.6</td>
<td>166.7</td>
<td>149.8</td>
<td>195.1</td>
<td></td>
</tr>
<tr>
<td>(V\textsubscript{ss-coli} (mL.kg\textsuperscript{-1})\textsuperscript{a}</td>
<td>477.0</td>
<td>447.0</td>
<td>633.9</td>
<td>329.8</td>
<td>677.9</td>
<td></td>
</tr>
<tr>
<td>CMS prop RUV (CV%)</td>
<td></td>
<td>52.9</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Coli prop RUV (CV%)</td>
<td></td>
<td>24.7</td>
<td></td>
<td></td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>Coli add RUV (mg/L)</td>
<td></td>
<td>0.018</td>
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<td>39</td>
</tr>
<tr>
<td>CMS Common RUV (mg.L\textsuperscript{-1})</td>
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<td>0.308</td>
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<td>11</td>
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<tr>
<td>Coli Common RUV (mg.L\textsuperscript{-1})</td>
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<td>0.220</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Derived parameters from the K\textsubscript{p} values and the physiological tissue volumes.

\textsuperscript{b} IIV was shared between the slopes and exponents estimates both for CMS hydrolysis clearance and colistin non-renal clearance.

\textsuperscript{c} A separate slope was estimated for each species therefore the RSE\% is reported between parentheses for each estimate.
Table 9. CMS and colistin PK parameter estimates together with their IIV and the RUV for model C (Paper II).

<table>
<thead>
<tr>
<th>Objective function value</th>
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</thead>
<tbody>
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<td><strong>model C</strong></td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Parameter</th>
<th>Mouse</th>
<th>Rat</th>
<th>Rabbit</th>
<th>Baboon</th>
<th>Pig</th>
<th>RSE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope&lt;sub&gt;CMS&lt;/sub&gt; (no unit)</td>
<td>0.88</td>
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</tr>
<tr>
<td>Slope&lt;sub&gt;hydr-CMS&lt;/sub&gt; (h^-1)</td>
<td>0.135</td>
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<td></td>
<td>31</td>
</tr>
<tr>
<td>EXP&lt;sub&gt;hydr-CMS&lt;/sub&gt; (no units)</td>
<td>1.07</td>
<td></td>
<td></td>
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<td></td>
<td>18</td>
</tr>
<tr>
<td>Slope&lt;sub&gt;nr-coli&lt;/sub&gt; (h^-1)</td>
<td>12.7</td>
<td>1.24 (12)</td>
<td>0.514 (16)</td>
<td>0.198 (12)</td>
<td>0.503 (17)</td>
<td>c</td>
</tr>
<tr>
<td>EXP&lt;sub&gt;nr-coli&lt;/sub&gt; (no unit)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ka (h^-1)</td>
<td>1.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.052</td>
</tr>
<tr>
<td>F&lt;sub&gt;0&lt;/sub&gt; (%)</td>
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</tr>
<tr>
<td>IIV Slope&lt;sub&gt;CMS&lt;/sub&gt; (CV%)</td>
<td>130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>IIV Slope&lt;sub&gt;hydr-CMS&lt;/sub&gt; (CV%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>IIV EXP&lt;sub&gt;hydr-CMS&lt;/sub&gt; (CV%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>IIV Slope&lt;sub&gt;nr-coli&lt;/sub&gt; (CV%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIV EXP&lt;sub&gt;nr-coli&lt;/sub&gt; (CV%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIV Coli RUV (CV%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>CL&lt;sub&gt;hydr-CMS&lt;/sub&gt; (mL.min^-1.kg^-1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7</td>
<td>4.8</td>
<td>3.7</td>
<td>2.5</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>CL&lt;sub&gt;hydr-CMS&lt;/sub&gt; (mL.min^-1.kg^-1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.3</td>
<td>0.8</td>
<td>2.1</td>
<td>2.0</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>CL&lt;sub&gt;nr-coli&lt;/sub&gt; (mL.min^-1.kg^-1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>211.0</td>
<td>234.3</td>
<td>9.1</td>
<td>3.4</td>
<td>8.4</td>
<td>-</td>
</tr>
<tr>
<td>V&lt;sub&gt;s-CMS&lt;/sub&gt; (mL.kg^-1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171.5</td>
<td>288.9</td>
<td>171.9</td>
<td>146.9</td>
<td>206.7</td>
<td>-</td>
</tr>
<tr>
<td>V&lt;sub&gt;s-coli&lt;/sub&gt; (mL.kg^-1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>731.0</td>
<td>432.6</td>
<td>628.1</td>
<td>401.7</td>
<td>773.9</td>
<td>-</td>
</tr>
<tr>
<td>CMS prop RUV (CV%)</td>
<td>54.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Coli prop RUV (CV%)</td>
<td>45.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Coli add RUV (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>CMS Common RUV (mg.L^-1)</td>
<td>0.183</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>Coli Common RUV (mg.L^-1)</td>
<td>0.131</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>61</td>
</tr>
</tbody>
</table>

<sup>a</sup> Derived parameters from the K<sub>p</sub> values and the physiological tissue volumes.

<sup>b</sup> IIV was shared between the slopes and exponents estimates both for CMS hydrolysis clearance and colistin non-renal clearance.

<sup>c</sup> A separate slope was estimated for each species therefore the RSE% is reported between parentheses for each estimate.
Interspecies WBPBPK model: prediction of colistin and CMS disposition in human from five animal species

The 95% confidence interval (CI) of the predicted median concentration-time profiles of CMS and colistin in human from models A, B and C are illustrated in Figure 9. Predicted CMS median profiles were similar in all models and described human data adequately. Predicted colistin median plasma profiles described less satisfactorily the human data and demonstrated some differences across models. Colistin elimination was underpredicted in models A and B, and, overpredicted in model C. Up to 3h post-dose, colistin concentrations were underpredicted in models A and C but fairly well described in model B.

**Figure 9.** WBPBPK model predictions of CMS and colistin PK profiles in healthy volunteers receiving a single dose of CMS sodium 80 mg through a 1-h i.v. infusion. The blue circles represent the observed data from Couet et al. (21), the black line the median of the observations, the grey shaded area the 95% confidence interval around the median model predictions when simulations include IIV, RUV and uncertainty in the estimated parameters. Predictions and observations at 15 and 18 h post-dose were omitted for visualization purposes.
Characterization of urine disposition of colistin and CMS in healthy volunteers (Paper III)

The UT submodel consisted of three distinct physiological compartments featuring kidney tubules, collecting system and urinary bladder. The compartments were defined by their physiological volume and the mass transfer of colistin and CMS was via the urinary flow rate (UFR) calculated from the data (21). The UT submodel was linked to the WBPBPK model via CL_{r,CMS} for CMS mass transfer and via CL_{rea-coli} in the case of colistin. A non-linear binding relationship was added to the UT submodel to account for the ability of colistin to bind to cell membranes. The full model, i.e. generic WBPBPK model together with the UT submodel, adequately described the PK of colistin and CMS in plasma and in urine (Figure 10).

Model parameter estimates are presented in Table 10. A faster hydrolysis in plasma was estimated ($k_{hyd-CMS} = 0.096$ h$^{-1}$) than in urine ($k_{hyd-CMS_{-urine}} = 0.052$ h$^{-1}$). The elimination rate constant of colistin in urine always approached zero when estimated. Tubular reabsorption of colistin was described by a non-linear relationship (Table 3) where only the affinity constant ($K_M$) was estimated. $K_M$ was estimated low (0.0053 mg.L$^{-1}$), signifying a high affinity of colistin for tubular transporters.

The $K_p$ of CMS and colistin for kidney was estimated higher than in other tissues, illustrating the accumulation of colistin in renal tissue. The volume of distribution at steady state ($V_{ss}$) was higher for colistin (24.1 L) than for CMS (17.7 L). The precision of the estimated parameters was satisfactory.
Figure 10. Visual Predictive Checks (VPC) of the plasma concentration-time profiles and urinary excretion rate of colistin and CMS in healthy volunteers who received 1 MU (80 mg) CMS sodium. The blue symbols represent the observed data; the black solid line represents the median of the observed data and the dashed red lines the 5th and 95th percentiles of the predictions. The yellow and blue shaded areas represent the 90% confidence interval of the median and the 5th and 95th percentiles of the predictions.
Table 10. CMS and colistin PK parameters, IIV and RUV estimates (Paper III).

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>CMS</th>
<th>Colistin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Typical value (RSE %)</td>
<td>IIV&lt;sup&gt;b&lt;/sup&gt; (RSE %)</td>
</tr>
<tr>
<td>Slope&lt;sub&gt;CMS (no units)&lt;/sub&gt;</td>
<td>1.34 (9)</td>
<td>23 (20)</td>
</tr>
<tr>
<td>k&lt;sub&gt;hyd-CMS (h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>0.096 (10)</td>
<td>26 (18)</td>
</tr>
<tr>
<td>k&lt;sub&gt;hyd-CMS-urine (h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>0.052 (15)</td>
<td>54 (20)</td>
</tr>
<tr>
<td>k&lt;sub&gt;nr-coli (h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>0.104 (6)</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;M (mg.L&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>0.0053 (19)</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;ON (h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>2.02 (12)</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;OFF (h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>0.87 (16)</td>
<td></td>
</tr>
<tr>
<td>F&lt;sub&gt;0 (%)&lt;/sub&gt;</td>
<td>6.6 (0.9)</td>
<td></td>
</tr>
<tr>
<td>CL&lt;sub&gt;r-CMS (L.h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>9.79</td>
<td></td>
</tr>
<tr>
<td>CL&lt;sub&gt;hyd-CMS (L.h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>6.73</td>
<td></td>
</tr>
<tr>
<td>CL&lt;sub&gt;hyd-CMS-urine (L.h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>CL&lt;sub&gt;nr-coli (L.h&lt;sup&gt;-1&lt;/sup&gt;)&lt;/sub&gt;</td>
<td>7.24</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;ss-CMS (L)&lt;/sub&gt;</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;ss-coli (L)&lt;/sub&gt;</td>
<td>24.1</td>
<td></td>
</tr>
<tr>
<td>Prop res err plasma (CV%)</td>
<td>44 (14)</td>
<td></td>
</tr>
<tr>
<td>Add res err plasma (mg.L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.049 (19)</td>
<td></td>
</tr>
<tr>
<td>Common res err plasma (mg.L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.20 (17)</td>
<td></td>
</tr>
<tr>
<td>Prop res err urine (CV%)</td>
<td>96 (12)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Clearances and volumes of distributions at steady state were derived from the estimated parameters (rate constants and K<sub>p</sub>) and physiological tissue volumes.

<sup>b</sup> IIV was expressed as a coefficient of variation (CV%).

Predictions of the impact of pathophysiological changes on tissue distribution of colistin and CMS (Paper III)

Critically ill patients often experience various organs and pathophysiological dysfunctions such as renal impairments failure and edema (9, 11), with consequences on exposure at target site they. In paper III, the developed WBPBPK model was employed to predict total and unbound colistin and CMS concentration time courses in tissues for typical individuals with renal failure (Figure 11) and for typical individuals with various interstitial volume fractions mimicking different degrees of edema (Figure 12). The more severe is the renal dysfunction, the higher is the tissue distribution of CMS and colistin. In contrast, when the interstitial volume fraction increases the exposure of CMS and colistin is reduced.
Figure 11. Typical WBPBPK model predictions of CMS and colistin total (blue and red solid lines respectively) and unbound (blue and red dashed lines, respectively) concentration-time profiles in tissue for subjects at different stages of renal dysfunction (CDK stages) and the median value of CrCL in the data after 1-h i.v. infusion of 1 MU CMS (80 mg). The black solid line represents the minimum inhibitory concentration (MIC=2mg.L⁻¹) for *Acinetobacter Baumannii*. 
Figure 12. Typical WBPBPK model predictions of CMS and colistin total (solid lines) and unbound (dashed lines) concentration-time profiles in tissues with different interstitial space volume fractions (1, 1.5, 1 and 3-fold the typical physiological value) after 1-h i.v. infusion of 1 MU (80 mg) CMS.
Predictions of colistin exposure in tissues after different dosing regimens (Paper III)

The predictions of the unbound concentration time course of colistin in tissues following a maintenance dose of 3 MU every 8 h or 4.5 MU every 12 h after loading doses of 6 MU, 9 MU, and 12 MU are shown for a typical subject in Figure 13. The solid horizontal black line represents the minimum inhibitory concentration for a wild type *Acinetobacter baumanii* (MIC=2 mg.L\(^{-1}\)). When no loading dose is given with the lowest maintenance dose (1 MU) the exposure of colistin is predicted below the MIC in all tissues except in kidney and brain. The dosing strategy that predicts the best coverage with an exposure above the MIC in tissue is when a loading dose (6, 9 or 12 MU) is administered to the patient with a maintenance dose of 4.5 MU starting at 12 h.

Predictions of the bacterial killing at target site (Paper IV)

Unbound colistin exposure in tissue was predicted applying the WBPBPK model developed in animal (Papers I and II). Model predictions of the colistin mediated bacterial killing were carried out in clinically relevant tissues or biological fluids for efficacy and toxicity concerns: plasma, kidneys, lungs and skin. Two dosing scenarios (I and II) were tested for the clearance of a susceptible *P. aeruginosa* (ATCC 27853) or a clinically isolated meropenem-resistant *P. aeruginosa* (ARU 552) in tissues. The ATCC 27853 strain was cleared from all tissues (at least a 2-log decrease), regardless of the dosing scenario. In contrast, ARU522 strain was only eliminated from kidney In Figures 14 and 15 are displayed the examples of bacterial killing in plasma and kidneys. Bacterial killing was faster for patients receiving loading doses (6 or 9 MU).
**Figure 13.** Typical WBPBPK model predictions of colistin unbound concentration-time profiles in tissues following different dosing regimens: no loading dose and maintenance dose of 1 MU (No LD & 1 MU), loading dose of 6, 9 or 12 MU with maintenance dose of 3 MU after 12 h (LD 12 h & 3 MU), loading dose of 6, 9 or 12 MU with maintenance dose of 3 MU after 24 h (LD 24 h & 3 MU) and loading dose of 6, 9 or 12 MU with maintenance dose of 4.5 MU after 12 h (LD 12 h & 4.5 MU). The black solid line represents the minimum inhibitory concentration (MIC=2 mg/L) for *Acinetobacter Baumannii.*
Figure 14. Model predicted colistin unbound concentration-time profiles for the dosing scenarios I with the corresponding bacterial killing of wild-type *Pseudomonas aeruginosa* (ATCC 27853) in plasma and model predicted colistin unbound concentration-time profiles for the dosing scenarios I and II with the corresponding bacterial killing of meropenem-resistant *Pseudomonas aeruginosa* (ARU 552) in plasma.
Figure 15. Model predicted colistin unbound concentration-time profiles for the dosing scenarios I with the corresponding bacterial killing of wild-type *Pseudomonas aeruginosa* (ATCC 27853) in kidney model predicted colistin unbound concentration-time profiles for the dosing scenarios I and II with the corresponding bacterial killing of meropenem-resistant *Pseudomonas aeruginosa* (ARU 552) in kidneys.

Modeling of the immune response to endotoxin exposure (Paper V)

Endotoxin toxicokinetic model

The concentration-time profile of ETX in plasma was best described by a one-compartment model with a nonlinear saturable elimination (Figure 16). Neither the addition of extra compartments nor different elimination models (e.g. linear or combined linear plus nonlinear) improved the fit to
the data. The pcVPC plots are displayed in Figure 18. ETX plasma concentrations at baseline and in the control groups were adequately depicted by a constant estimated baseline ($S_{0,ETX}$) reflecting the endogenous ETX level. The high baseline levels of ETX observed in the control groups were handled by initializing the ETX PK compartment with an estimated amount (Cont.) relating to some initial contamination. The decay of Cont follows the same kinetics as any infused ETX.

The IIV was estimated on the maximum clearance capacity ($V_{\text{max}}$) and the volume of distribution ($V_C$) with a correlation of -0.18. IIV was also estimated $S_{0,ETX}$ and Cont. The correlation between these two parameters was +0.78. A bioavailability fraction ($F$) included in model fixed to 1 associated to an estimated IIV in order to explain part of the analytical method and to take into account for the potential variability in ETX doses. An IIV was estimated for the residual error component of the model allowing for differences in RUV between individuals. The parameter estimates are displayed in Table 11.

![Figure 16](image.png)

**Figure 16.** Prediction corrected visual predictive check for endotoxin measurements, assessing the models’ ability to describe both the variability and mean tendency in the data. The data shown are those arising from studies A (A) and B (B). The circles represent observed data, the red solid lines represent the median of the observed data and the dotted blue lines represent the median of the 2.5 % and 97.5 % outer observations. The transparent orange field represents a simulation-based 95% confidence interval for the median and the transparent blue fields represent a simulation-based 95% confidence interval for the 2.5 % and 97.5 % model predicted percentiles.
Exposure response model for IL-6 and TNFα

All data were included in the model development including BQL data. Different models were tested in order to characterize the effect of ETX concentration on the release of IL-6 and TNF-α in plasma as implemented in the indirect response model (linear, $E_{max}$, sigmoidal $E_{max}$). The plasma concentrations versus time profiles for IL-6 and TNF-α were described well by a standard $E_{max}$ model with two transit compartments. The optimal number of transit compartments was determined in a step-wise manner evaluating the change in OFV ($dOFV$) and the individual fits as goodness of fit diagnostics. Furthermore, a study-dependent baseline was estimated for TNF-α leading to a 7-fold difference in the baseline estimates between the two studies even though both values remain below 10 ng L$^{-1}$. IIV was estimated for $MTT$, $S_0$TNFα and $S_0$IL6, with a correlation of $+0.83$ correlation in the case of IL-6.

Tolerance model

Several tolerance models were assessed to investigate the tolerance development to ETX challenge observed in the data. The model fit to IL-6 and TNF-α plasma data was improved $EC_{50}$ (i.e. potency) was increasing exponentially with time. The addition of this empirical tolerance model adjusted the performance and predictions of the model as judged by $dOFV$ ($\geq 3.84$), the goodness-of-fit plots and the pcVPC plots (Figures 17 and 18). The parameter estimates are reported in table XII.

Noticeably, for the exposure-response model of IL-6, all parameters were shared the two studies. However, TNF-α data were more difficult to describe because of the rapid burst 1 h after start of the experiment. The apparent rapid tolerance development explains the high estimate of $ktol$ toward high values (>100 h$^{-1}$). In order to circumvent this issue and stabilize the model, the estimate of $ktol$ was fixed to 25 h$^{-1}$, which depicts a rapid tolerance development without a significant $dOFV$. 
Figure 17 Prediction corrected visual predictive check for interleukin-6, assessing the models ability to describe both the variability and mean tendency in the data, as well as the proportion of samples below the limit of quantification. The data shown are that arising from study A (A) and B (B).

Top panel: The circles represent observed data, the red solid line represents the median of the observed data and the dotted blue lines represent the median of the 2.5% and 97.5% outer observations. The transparent orange field represents a simulation-based 95% confidence interval for the median and the transparent blue fields represent a simulation-based 95% confidence interval for the 2.5% and 97.5% model predicted percentiles.

Lower panel: The circles represent the observed proportion of samples below the limit of quantification at a given time and the transparent blue field represents a simulated-based 95% confidence interval for the proportion of simulated values below the limit of quantification.

Figure 18. Prediction corrected visual predictive check for tumor-necrosis factor-α, assessing the models ability to describe both the variability and mean
tendency in the data, as well as the proportion of samples below the limit of quantification. The data shown are that arising from study A (A) and B (B).

**Top panel:** The circles represent observed data, the red solid line represents the median of the observed data and the dotted blue lines represent the median of the 2.5% and 97.5% outer observations. The transparent orange field represents a simulation-based 95% confidence interval for the median and the transparent blue fields represent a simulation-based 95% confidence interval for the 2.5% and 97.5% model predicted percentiles.

**Lower panel:** The circles represent the observed proportion of samples below the limit of quantification at a given time and the transparent blue field represents a simulated-based 95% confidence interval for the proportion of simulated values below the limit of quantification.
Table 11. Parameter values from the analysis of ETX, TNF-α and IL-6.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Parameter Definition</th>
<th>Population Value (RSE%)</th>
<th>Inter-Individual Variation CV% (RSE%) [SHR%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$ (EU h$^{-1}$)</td>
<td>ETX maximum elimination capacity</td>
<td>1.620.000 (7)</td>
<td>61.7 (89) [33]</td>
</tr>
<tr>
<td>$K_m$ (EU L$^{-1}$)</td>
<td>ETX where 50 % of $V_{\text{max}}$ is reached</td>
<td>9140 (4)</td>
<td>-</td>
</tr>
<tr>
<td>$V_C$ (L)</td>
<td>Volume of distribution</td>
<td>204 (25)</td>
<td>51.3 (111) [38]</td>
</tr>
<tr>
<td>$S_{0,\text{ETX}}$ (EU L$^{-1}$)</td>
<td>ETX baseline</td>
<td>165 (21)</td>
<td>-</td>
</tr>
<tr>
<td>Cont (EU)</td>
<td>ETX higher $S_{0,\text{ETX}}$ measurement</td>
<td>24300 (5)</td>
<td>88.0 (25) [7]</td>
</tr>
<tr>
<td>F</td>
<td>Bioavailability</td>
<td>1 (FIX)</td>
<td>81.9 (46) [21]</td>
</tr>
<tr>
<td>EPS</td>
<td>Additive residual error</td>
<td>0.223 (11)</td>
<td>38.7 (14)</td>
</tr>
<tr>
<td>$S_{0,\text{TNF}}$ (ng L$^{-1}$)</td>
<td>TNF-α baseline (study A)</td>
<td>1.09 (47)</td>
<td>64.5 (34) [32]</td>
</tr>
<tr>
<td>$S_{0,\text{TNF}}$ (ng L$^{-1}$)</td>
<td>TNF-α baseline (study B)</td>
<td>7.38 (36)</td>
<td>-</td>
</tr>
<tr>
<td>MTT (h)</td>
<td>Mean transit time</td>
<td>1.16 (7)</td>
<td>26.6 (31) [19]</td>
</tr>
<tr>
<td>$E_{50}$ (EU L$^{-1}$)</td>
<td>TNF-α where 50 % of $E_{\text{max}}$ is reached</td>
<td>67.4 (24)</td>
<td>-</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (h$^{-1}$)</td>
<td>TNF-α maximum increase in $K_{\text{in}}$</td>
<td>80800 (9)</td>
<td>-</td>
</tr>
<tr>
<td>EPS</td>
<td>Additive residual error</td>
<td>0.961 (27)</td>
<td>-</td>
</tr>
<tr>
<td>$S_{0,\text{IL6}}$ (ng L$^{-1}$)</td>
<td>IL-6 baseline</td>
<td>8.41 (24)</td>
<td>91.4 (13) [6]</td>
</tr>
<tr>
<td>MTT (h)</td>
<td>Mean transit time</td>
<td>2.46 (13)</td>
<td>31.8 (15) [10]</td>
</tr>
<tr>
<td>$E_{50}$ (EU L$^{-1}$)</td>
<td>IL-6 where 50 % of $E_{\text{max}}$ is reached</td>
<td>28.4 (76)</td>
<td>-</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (h$^{-1}$)</td>
<td>IL-6 maximum increase in $K_{\text{in}}$</td>
<td>2818 (38)</td>
<td>-</td>
</tr>
<tr>
<td>EPS</td>
<td>Additive residual error</td>
<td>0.491 (11)</td>
<td>-</td>
</tr>
</tbody>
</table>

CV: coefficient of variation; ETX: endotoxin; EU: endotoxin units; IL-6: interleukin-6; RSE: residual standard error; SHR: shrinkage; TNF-α: tumour-necrosis factor-α.
Discussion

WBPBPK model for colistin and CMS (Papers I-IV)

This thesis primarily describes the development of a WBPBPK model for colistin and CMS. The model structure was successfully applied to depict the disposition of these compounds in five animal species as well as in healthy volunteers and critically ill patients. Despite the knowledge gaps on colistin and CMS PK, the WBPBPK model was able to characterize colistin and CMS concentration-time profiles in plasma and urine. The model could be used to predict the PK of both molecules in tissue under clinically relevant pathophysiological circumstances and following different dosing rationales. The model predicted exposure in tissue was also combined with a published semi-mechanistic PKPD model (22) to simulate bacterial count time courses at potential sites of infection for two bacterial strains (*Pseudomonas aeruginosa* ATCC 27853 and ARU 552) and following different dosing regimens.

The WBPBPK model developed in rat (Paper I) has enhanced the understanding of colistin and CMS tissue distribution by combining the use of experimental tissue data with WBPBPK modeling. Noticeably, this is the first study in which colistin and CMS tissue concentrations have been experimentally measured employing LC-MS/MS methods (19, 20). Most of the PK studies performed in biological fluids beside plasma (9, 10, 21) or urine (21) were carried out in epithelial lining fluid (ELF) (80, 81, 104) or cerebrospinal fluid (CSF) (105, 106) or using microbiological analytical methods (101).

This is the first WBPBPK model characterizing the disposition of a polymyxin antibiotic; it was constructed combining a “top down” and a “bottom up” modeling approach (56, 57), using literature, *in silico*, *in vitro* and *in vivo* data. In Paper I, three strategies for incorporating prior information on tissue distribution of colistin and CMS were compared: estimating $K_p$ using $K_p$ priors predicted *in silico* (scenario I), estimating $K_p$ using experimental prior values from rat tissue homogenates (scenario II) and fixing $K_p$ to the experimental values (scenario III). In all scenarios, the plasma PK of CMS was well described. The PK of colistin in plasma was well characterized in scenarios II and III while it was under-
predicted in scenario I. This demonstrates the superiority of the use of experimental $K_p$ over *in silico* $K_p$ as priors in the case of colistin. The $K_p$ prior values from the *in silico* model apply only to the passive transport processes involved in tissue distribution (54) and fail to describe the active tubular reabsorption processes such as via PEPT2 transporters in human (107) and rat kidney (26). This is particularly important to increase the accuracy of model predictions with respect to colistin induced nephrotoxicity. The experimental $K_p$ priors of colistin and CMS used in the WBPBPK model were obtained from rat tissue homogenates by a rigorous experimental procedure. The freshly excised organs were cleaned and tissue homogenization was carried out on dried ice to reduce post-excision hydrolysis of CMS. The use of tissue homogenates was justified by the impossibility of using microdialysis techniques because of the high colistin non-specific binding affinity for lab material (108, 109).

For all studies, the derived volumes of distribution at steady state ($V_{ss}$) for CMS were close to the extracellular fluid volume (110), which is consistent with the fact that it does not penetrate into the cells but distributes only into interstitial fluid (21, 24). Colistin $V_{ss}$ was higher than CMS $V_{ss}$ in all scenarios, which could be explained by the non-specific binding of colistin to membrane phospholipids (101, 111, 112) of the cells and the accumulation in renal tubular cells (107). Colistin and CMS clearances in rat and human derived from the WBPBPK model were consistent with literature values (9, 11, 21, 24, 80, 113). The estimated non-renal clearance of colistin was in the same range as those reported in publications using non-compartmental or classical compartmental analyses (101). Currently, no evidence for a clear elimination pathway of colistin is available except old studies reporting that a minor fraction of colistin possibly undergoes glucuronidation in urine and bile (80, 82), and that colistin is not spontaneously hydrolyzed *in vitro* in buffer at physiological temperature ($37^\circ$C) (82). Therefore, the clearance of colistin was assumed to occur in every tissue at the same rate.

The WBPBPK model developed in rat was applied and expanded to describe plasma and urine PK of colistin and CMS in healthy human subjects (21) by the addition of a physiologically based urinary tract (UT) submodel (Paper III). The full model (generic WBPBPK model plus UT submodel) well described the observed urine and plasma concentration-time profiles of colistin and CMS (Figure 10). The hydrolysis of CMS in urine was estimated to be faster than in plasma and tissue ($k_{hyd-CMS}=0.096$ h$^{-1}$ versus 0.052 h$^{-1}$) which is in agreement with previously reported results (18). There was no impact of pH on hydrolysis kinetics even though in theory water and urine are more acidic than plasma and therefore
should catalyze CMS conversion to colistin. No elimination of colistin was estimated in urine, suggesting that colistin was either eliminated via urinary excretion or reabsorbed into kidney tubular cells. The latter was supported by a recent study that demonstrated colistin accumulation in the renal cortex cells (114). Tubular reabsorption of colistin was modeled as a saturable process with a low affinity constant estimate \(K_M = 0.0053 \text{ mg.L}^{-1}\). The model included non-specific binding compartments linked to each colistin UT compartment as supported by several publications in different media and to various surfaces (17, 19, 20, 108, 111). The association and dissociation first order rate constants \(K_{ON}\) and \(K_{OFF}\) were estimated and the binding was faster than the release. The UT submodel was built on a physiological basis and could be refined when new data and knowledge on the mechanisms driving colistin disposition in urine are available.

**Interspecies WBPBPK model: prediction of colistin and CMS disposition in human from five animal species (Paper II)**

The WBPBPK model in rat (Paper I) was successfully applied to simultaneously fit the plasma concentration time courses of colistin and CMS and colistin in mice, rats, rabbits, baboons and pigs. CMS and colistin \(K_p\) were estimated using the same experimental \(K_p\) priors which were corrected by the intrinsic clearances (i.e. \(CL_{\text{hyd-CMS-int}}\) and \(CL_{\text{nr-coli-int}}\)) in each tissue. This assumption was made to limit the number of estimated parameters.

Colistin and CMS clearances were estimated. All tested scaling models (A, B and C) described fairly well the time courses of colistin and CMS concentrations in the animal species, except colistin in mice for model A. Noticeably, the model interspecies scaling was more robust for CMS than for colistin. The knowledge gap on colistin elimination pathway and protein binding in tissue, which appears to involve processes not fully captured by allometry, might explain this difference.

Regarding the variability, estimated IIV did not discriminate between interspecies and interindividual variability because of the limited number of animals in most species.

The interspecies WBPBPK model was then extrapolated to human, which stressed the strengths and the limitations of the scaling strategies. The PK of CMS appeared easily scalable from animals to humans, which was not unexpected as the scalability between animal species had been
good. This was not the case for colistin, for which the PK proved less scalable from animals to humans. Colistin concentrations were predicted in the right range, but the predicted changes in concentrations over time were either too fast or too slow. This mismatch was attributed to a misprediction of CL_{nr-coli}. None of the considered scaling alternatives were fully adequate even though model B appeared to have better interspecies scaling features than the other models. This model was based on allometric scaling to the physiological volume of the tissues and the maximum lifespan potential of each species. These results strengthen the credibility of model B for predicting colistin and CMS exposure in tissues, both in animals and in humans. As there is an urgent need to develop new antibiotics with a better comprehension of their PKPD features, such interspecies WBPBPK models might be useful to understand the disposition of a new drug candidate and to predict its PK in human.

**Exposure predictions in tissue (Papers I-IV)**

Most bacterial infections occur in the interstitial fluid (60) which advocates for a better understanding of tissue exposure of colistin in order to understand and predict bacterial killing. Model predictions of the total and free concentration-time profiles of colistin and CMS in tissue were performed. The binding of colistin and CMS to plasma and tissue proteins was accounted for, as well as their distribution to interstitial space only. The difference in predicted unbound concentrations between plasma and tissues are driven by their in silico predicted unbound fraction of CMS and colistin in tissue (\(f_{ut}\)), which accounts only for the binding to serum albumin (97). Predicted tissue concentrations were used to evaluate dosing strategies, to investigate the impact of pathophysiological changes and to predict bacterial killing at potential target sites.

**Application of the WBPBPK model to investigate the impact of pathophysiological changes (Paper III)**

WBPBPK models are particularly well suited to examine the influence of pathophysiological disturbances on the PK of a drug. This feature is specifically valuable for colistin as it stands as one of the last therapeutic options for patients infected with multidrug resistant Gram-negative bacteria (9, 10, 24, 115, 116). These patients often experience multiple organ dysfunctions (e.g. renal or hepatic impairment) as well as fluid and electrolyte disorders (e.g. edema) due to inflammation and denutrition, which
impact the disposition of antibiotics (117-121). The WBPBPK model developed in Paper III was used to predict colistin exposure under different degrees of renal dysfunctions (5 CKD stages, www.renal.org) and different degrees of edema (Figures 11 and 12). In the renal dysfunction scenario, colistin and CMS distribution in tissue was augmented when renal function decreased because of a reduced CL_{r-CMS}. In the edema scenario, different interstitial volume fractions (IVf) were used to correct the tissue concentrations to mimic fluid shift phenomena occurring in severely ill patients (9, 11, 12, 119). Predicted exposure of colistin in tissue was increased with lower IVf. Accounting for these changes in pathophysiology is important when administering an antibiotic as colistin because overdosing these patients may lead to unwanted nephrotoxic side effects, while suboptimal concentrations could trigger treatment failure and in the long term emergence of bacterial resistance (60).

Application of the WBPBPK model to investigate different dosing strategies (Paper III)
The full model developed in healthy volunteers including the generic WBPBPK model structure linked to the UT submodel was used to predict colistin exposure in tissue following different dosing regimens (Figure 13). The original dose in the healthy volunteer study design was used as a reference. The investigated dosing schemes had already been by Mohamed et al (11). Colistin exposure after administration of 1 MU q8h was predicted to be below the breakpoints of EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical and Laboratory Standards Institute) for Acinetobacter baumannii for most tissues such as the skin which is a potential site of infection (e.g. fasciitis necrosis) (116). A more suitable exposure, i.e. above the MIC in most tissues, was obtained when a loading dose (6, 9 or 12 MU) was given with a maintenance therapy of 4.5 MU starting at 12 h.

Application of a WBPBPK-PD model for colistin to predict the clearance of P. aeruginosa at potential infection sites (Paper IV)
The WBPBPK model developed in rat (Paper I) was successfully applied to describe plasma PK of CMS and colistin in critically ill patients receiving multiple doses of colistin (9, 11). The WBPBPK model was then employed to predict the unbound concentration time course of colistin in
potential target tissues infected with *P. aeruginosa*: skin, lungs, blood and kidneys (9, 11, 116). Two dosing scenarios were simulated: the original dosing schedule (scenario I) and the original dosing schedule with a loading dose of 9 MU (scenario II) instead of the first dose (typically 3 or 6 MU). Predictions of colistin concentrations in target tissues were used to predict the bacterial killing of a susceptible *P. aeruginosa* strain (ATCC 27853) and a clinical meropenem-resistant *P. aeruginosa* strain (ARU 552).

In all tissues, regardless of the dosing scenario, ATCC 27853 bacteria were cleared rapidly whereas ARU 552 was only eliminated from kidney but not from any other tissue. Bacterial killing appeared much faster for ATCC 27853 when a loading dose of 9 MU was given which will likely lead to a faster resolution of the infection in the patients.

**Modeling of the immune response to endotoxin exposure**

**Endotoxin toxicokinetic model (Paper V)**

A toxicokinetic (TK) model was developed to describe plasma concentrations of *E. coli* ETX over time after various doses and infusion lengths. A one-compartment TK model with non-linear elimination was able to describe the observed data. A two-compartment model had previously been fitted to endotoxin data in rhesus monkeys (122) but in the current study no improvement was observed when a two-compartment model was used. The non-linear mechanism for the elimination of ETX was in agreement with the saturable uptake experimentally observed in early studies (123) and a more recent study using PBPK models (124). The largest amounts of recovered radioactive labelled ETX were found in plasma, liver and spleen. The latter two are part of the reticuloendothelial system responsible for the saturable elimination of ETX (94).

**Effect of endotoxin exposure in plasma on the release of IL-6 and TNFα (Paper V)**

The release of the pro-inflammatory cytokines TNFα and IL-6 was best described by indirect-response model coupled to a three-compartment transit chain which described the rapid build-up of the cytokine plasma profiles. An E_max exposure-response relationship well described the stimulation of the production rate of TNFα and IL-6 by ETX concentrations.
Various mechanism-based models (95) were tested to describe tolerance phenomena to ETX. However, none of these models proved superior to a simple empirical model describing tolerance as a time-dependent exponential increase in ETX potency ($EC_{50}$). The model predicted that the ability of ETX to induce a response went drastically down with time since start of ETX infusion. The developed exposure-response model for ETX and cytokines could be further applied in longitudinal studies and could be refined by incorporating physiologically and biologically based components.
Conclusion

In this thesis, physiologically based pharmacometric models were developed to provide a better understanding of the pharmacokinetics of colistin and its prodrug colistin methanesulfonate (CMS) in animal and human, as well as to describe the early response of the immune system to Gram-negative bacterial infections. The value of whole-body pharmacokinetic (WBPBPK) models was evaluated to predict colistin and CMS distribution in tissue under different circumstances. The methods used in this thesis add to the development of an integrated modeling framework including physiology, drug distribution, bacterial susceptibility and resistance as well as innate immunity.

Specifically:

- A WBPBPK model was developed to describe plasma pharmacokinetics of colistin and CMS in rat. Three approaches were evaluated in order to incorporate prior information on tissue distribution of colistin and CMS. It has been demonstrated that $K_p$ obtained from rat tissue homogenates were well suited to predict tissue distribution accounting colistin the accumulation in kidney.

- The WBPBPK model was successfully applied to describe simultaneously the plasma concentration-time profiles of colistin and CMS in five animal species. The interspecies WBPBPK model was then used to scale up plasma PK from animal to human, providing knowledge on the strengths of the scaling properties of the model for colistin and CMS as well as its limitations partly due to the lack of knowledge on the mechanism driving the disposition of colistin.

- The WBPBPK model was expanded with the development of a physiologically based urinary tract (UT) submodel that well described colistin and CMS concentrations in human urine and plasma.
• As colistin is an antibiotic used in clinical practice to treat severely ill patients, the developed WBPBPK model was employed to predict colistin and CMS exposure in human tissues investigating the effects of clinically relevant pathophysiological changes and the suitability of different dosing strategies.

• The model predicted concentration time course of colistin in target tissues was used to drive the bacterial killing for a susceptible and a meropenem-resistant strain of \textit{P. aeruginosa} following different dosing schedules. The susceptible \textit{P. aeruginosa} was cleared from all tissues regardless of the dose whereas the resistant one was only cleared from kidney.

• A toxicokinetic model was developed to describe the plasma time course of \textit{E. coli} endotoxin and its effects on the release the pro-inflammatory cytokines TNF-\textgreek{a} and IL-6 accounting for the tolerance development.
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