A Generic Physiologically Based Pharmacokinetic Model
to Predict Drug Distribution in Plasma, Red Blood Cells, Interstitial and Cellular Space with Application to the Pharmacokinetics of Tolbutamide
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0.1 Abstract

In this thesis, a novel, general whole-body physiological based pharmacokinetic (PBPK) model will be presented. The derivation of the current mathematical framework and its parameters will be particularly emphasized. The main goal was to develop a model that allows prediction and analysis of drug distribution. The model has been tested with application to tolbutamide pharmacokinetics. The present PBPK model is able to sufficiently predict concentration-time profiles in human erythrocytes, plasma, interstitial and cellular compartments of various organs. Its value for therapeutic risk analysis has been tested, implementing genetic polymorphisms in metabolizing enzymes, as well as illness-induced physiological changes. Furthermore, in this thesis, we will demonstrate the general applicability of the developed model by simulating and comparing drug distribution in two different species (human and rat). Comparison of our results with experimental data [27, 53] showed good agreement (visual comparison), indicating that the developed PBPK model is able to sufficiently characterize drug-transfer kinetics and to satisfactorily predict drug distribution with experimentally verified parameters. We infer that the generic mathematical framework provides a stable characterization of the processes involved in drug disposition, allowing interpretation beyond distribution processes.

0.2 Introduction

In the drug discovery business, computational scientists frequently have to justify their work, when lab-scientists come along, asking the famous question: "is there really a case where a drug that’s on the market has been designed by a computer?", sensing the desired answer is "no". The causes of the threateningly competitive feelings between lab- and computational scientists, however, remain a mystery. Both disciplines are nowadays interwoven to such a degree, that ignoring one of these disciplines will make it a lot harder to sustain the institutions positions on the market. Experimental research is needed in order to derive a mechanistical view of biological processes. To this end computation gives experimental results additional meaning, by positioning them into a larger frame. Computation, on the other hand, can help asking the right questions and therefore direct and accelerate experimental discovery.

The reality in drug discovery nowadays is that the use of computers and computational methods permeates all aspects of drug discovery [23]. Those who are most proficient with the computational tools have the advantage of
delivering new drug candidates more quickly and at lower costs than their competitors. Cost-efficient modern drug discovery has a substantial statistical component in it: Envelope pushers need to have the resources to take higher risks.

Most drugs now arise through discovery programs that begin with identification of a biological target of potential therapeutic value through biological studies (e.g. knockouts). A multidisciplinary project team is then assembled with the goal of finding clinical candidates, i.e. drug-like compounds that are ready for human clinical trials. Typically these compounds bind selectively to their molecular target and interfere in its activity.

The cornerstones of computation in drug discovery are library screening (e.g. virtual screening and docking), advanced treatments of protein-ligand binding through MC (Monte Carlo methods) and MD (Molecular Dynamics) techniques and the prediction of properties and drug-likeness. The later include pharmacokinetics.

The main principles of drug-likeness have been derived from the action of hormones. Oral drugs are usually small, fatty weak acids or basis. Chemically, they are relatively stable. Hydrophobic compounds have relatively poor solubility and high serum protein binding, whereas the opposite is true for the hydrophilic compounds. This dichotomy is responsible for the classic lead optimization struggle of solubility versus permeability. Lipinski [30] has states some rules, that are considered to describe drug-likeness. The principles of drug-likeness will be discussed in more detail.

**Pharmacokinetics.** Pharmacokinetics is defined as the study of the movements of drugs within biological systems (concentration-time), as affected by absorption/uptake, distribution, metabolism and elimination (ADME). Pharmacology on the other hand is defined as the study of how chemical substances interfere with living systems (response-concentration). Strict separation of these two fields, however, is hardly possible. The movement of a drug and therefore the concentration at a target site is responsible for the intensity of a drug response. Precise pharmacokinetic modelling may therefore help to understand a compounds pharmacology. Furthermore precise pharmacokinetic models can predict the time, at which a certain drug concentration has reached a therapeutic limit in the target tissue, which makes them useful in therapeutics, when we are interested in the response-time profile of a drug.

The absorption processes and the first-pass clearance of a compound determine its bioavailability. The bioavailability of a compound is a core criteria, since it describes which fraction of the initially administered drug will enter the system. Therefore, this value can be correlated with the dose regimen
and answer the question of 'how much' drug to administer in order to cause an effect. The distribution characteristics (e.g. volume of distribution) on the other hand, together with the drug’s elimination (clearance) determine the drug’s half-life. The half-life describes the decline in drug concentration after absorption. Depending on the drug’s half-life, the drug’s elimination will cause the concentration be too low, at a certain point, to cause significant responses. Therefore the drug’s half-life helps answering the question of 'how often' to administer a drug.

**Why is in silico ADME needed?** Traditionally, drugs were discovered by testing compounds synthesized in time-consuming multi-step processes against a battery of in vivo biological screens. Promising compounds were then further studied in development, where their pharmacokinetic properties, metabolism and potential toxicity were investigated. Adverse findings were often made at this stage, with the result that the project would be halted or restarted in order to find another clinical candidate — an unacceptable burden on the research and development budget of any pharmaceutical company. Waterbeemd at al. [60] analyzed the main reasons for attrition in drug development and came to the conclusion, that poor pharmacokinetics and animal toxicity account for half of all failures in drug discovery.

As the capacity for biological screening and chemical synthesis have dramatically increased, so have the demands for large quantities of early information on absorption, distribution, metabolism and excretion (ADME) data. Various medium and high-throughput in vitro ADME screens are now in use. In addition, there is an increasing need for good tools for predicting these properties to serve two key aims in drug discovery: First, at the design stage of new compounds and compound libraries so as to reduce the risks of late stage attrition; and second, to optimize the screening and testing by looking only at the most promising compounds [60].

In the present thesis, a physiologically based pharmacokinetic (PBPK) model will be presented. It is conceptionally represented in figure 2.1 and 2.2. The model derivation will be particularly emphasized. Adequate approximation of species-physiology, together with the use of drug-specific in vitro data is aimed to provide applicability to a large number of substances. Physiological settings can be changed in order to ask questions towards therapeutics, pharmacology and to evaluate toxicology analysis. The model integrates experimentally derived in vitro data and provides simulations of cell, interstitium, erythrocyte and blood plasma kinetics, approximating in vivo conditions. It can be used to predict and analyze concentration-time profiles of drug candidates in cellular-, interstitial-, erythrocyte- and
plasma compartments, prior to \textit{in vivo} studies in mammals. By changing physiological data (e.g. allometric scaling, clearance), evaluation of risk analysis and prediction of drug dosing regimen (e.g. patient optimized drug treatments) can be performed. PBPK models can serve as diagnostic tools, helping to reveal new processes involved in drug absorption, distribution, metabolism and elimination (as shown by Takanaga et al. [54] and Sugita et al. [53]), by taking seemingly \textit{false} ADME/Tox predictions as starting points for experimental analysis. So far, however, parametrization remains the critical point in developing a physiologically based pharmacokinetic model. Each drug under investigation requires essential drug-specific input parameters. In the present thesis we will test the developed PBPK model in application to tolbutamide, an insulinotropic sulfonyleurea used in diabetes II treatment. We chose tolbutamide mainly, because it is a well studied drug and many drug-specific parameters can be found in the literature.
Chapter 1

Pharmacokinetics in Drug Design

Pharmacokinetics continue to play an increasingly important role in drug development, starting with drug discovery and lead optimization, pharmacology and safety evaluation, continuing into clinical development and finally helping to position the product in marketplace. The successful development of a drug candidate depends on a collaborative interaction of pharmacokinetics with a number of disciplines. The goals of in vitro and nonclinical studies are to demonstrate, directly or indirectly the biological activity against the target disease, to provide data for toxicology and safety evaluation and to provide pharmacokinetic and pharmacodynamic data, that may be helpful in developing dose regimens and dose escalation strategies in clinical trials. Parameters like the drug’s halflife ($t_{1/2}$), its concentration-time profile and the bioavailability (F) are of particular interest. The drug’s halflife determines the frequency of drug administration and is an interplay of the drug’s distribution and its elimination from the organism. The bioavailability answers the question of ‘how much’ drug to administer. The bioavailability is an interplay of the drug’s elimination during first-pass and its absorption. In order to get these parameters, we need to predict the drug’s absorption, distribution, metabolism and excretion (ADME).

Analytical methods that quantify the compound and its metabolites in relevant biological fluids are a prerequisite for conducting robust pharmacokinetic studies. During the early discovery and development of a novel compound, analytical methods may not always be available and it may not be prudent to invest a lot of resources to develop specific analytical methods. At this stage, limited pharmacokinetic data may be obtained using biological assays or radio-labelled material.
There are different approaches to model drug pharmacokinetics. Figure 1.1 gives an overview over the diverse models. Each of these approaches is more or less qualified for the targeted study objective and each approach requires a different set of input parameters. Therefore, the available data and the study objective designates the pharmacokinetic model.

A non-compartment model (figure 1.1) allows problems to be approached in a more general way, that means, there are fewer assumptions that can be verified easily. It is also possible to focus on a single process (e.g. absorption, distribution) through this approach. This approach attempts to model the response rather than the structure of a process. Therefore it does little to explain why a certain compound exhibits a certain profile.
If model limitations, like verifying assumptions, are met, a 'traditional' compartment model may be useful. However, there is little physiological and anatomical relevance of the compartments (figure 1.1). This approach follows the principle, that 'simpler is better'. The researcher starts with a minimum number of compartments for the entities studied. Additional compartments are included, only if the data suggests that they are necessary. Thus, a one-compartment model will be used unless the data can be shown to "require" a more complex model, such as a two-compartment model. Although the analyses are more involved than non-compartmental analysis, the developed compartmental models can be useful in extrapolating dosage regimen calculations and in the investigation of mechanisms of drug absorption and disposition. 'Traditional' compartmental analysis provides a middle ground between non-compartmental analysis and physiologically based pharmacokinetic analysis.

Physiologically based pharmacokinetic (PBPK) models are specific compartmental models. The PBPK compartments represent physiological compartments, such as organs (see figure 1.1). PBPK models include blood flow, distribution and elimination from individual organs and tissue groups. Therefore, they are more realistic and meaningful than the other previously discussed model approaches, but they also require an improved theoretical basis for predicting pharmacokinetic effects. Because of the complexity of this approach, many parameters and assumptions may not be verified. This approach is most useful to explain the pharmacokinetics of a compound and to identify the kinetics and actions of a new compound. PBPK models are very useful in answering 'what if' questions (e.g. regarding physiological issues).

In this thesis a novel physiologically based pharmacokinetic model will be presented. The developed model is conceptionally represented in figure 2.1 and 2.2. The following chapter provides an overview of physiologically based pharmacokinetics with special emphasis on ADME processes and their mathematical formulations.

1.1 Physiology

During the testing of new compounds, animal models as well as healthy, young, male volunteers (or fatally ill volunteers) are being tested for their drug response. Taking into account that most drugs target different groups than those tested, namely diseased humans of all ages and both sexes, it is important to consider inter-individual [28,46] and inter-species [28] variation on drug response. Those variations in drug response mainly correspond to different morphologies between the individuals.
A major step in the developing of new compounds is the shift from animal testing, which is obligatory in order to get a compound approved, to clinical testing in human. Since it is absolutely unacceptable to risk human life for compound testing, the results from previous animal testing have to be used to calculate human dose regimen. This task is by far not simple and requires absolute preciseness and awareness of all important factors. Once the drug response has been tested in human, the next obstacle is to consider how gender, age and body size may influence the drug response. A challenging point hereby is to account for the different metabolic capabilities during individual development. A child, for example, due to its different ontology, may not be treated like a small adult [5] in pharmacokinetics. The same principle accounts for different genders, chronic diseases (e.g. alcohol abuse) and any age-related differences, since many processes diminish with their demands. Besides different metabolic capacities, organ sizes have to be considered. It has been recently shown [27] that genetic polymorphisms, not noticeable in everyday life, may have severe influences on drug kinetics, especially if the drug-metabolizing enzymes are affected.

It is worth mentioning, that the basic tissue composition (fraction of water, neutral lipids, phospolipids and proteins) of organs is assumed to vary little between species and individuals. In some cases, however, differences may occur, if we consider diseased organs and gender-specific differences (women generally have a higher sub-cutaneous fat content). These differences are small, however, and may be negligible in the early stages of the drug design process.

1.1.1 Species Differences

Most drugs are being developed in order to be used in human. Nonetheless, it is mandatory to test the final drug candidates in animals prior to clinical testing in human. Animal testing may produce important drug-specific in vivo data. This data can be decisive in choosing the final drug from the developed candidates. Nonetheless, every now and then drugs show different absorption-, distribution-, metabolism-, excretion- and effect characteristics (ADME+effect) in humans compared to the ADME+effects in the tested animals.

Evolutionary divergence has supplied all species with different features, regarding their physiology, in order to adapt to environmental circumstances. Some of those may be obvious, like the body size, if we compare a mouse and a human. The organism and thus the organs of a species, however, are adapted to their demands. Since these demands may vary, the organs of a species are assumed to differ in relative size and performance towards a
certain function. Differences in protein expression and appearance are assumed to occur. Unlike natural (species-specific) effector-proteins, drugs are not designed in accordance to these differences. The differences in protein expression and appearance between species might, however, effect regulatory networks that are involved into the ADME+effect of a drug. By this means, side effects of drugs might occur in human, but not in the tested animals (or vice versa). The intensity of the drug effect between species might also vary. Differences in protein expression and appearance are generally assumed to influence processes involved in drug absorption, -distribution, -metabolism, -excretion and -effect as described in more detail later. While it is possible to roughly approximate organ sizes between two well studied species like rats and humans [19,25], differences in ADME+effect of a compound are rather difficult to predict.

1.1.2 Inter-Individual Differences

When it comes to drug treatment, inter-individual differences have to be considered. Physiologically important sizes are determined by a diversity of factors such as gender, age, body size, genetic limitations, environmental influences, chronic diseases and racial differences. Except for inevident genetic factors, these differences can somehow be related to each other and therefore be integrated into a physiologically based pharmacokinetic model.

**Gender, Age and Size.** Allometric scaling approaches [7, 46] have done a great deal in describing age, body size (weight, height) and gender-specific differences. The main principle of the allometric scaling theory is an exponential correlation between different body sizes. The general form of an allometric equation is:

\[ Y = aX^b, \]

where \( X \) is a known parameter, \( a \) is a scaling factor and \( b \) is the exponential factor, typically around 0.75 [18]. \( Y \) is the unknown quantity. These allometric scaling approaches can take different correlations between body sizes for infants, for example, into account by applying distinct equations for infants and non-infants. Normally, these equations are derived by regression analysis of huge data sets. By implementing these equations into a PBPK model, it is possible to simulate inter-individual variations based on the above mentioned considerations.

**Genetic Polymorphisms.** Genetic polymorphisms may be hard to detect, if they have no every day life effect. Some, however, may result in different drug
responses compared to the major genotype. The responsible mutations will alter drug-specific interactions with the organism, such as absorption, protein binding, drug metabolism or drug-specific effects. Genetic influences can be simulated with a physiologically based pharmacokinetic model: A genetic variation in the absorption mechanisms will change the rate of absorption and can therefore be simulated by an equivalent change in the corresponding parameters. Changes of the drug-specific protein binding will alter the fraction of unbound drug and therefore change the concentration of interactive drug. Alterations in drug-specific metabolism can be modelled by changing the corresponding drug-specific clearance \[27\] (theoretical volume that is cleared of drug within a certain period of time). A change in the effectiveness of the drug can be simulated by correcting the corresponding drug-effect parameters.

Generally, the effects of genetic polymorphisms correspond to either a change in the concentration of the interacting proteins (involved in absorption, protein binding, drug metabolism or drug-specific effects) or to a change in the effectiveness of the interaction. These kinds of effects can be simulated like a competitive, un-competitive or mixed inhibition, as discussed later.

Others. Other factors that may change the drug response are environmental influences, chronic diseases and the patients lifestyle. Those factors could in principle be included as well, but are not considered in the present study.

1.2 Pharmacokinetics

Drugs have different features, like their solubility, their size and their ability to interact with other compounds in the body. The fundamental characteristics of a drug can be divided into two groups: Characteristics that influence the drug’s distribution (ADME; Absorption, Distribution, Metabolism and Excretion) in the organism and characteristics which describe the drug’s effects (pharmacodynamics) within the body. In a drug discovery setup, the compounds are tested for their effectiveness \textit{in vitro}, determined by their \(IC_{50}\) value. —the value at which 50% of the drug’s target is inhibited (by the drug). Furthermore, the most promising compounds are being optimized with regard to their solubility characteristics [33]. Here, the Lipinski rule-of-five [30,31] is frequently used as a guideline. In summary, through application of these rules the drug is aimed to be provided with the ability to be water-soluble and to be able to cross a cellular bilayer membrane by free diffusion, so that the drug can reach its intended destination in the body. Thus, the distribution characteristics influence the \textit{in vivo} effect of the compound.
The absorption processes in the gastro-intestinal tract determine the bioavailability, which is the fraction of drug that enters the system. These processes also determine the time after administration when we will have to face the drug’s effect. The amount of absorbed drug corresponds to the presence of transporter proteins as well as the lipophilicity of a drug.

The gastro-intestinal (gI) tract is a medium with gradually changing pH-value, so that the pK-value of a drug determines its ionization level (see equation 1.1 and 1.2). A pK value of 3 means that 50% of the compound is ionized, when dissolved in a medium with a pH-value of 3. The ionization has a major influence on the ability of a drug to cross bilayer membranes and therefore the ionization has a major influence on the absorption of a drug. In summary, it can be said that the pK-value of a drug determines the time, when most of the drug is going to be absorbed (either through free diffusion or transporter-mediated transport). The underlying mechanism is called ‘ion-trapping’.

Within the human body, changes in the pH-value of major compartments are assumed to be relatively small compared to the pH differences in the gI-tract. For non-basic compounds, ‘ion-trapping’ processes are assumed to have small influences on the distribution characteristics within the major compartments of the organism. Lysozymes (small cellular components for bio-degradation), however, exhibit an acidic profile. Basic compounds may be trapped within the lysozymes by ‘ion-trapping’ processes.

The main diffusion processes are passive diffusion and transporter-mediated diffusion. The passive diffusion processes are assumed to be solely determined by the drug’s solubility and the composition of the membrane. Transporter mediated diffusion processes may vary from one cell type to the other and are difficult to predict. PBPK models may, however, give indications for the presence of these transporters. These indications should then be analyzed and verified (or falsified) by an experimental setup.

The ‘free-drug’ theory states that only unbound drug is able to interact with the organism. Therefore, protein binding determines the fraction of drug that is available to diffusion, metabolism and drug-target interaction (effect). While the fraction of bound drug can be determined in the blood plasma, protein binding within the cells remains a challenging issue. If a drug is bound in the cells, the presence and amount of binding proteins may vary from one cell type to the other. Because it is too time (and money) consuming to determine these factors, cellular protein binding is frequently neglected. However, the fraction of compound, that is unbound in the cellular space, can be estimated by the drug’s membrane affinity and by the composition of the corresponding tissues [43].

There are different routes of drug elimination in the organism. Compounds
may directly be excreted and therefore cleared from the organism. They might also be degraded within the organism, in order to facilitate their excretion and elimination.

Drug metabolism may, by changing the appearance of a drug, result in various outcomes. Drug conversion may result in ineffective metabolites. The speed of metabolism, therefore, corresponds to the clearance of parent compound from the body. Examples are warfarin and tolbutamide. In other cases the drug metabolite is active, while the administered compound is inactive. In this case, the rate of metabolism determines the amount of active drug. An example is Ximelagatran (activation through reduction to melagatran). Taking these assumptions together, the drug metabolism determines the overall concentration-time profile of active drug by limiting its breakdown, its clearance or its production. Most drugs are assumed to be metabolized in the liver. The rate of metabolism can be determined experimentally by *in vitro* experiments.

Drug effectiveness can be correlated to the previously performed high throughput screening experiments. However, since an *in vitro* experiment represents a simplified system, side-effects will be revealed by *in vivo* experiments only.

In summary, the main pharmacokinetic processes of a drug involve absorption, distribution, metabolism, excretion and the processes involved in drug effects (ADME+effect).

### 1.2.1 Routes of Administration

Depending on the drug under consideration, there are several possible routes of administration. These include:

- intra-venous (i.v)
- intra-muscular (i.m.)
- subcutaneous (s.c)
- by inhalation
- mucous(e.g.intranasal)
- transdermal
- oral (p.o.)

Certain drugs take certain routes only. However, the most important routes are oral administration (p.o.) and i.v., since almost all drugs can be administered intra-venously. Intra-venous administration can be realized mathematically by increasing the concentration in the vein with the amount of
administered drug. This model implies that the venous blood is well stirred. Most drugs are optimized, so that they can be administered as tablets in order to take the oral route into the body. The processes involved in oral absorption are far more complex than those of i.v. administration and will therefore be discussed in more detail.

**Oral Absorption**

The underlying mechanisms of drug absorption (after oral administration) in the gI-tract are quite similar to the processes involved in diffusion, which are basically free diffusion through the bilayer membranes and transporter-mediated diffusion. Diffusion processes are described in detail in the subsection 1.2.2 on page 15. Since the gastro-intestinal (gI) tract is a medium with gradually changing pH-value, another factor gains importance: Most drugs are small, weak electrolytes. However, hydrophobic cellular membranes are more permeable to non-ionized compounds (figure 1.2). The fraction of non-ionized compound (and therefore the permeative performance of the compound) depends on the pK-value of the compound as well as the pH-value of the surrounding biophase. Henderson and Hasselbalch [17,51,52] formulated an equation which describes this correlation:

In the case of weak acids:

$$\log_{10} \frac{[\text{ionized}]}{[\text{non-ionized}]} = pH - pK$$

(1.1)

and in the case of weak bases:

$$\log_{10} \frac{[\text{non-ionized}]}{[\text{ionized}]} = pH - pK$$

(1.2)

where pK is the negative logarithm to the basis 10 of the equilibrium constant (dissociation constant) K. Generally, we can say that drugs appear in their ionized form, if they are exposed to an environment that corresponds to their pH-opposite. Acids are ionized to a higher degree if they are exposed to a high pH-milieu (basic environment) and bases are ionized to a higher degree if they are exposed to a low pH-milieu (acidic environment). The processes involved in drug absorption can be implemented into a mathematical model in different ways. One way is to develop an explicit mathematical model of the gI-tract with a time dependent pH-gradient [3,26,64], where the time dependence simulates the digestion process. Site-specific transporters will be included at their corresponding locations. This explicit model does not require \textit{in vivo} data.
Another model considers the GI-tract as a black box. Therefore, this model depends on appropriate \textit{in vivo} data, such as the mean absorption time (MAT) and the total fraction of absorbed drug, called bio-availability (F). Hereby, the parameter for the mean absorption time implicitly includes the time dependent ionization and absorption characteristics of a drug. An implicit model for the oral drug absorption has been proposed by M. Weiss [63]. The fraction of absorbed drug at a given time $f_A(t)$ is calculated using the inverse Gaussian distribution as a model of the input time distribution:

$$f_A(t) = F \sqrt{\frac{\text{MAT}}{2\pi \text{CV}_A^2 t^3}} e^{-(t-\text{MAT})^2/2\text{CV}_A^2 \text{MAT} t}$$  \hspace{1cm} (1.3)

The parameter $\text{CV}_A^2$ is the normalized variance of the distribution. By multiplying the absorbed fraction at time $t$ ($f_A(t)$) with the initially, orally administered amount of drug, we receive a mass flow equation. In the present study we have used this model to simulate oral drug absorption.

![Figure 1.2: 'Ion-trapping'. The pH-value on each side of the membrane determines the steady state between ionized and unionized drug.](image-url)
1.2.2 Distribution

Three major processes are involved in the distribution of a compound: protein binding, passive diffusion and carrier-mediated diffusion (figure 1.3). Drugs are usually optimized in order to increase their ability to passively permeate solid, hydrophobic membranes, since carrier-mediated transport and some aspects of protein binding are rather hard to predict. Lipinski has postulated a few features in 1997 [30, 31], that are commonly found in orally active drugs. These features increase the drug’s ability to passively diffuse through membranes. Lipinski’s concept is called the rule-of-five: A drug should have:

- not more than 5 hydrogen bond donors (OH and NH groups)
- not more than 10 hydrogen bond acceptors (notably N and O)
- a molecular weight below 500
- a LogP below 5.

The first two rules guarantee that the drug is not too water-soluble. This feature is important for the drug’s ability to cross hydrophobic membranes passively (these rules can be seen as a lower bound for its lipophilicity). The third rule minimizes steric hindrance, and therefore enables the drug to diffuse through more loose membranes, such as the capillary membrane. The fourth rule guarantees that the drug will not be stuck in the membrane. The partition coefficient LogP between a lipophilic solvent (commonly octanol) and a hydrophilic solvent (normally water) is an indicator for the drug’s lipophilicity and thus its ability to cross a lipophilic barrier, such as the bilayer membrane of cells (this rule describes an upper bound for the drug’s lipophilicity).

**Protein Binding.** Drugs can be bound to macromolecules. Protein binding defines the fraction of a drug (within a certain compartment in the body), that is free to diffuse, interact and interfere in the metabolism, since the bound drug is coated. The amount of binding proteins and their affinity to the drug determine the concentration discrepancy between two membrane-separated compartments in the equilibrium, since only the unbound fraction ($f_u$) of drug is able to passively diffuse through a membrane, or to be transported through a membrane (see figure 1.3). This principle can be expressed mathematically:

$$f_u = \frac{C_u}{C}$$

(1.4)
Figure 1.3: Diffusion processes. Unbound drugs can either be transported through membranes or diffuse passively. Therefore, drug binding, as well as the net flux of the transport systems will determine the concentration discrepancy between the two sides of a membrane in the equilibrium.

Where \( C \) denotes the total concentration of a drug and \( C_b \) and \( C_u \) are the concentrations of bound and unbound drug respectively. It follows, that:

\[
\Rightarrow f_u = \frac{C_u}{C_u + C_b}
\]  

(1.5)

In order to compute the unbound fraction independently from the concentration of the protein drug complex, we introduce \( K_D \), the dissociation constant for a particular binding process. If we consider the most simple case, where we have one binding protein with one binding site, \( K_D \) becomes:

\[
K_D = \frac{C_u C_{bp}}{C_b}
\]

Where \( C_{bp} \) denotes the concentration of the binding protein (bp). From that, we can form:

\[
C_b = \frac{C_u C_{bp}}{K_D}
\]
and fit it into equation (1.5):

\[ f_u = \frac{C_u}{C_u + \frac{C_u C_{bp}}{K_D}} \]

From here we get:

\[ f_u = \frac{K_D}{K_D + C_{bp}}. \]  \hspace{1cm} (1.6)

Hence, for the determination of the unbound fraction of a drug, we need to know two parameters: The concentration of the binding protein, \( C_{bp} \), and the binding affinity of the protein-drug interaction (here expressed in terms of the dissociation constant \( K_D \)).

Some (transporter-) proteins have more than one binding site for a certain drug. In the case of the currently studied drug, tolbutamide, we have three distinct binding sites with comparable affinity [2] on the corresponding transporter-protein, albumin. A mathematical formulation for this circumstance is given by:

\[ C_{bp1} + C_{bp2} \rightleftharpoons C_{b1} \]

\[ C_{bp1} + C_{bp2} \rightleftharpoons C_{b2} \]

\[ C_{bp1} + C_{bp2} \rightleftharpoons C_{b3} \]

where \( C_{bp1}, C_{bp2}, C_{bp3} \) are the respective binding proteins and \( C_{b1}, C_{b2}, C_{b3} \) are the respective concentrations of the drug-protein complexes. If the binding occurs with the same affinity and the binding sites are located on the same protein, as for tolbutamide-albumin binding, we have:

\[ C_{bp1} = C_{bp2} = C_{bp3} \]

and

\[ C_{b1} = C_{b2} = C_{b3} \]

with respect to equation 1.5, we write:

\[ f_u = \frac{C_u}{C_u + C_{b1} + C_{b2} + C_{b3}} \]

\[ = \frac{C_u}{C_u + 3C_{b1}} \]

which is

\[ f_u = \frac{C_u}{C_u + 3\left[\frac{C_u C_{bp}}{K_D}\right]} = \frac{K_D}{K_D + 3C_{bp}}. \]  \hspace{1cm} (1.7)
Passive Diffusion. In the late 1800's, E. Overton discovered that substances that dissolve in lipids pass more easily into the cell than those that dissolve in water [4]. This was some of the first evidence that cells are surrounded by a lipid membrane. The phospholipid membrane can greatly modify the permeation of molecules into a cell. The membrane acts as a barrier to passive diffusion of water-soluble molecules. The correlation between permeability and solubility in lipids is appropriately named Overton's Rule [4]. Passive diffusion is generally assumed to be a non-saturable process (figure 1.4) within the expectable drug concentration ranges. Passive diffusion is a random movement of molecules but has a net direction towards regions of lower concentration. It is assumed that passive diffusion tunes into an equilibrium. The rate of diffusion is affected by properties of the cell, the diffusing molecule and the surrounding solution. The rate of diffusion, named \( \frac{dn}{dt} \), is the change in the number of diffusing molecules inside the cell over time. The net movement of diffusing molecules depends on the concentration gradient of molecules, that are able to pass the barrier. For drugs it is assumed that binding to macromolecules or non-dissolved structures limits the rate of diffusion. Unbound drug, however, is able to permeate a membrane. Therefore we assume, that the rate of diffusion is directly proportional to the concentration gradient, \( \Delta C_u \), of unbound drug across the membrane. The concentration gradient is the difference in molecule concentration inside and outside of the cell across a cell membrane with a certain width. This is equivalent to \( C^x_u - C^y_u \) where \( C^x_u \) and \( C^y_u \) are the concentrations of unbound substrate outside and inside the cell. When the concentration outside the cell, \( C^x_u \), is larger than inside the cell, \( C^y_u \), the concentration gradient \( \Delta C_u \) will be positive, and net movement will be into the cell (positive value of \( \frac{dn}{dt} \)). We can describe the rate of diffusion as directly proportional to the concentration gradient by the following equation:

\[
\frac{dn}{dt} = P \times A \times \Delta C_u
\]

where \( A \) is the membrane area and \( P \) is the permeability constant. \( P \) is a constant relating the ease of entry of a molecule into the cell to the molecule’s size and lipid solubility. If we call \( P \times A \) the diffusion coefficient \( D^{xy}_{\text{drug}} \) of a drug between the compartments \( x \) and \( y \), we derive the mass flow equation:

\[
\frac{dn}{dt} = D^{xy}_{\text{drug}} (C^x_u - C^y_u)
\]

(1.8)

Since we assume, that only the unbound fraction \( (f_u) \) of the drug is able to pass the membrane, the equilibrium for the drug concentration between both sides of the membrane is determined by the amount of bound/unbound drug on each side of the barrier at some steady state (ss). This means, that
if the compound is able to cross the membrane for passive diffusion, the amount of binding proteins and their affinity to the compound determines the total concentration of drug on each side of the membrane (figure 1.4) in the equilibrium. The parameter which describes this steady state condition is called the partition coefficient $P$:

$$P^{x:y} = \frac{C^{ss,x}}{C^{ss,y}}$$

where $x$ and $y$ represent the two sides of the membrane and $C^{ss,x}$ and $C^{ss,y}$ are the total concentrations (bound and unbound) of the compound on each side of the barrier in the steady state. Considering only passive diffusion, a net flux of unbound drug will be towards the region of lower concentration, as discussed before. Therefore, in a steady state, we have:

$$C^{ss,x} = C^{ss,y}$$

where $C^{ss,x}_u$ and $C^{ss,y}_u$ are the concentrations of unbound compound on the corresponding sides $x$ and $y$ of the membrane at steady state. If we consider linear protein binding, it follows that:

$$f^x_u C^{ss,x} = f^y_u C^{ss,y}$$

where $f^x_u$ and $f^y_u$ are the fractions (of the total compound concentration) of unbound drug.

$$\Rightarrow \frac{C^{ss,x}}{C^{ss,y}} = \frac{f^y_u f^x_u}{f^y_u f^x_u} = \frac{f^y_u}{f^x_u} = P^{x:y}. \quad (1.9)$$

This means that, if the drug under consideration is less bound on side $x$ of the membrane than on side $y$, the total concentration of the drug will be less on side $x$ than on side $y$ at steady state and vice versa. Taking these considerations (equations 1.4, 1.8 and 1.9) together, we derive the following equation for the rate of diffusion:

$$\frac{dn}{dt} = D^{x:y}_{\text{drug}} \left( f^x_u C^x - f^y_u C^y \right) \quad (1.10)$$

where $\frac{dn}{dt}$ is the flow of permeating drug, $D^{x:y}_{\text{drug}}$ is the diffusion coefficient for the drug under consideration, between the two sides of the membrane $x$ and $y$. $C^x$ is the concentration of drug on side $x$ and $C^y$ is the concentration of drug on side $y$ of the membrane. $P^{y:x}$ is the partition coefficient between $y$ and $x$ for the drug under consideration.

Membranes are assumed to be more or less homogeneous. For a loose membranes, the passive diffusion processes involve absorption along with bulk
flow of water. This is the major mechanism across capillary endothelial cell membranes. The drug’s molecular size should be less than 100 to 200 dalton and only unbound drug is absorbed. In the present model, we refer to this kind of diffusion as diffusion from plasma to the interstitial space, $D_{p:i}$. If this membrane does not form a barrier, the diffusion coefficient will not be a limiting factor; the rate of diffusion will be high (blood flow limited). Passive diffusion through a solid membrane is limited by the drug’s lipophilicity and therefore by its ability to cross the membrane. Solid membranes form barriers between the interstitial and cellular space, as well as between the red blood cells and the plasma. Diffusion through these barriers will be denoted $D_{i:c}$ and $D_{c:p}$ in the present model.

**Transporter-mediated Diffusion.** Carrier-mediated transport is a saturable diffusion process. Unlike passive diffusion, facilitated diffusion involves a limited number of carrier proteins. At low concentrations, molecules pass through the carrier proteins in a way similar to that of passive diffusion. At high solute concentrations, however, all the proteins are occupied with the diffusing molecules. Increasing the solute concentration further will not change the rate of diffusion. In other words, there is some maximum rate of diffusion ($V_{max}$) when all carrier proteins are saturated. Therefore, we can not use a simple linear equation to describe the rate of diffusion. The rate of diffusion will increase with increasing solute concentration, but must asymptotically approach the saturation rate, $V_{max}$ (figure 1.4). How quickly the carrier proteins become saturated can be described by the variable $K_m$, the concentration, at which the rate of diffusion is $1/2 V_{max}$. The underlying kinetic modelling approach is called Michaelis-Menten kinetic. $K_m$ and $V_{max}$ depend on properties of the diffusing molecule, such as its permeability ($P$), as well as the surface area ($A$) of the cell, but for simplification we give the equation as:

$$\frac{dn}{dt} = \frac{V_{max}}{1 + \frac{K_m}{\Delta C}}$$

Where $\frac{dn}{dt}$ is the net movement of the drug, $V_{max}$ is the maximum rate of diffusion, $K_m$ is the compound concentration at which the rate of diffusion is $1/2 V_{max}$ and $\Delta C$ is the concentration gradient of drug. Thus the equation can be reformulated:

$$\frac{dn}{dt} = \frac{V_{max}}{1 + \frac{K_m}{C^x - C^y}}$$

Where $C^x$ and $C^y$ are the concentrations on the respective sides of the membrane (outside and inside). If we consider, as assumed for free diffusion, that
only unbound drug is able to be transported, this equation becomes:

\[
\frac{dn}{dt} = \frac{V_{\text{max}}}{1 + \frac{K_m}{C_{xu} - C_{yu}}}
\]

(1.11)

where \(C_{xu}\) and \(C_{yu}\) are the concentrations of unbound drug on the respective sides of the membrane. If we have two independent transport mechanisms, one that accounts for the influx and one that accounts for the efflux transport, the equations can be transformed into classical Michaelis-Menten equations:

\[
J^{x:y} = \frac{V^{x:y}_{\text{max}} C^{x}_{u}}{K^{x:y}_{m} + C^{x}_{u}}
\]

(1.12)

and

\[
J^{y:x} = \frac{V^{y:x}_{\text{max}} C^{y}_{u}}{K^{y:x}_{m} + C^{y}_{u}}
\]

where \(J^{x:y}\) and \(J^{y:x}\) are the respective fluxes and \(V^{x:y}_{\text{max}}\) and \(V^{y:x}_{\text{max}}\) their corresponding maximum velocities.

1.2.3 Metabolism

Metabolism is the enzymatic biotransformation of drugs. Metabolites are generally more polar and less lipid-soluble than the parent drug. As a result, tissue penetration and renal tubular resorption are decreased and elimination is enhanced. Metabolites are usually inactive or less active than the parent compound. However, some drugs are converted to active derivatives. Although biotransformation may occur at several sites throughout the body, the liver is the primary site of conversion. Hepatic metabolism occurs via phase I or phase II reactions. Phase I reactions are classified as non-conjugative and can produce active or inactive metabolites. The reactions include oxidation, hydrolysis, and reduction. The cytochrome P450 enzymes are responsible for most oxidation reactions. Phase II reactions are conjugative and generally produce water soluble, inactive compounds that are renally eliminated. In the present example we consider phase I reactions only. In the present model these reactions can directly be related to the inactivation of the compound and thus to the elimination of the compound.

Clearance. The metabolic clearance describes the theoretical volume that is cleared of drug in a given time period and is expressed in volume/time. It determines the steady-state concentration for a given dose.

Hepatic Clearance. Hepatic (‘in vivo’) clearance (\(\text{Cl}_{h}\)) is determined by the
Figure 1.4: Saturable and non-saturable diffusion processes. The left picture shows a graph, where the rate of diffusion is a simple linear function of the concentration gradient, such as in passive diffusion processes. Notice, that the rate of diffusion increases as the concentration gradient increases. If the concentration of molecules outside the cell is very high relative to the internal cell concentration, the rate of diffusion will also be high. If the internal and external concentrations are similar (low concentration gradient) the rate of diffusion will be low. The right picture shows saturable diffusion kinetics. By graphing the corresponding equation, we see that at low concentrations of solute, the rate of diffusion into a cell occurs almost linearly, like simple diffusion. Notice that at low solute concentrations, the slope is much steeper than that of simple diffusion. Facilitated diffusion can increase the rate of diffusion of particular molecules at low concentrations. However, the rate of facilitated diffusion levels off with increasing solute concentration. Additional increases in external solute concentration cannot increase the rate of diffusion once carrier proteins are saturated.

Liver blood flow (\(Q_h\)) and the efficiency of the liver in extracting drug from the bloodstream. Hepatic ('in vivo') clearance is affected when blood flow is altered or when the extraction ratio changes. The extraction ratio increases when enzyme inducers increase the amount of drug-metabolizing enzyme. It decreases when enzyme inhibitors inhibit drug-metabolizing enzymes or necrosis causes loss of parenchyma (loss of functional liver tissue). Thus, extraction ratios are determined by the drug-metabolizing capacity of hepatic enzymes. Likewise for the previously described processes, it is assumed that
only unbound drug can enter the hepatic cells and be metabolized. Therefore, protein binding limits the extraction ratios as well. The extraction ratios are classified as high (>0.7), intermediate (0.3-0.7), and low (<0.3).

High-extraction drugs usually have a high intrinsic clearance (Cl\textsubscript{int}). The hepatic enzymes are very efficient and have a high capacity to metabolize drug. In this case, the drug clearance primarily depends on the blood flow to the metabolizing organ (Cl\textsubscript{h} \sim Q\textsubscript{h}) and the drug’s ability to diffuse into the organ. Examples include propranolol, verapamil, morphine and lidocaine.

Low-extraction drugs usually have a low intrinsic clearance. Hepatic enzymes have a low capacity to metabolize drug and therefore drug clearance primarily depends on the intrinsic ability of the organ to clear unbound drug from the blood (Cl\textsubscript{h} \sim Cl\textsubscript{int}). Examples include diazepam, tolbutamide, warfarin, theophylline and phenobarbital.

In our PBPK model physiological influences on the hepatic clearance, such as hepatic blood flow are explicitly included in the PBPK model. Therefore, we use the intrinsic clearance, as described below, to simulate liver metabolism.

**Intrinsic Clearance.** Intrinsic clearance is a pure measure of enzyme activity towards a drug and is not influenced by other physiological determinants of liver clearance such as hepatic blood flow or drug binding within the blood matrix. As with all clearance terms, it has units of volume rate (e.g. L/min) and acts as a proportionality constant to describe the relationship between rate of metabolism of a drug and its concentration at the enzyme site (C\textsubscript{e}) [20,21,41].

\[
\text{Rate}_{\text{metab.}} = \text{Cl}_{\text{int}} C_e
\]

The free concentration of drug (C\textsubscript{u}) within the liver is assumed to equate with C\textsubscript{e}.

\[
\text{Rate}_{\text{metab.}} = \text{Cl}_{\text{int}} C_u \tag{1.13}
\]

From a biochemical viewpoint, Cl\textsubscript{int} can be considered in terms of the enzyme parameters of the Michaelis-Menten relationship shown in equation (1.14)

\[
\nu = \text{Rate}_{\text{metab.}} = \frac{V_{\text{max}} C_u}{K_m + C_u} \tag{1.14}
\]

Where Rate\textsubscript{metab.} (\(=V\frac{dC_{\text{met}}}{dt}\)) describes the decrease of drug over time, V\textsubscript{max} is the maximum reaction velocity and K\textsubscript{m} is the Michaelis-Menten parameter. C\textsubscript{u} is the concentration of unbound drug.

Taking together equation(1.13) and equation(1.14) Cl\textsubscript{int} can be expressed by the Michaelis-Menten parameters:

\[
\text{Cl}_{\text{int}} = \frac{V_{\text{max}}}{K_m + C_u} \tag{1.15}
\]
Under physiological conditions $C_u$ might be 10 % or less of $K_m$, thus we retrieve linear conditions for the rate of metabolism. Equation (1.14) reduces to

$$
\text{Rate}_{\text{metab.}} = \frac{V_{\text{max}}C_u}{K_m}
$$

which is analogues to equation(1.13). Hence,

$$
\text{Cl}_{\text{int}} = \frac{V_{\text{max}}}{K_m}
$$

Thus, the commonly reported Michaelis-Menten parameters can be used to obtain an in vitro intrinsic clearance. In the case of hepatic microsomes, $\text{Cl}_{\text{int}}$ will be expressed in terms of $[\mu L/(\text{min} \cdot \text{mg microsomal protein})]$ and in the case of hepatocytes $[\mu L/(\text{min} \cdot 10^6 \text{ cells})]$ since rates of metabolism are usually measured as $[\text{pmol/min}]$ for micromolar drug incubation concentrations.

1.2.4 Excretion

The major route for the excretion of drugs is the renal excretion. The compounds excreted through the kidney most commonly have increased water-solubilities, like hydroxylated metabolites of a parent compound. The parameter, which describes excretion through the kidney is the renal clearance ($\text{Cl}_{\text{ren}}$). However, some drugs may be excreted in the feces. This route of excretion is called the biliary excretion, since the compounds are excreted together with the bile acid. The major organ for the excretion of gaseous and volatile substances is the lung. An example for this route is the excretion of alcohol. In summary, possible excretion routes are:

- renal excretion
- biliary excretion (into intestine)
- pulmonary excretion
- salivary excretion (swallowed into intestine)

As for the routes of administration, certain drugs will take certain routes only. Drugs are usually eliminated through the kidney by glomerular filtration or active tubular secretion. Many drugs are also secreted into the bile and subsequently pass into the intestine. If enterohepatic recirculation (cycle of biliary secretion and intestinal resorption) does not occur, fecal elimination follows.
Elimination by Metabolism. Many drugs may not be excreted, before they are transformed by enzymes. Therefore, biotransformation by the metabolism might be rate limiting for the elimination of a compound. The rate of drug clearance through biotransformation is described by the parameter $C_{\text{int}}$ and $C_h$. The processes involved in biotransformation are described in detail in section 1.2.3 on page 21.

1.2.5 Effect

Drugs usually effect the homeostatic metabolism. However, in an individual, there is a drug concentration below which the drug is ineffective (the minimum effective concentration), because its effects are levelled out by the organism. There might be a concentration above which the drug starts to have undesirable effects (the minimum toxic concentration). These boundaries define the therapeutic range: -the concentration range in which the drug is effective and save. Most people respond to drug concentrations in the same ranges. However, there is always a possibility that the range will be different in an individual patient.

One theory on the mechanism of drug-action is the occupation theory. It suggests that the intensity of a pharmacological response ($E$) is proportional to the concentration of a reversible drug-receptor complex. A mathematical description of the occupation theory, assuming complete and instantaneous drug distribution, yields to a Michaelis-Menten kinetic:

$$E = \frac{E_{\max}C_u}{EC_{\text{50}} + C_u}$$

(1.17)

where $E$ is the intensity of the pharmacological response, $E_{\max}$ is the maximum attainable value of $E$, $C_u$ is the concentration of free drug at the active complex and $EC_{\text{50}}$ is the concentration of substrate at the enzyme site, where the effect is half of its maximum.

Influences on Drug Effect. In some cases, especially along with long-term drug treatment, the body is able to recover its homeostatic metabolism. The drug-target interaction can be influenced by desensitization or by lowering the target-protein concentration. Desensitization will affect the drug’s ability to cause a pharmacological response, by lowering the affinity to the target. Though, $EC_{\text{50}}$ in equation 1.17 will be altered. Lowering the drug’s target concentration will affect the parameter $E_{\max}$ in equation 1.17, since more target proteins can attain more response.

Adaption processes, such as the levelling out of the drug-aimed response (e.g. by an increase of the production of the protein, which is aimed to be
decreased in concentration) can be simulated by changing the corresponding equations in a pharmacokinetic model (if considered).
Chapter 2

A Physiology Based Model

The idea of physiologically based pharmacokinetic models is to characterize drug distribution to specific organs in terms of relevant anatomical and physiological variables. Tissue compartments represent specific anatomical volumes, connected by the blood circulation. Drug uptake into organs is assumed to be a function of thermodynamic (passive diffusion) and membrane transport (facilitated transport). Individual compartments have either a blood flow limited or membrane-limited structure. Any compartment in the model is assumed to be a well-mixed homogenous space, yielding a single drug concentration.

Model predictions can be conducted under numerous conditions with driving force to answer questions of ‘what will happen’ if a certain parameter is altered. This type of probing questions can be useful in both clinical and preclinical cases that could assist the drug development process. Another application of PBPK models would be to assess novel drug delivery systems because of their ability to characterize drug disposition at cell level.

In this chapter we will particularly discuss the distinct anatomical regions and their interconnections, as implemented in the present PBPK model.

2.1 From Anatomy to Compartments

The present model is a multi-compartment whole-body model. The organs are regarded as functional units, thus integrated as independent units into our physiologically based pharmacokinetic (PBPK) model. The venous and arterial systems are considered as units with limited features. The derived mathematical framework is aimed to be completely general. Some organs, however, are not considered, but can be included, if it turns out that they are important in order to describe drug distribution. The underlying frame-
work of the generic PBPK model is conceptionally represented in figure 2.1 and mathematically described in section 4.1.2 on page 42. With respect to tolbutamide pharmacokinetics some drug-specific simplifications of the model, affecting the routes of administration and the routes of excretion can be applied. We assume that tolbutamide is solely metabolized by the liver. The unmetabolized drug is not excreted. Thus elimination of tolbutamide is modelled by biotransformation in the liver (Cl_{int}). With respect to tolbutamide, possible routes of administration are intravenous administration (i.v.) and oral administration (p.o.).

2.1.1 Organ Topology

The present model considers 12 tissues (lung, brain, heart, muscle, spleen, skeleton, skin, kidney, pancreas, adipose, liver and GI-tract) and 2 interconnecting compartments, namely the arterial blood and the venous blood, as shown in figure 2.1. The organs have been categorized in terms of their function in our model. Since the site, where the drug takes effect, varies for each drug under consideration (tolbutamide: pancreas; diazepam: brain; propanolol: heart, etc.) it has not been used as a category.

- storage: lung, brain, heart, muscle, spleen, skeleton, skin, kidney, pancreas, adipose
- metabolism: mainly liver
- connecting: artery, vein
- oral absorption: GI-tract

Interconnection of the Organs. The interconnection of the organs is derived from anatomical data [1,52] in order to provide a realistic setting. Exchange of drug between the organs occurs via the blood flow. The total blood input has to equalize the total blood output for every compartment in order to guarantee a closed system. The connectivity of the organs is conceptionally represented in figure 2.1. Generally spoken the brain-, heart-, muscle-, gut-, spleen-, bone-, skin-, kidney-, pancreas- and adipose compartments receive all of their input blood from the artery. The liver receives some fraction of its input blood flow directly from the artery, while it also receives blood from the gut, the pancreas and the spleen (portal vein, first pass effect).
The blood output of all compartments except for the gut, pancreas, spleen and lung is gathered in the vein-compartment. The vein itself serves as the sole input source of the lung. Therefore the lung’s blood in- and output will equalize the cardiac output. The lung emits its blood content into the artery. Hence, the cycle is closed. The mathematical relationships are explained in section 4.1.2 on page 42 for mathematical relationships concerning each compartment.

Figure 2.1: Conceptual representation of the generic PBPK framework. The connectivity of the compartments (via the bloodstream) is indicated by solid arrows. Possible routes of drug application are indicated by small yellow arrows. Possible routes of drug excretion are indicated by small red arrows. The metabolizing organ is highlighted in blue. See section 4.1.2 on page 42 for mathematical relationships concerning each compartment.
detail in section 4.1.2 on page 42.

2.1.2 Compartments

In PBPK models, organs are often represented by compartments. In the current model, however, we have implemented a more accurate segmentation. In order to be able to model some of the processes involved in drug disposition in detail, a segmentation of the implemented organs is utilized. In the current model, organs consist of cellular-, interstitial-, plasma- and erythrocyte compartments (see figure 2.2). The cellular fractions of the organs remain the largest part for all organs under consideration. The used segmentation strongly influences the distribution processes of drugs in the way, that it is simulating a more realistic setting. This means that certain transitions from one compartment to the other are forbidden or promoted, depending on our physiological assumptions. The segmentation of the organs is conceptionally represented in figure 2.2.

Organ Constituents: Vascular and Extravascular. The organs are assumed to consist of four major units. The cells, which make the major segment, are surrounded by the interstitial per. In the present model, the interstitial space provides an exchange route between the cells and the blood. The blood provides the exchange route between the different organs. The blood can further be partitioned into its solid constituents (mainly erythrocytes) and its liquid constituents (blood plasma).

Vascular. The blood stream is connecting the different organs and assures that compounds are distributed throughout the body.

Blood consists of liquids and solids. The proportion of solid constituents in the blood is specified by the hematocrit (hct). A hematocrit of 45 means, that 45% of the blood volume consists of solid constituents. The hematocrit is usually around this value of 45 in human. The liquid part of the blood consists of plasma and some dissolved plasma proteins, such as Albumin (60%) and α-, β- and γ-globulins (36%) [12,52], which are able to bind drugs. The solid constituents are erythrocytes, leucocytes and thrombocytes. The erythrocytes represent the biggest proportion of the solid constituents (10³ times more than the other two), so for simplification only they will be considered in the model.

Examining the pharmacokinetic value of erythrocytes in our model (namely distribution processes) erythrocytes obey like normal cells: They are surrounded by a lipid-bilayer membrane that encloses a certain intracellular volume. Drugs that pass into erythrocytes may be bound within the ery-
thromocytes. Thus, because of the different conditions inside and outside of the erythrocytes, a concentration discrepancy, caused by the processes shown in figure 1.3 may occur.

In summary, the vascular proportion of all considered organs consists of two compartments, namely the erythrocytes and the plasma compartment.

![Organ - Compartments](image)

**Figure 2.2**: Conceptional representation of the compartment interconnection. Solid arrows indicate possible transition routes between the sub-compartments with the bulk flow of water (holey membrane). Dashed arrows indicate diffusion processes through solid membranes. Block arrows indicate possible drug transport routes with the blood flow. See section 4.1.2 on page 42 for mathematical relations.

**Extravascular.** The extravascular compartments of an organ are not directly connected with other organs.

Besides the cellular volume, the space in between the cells, the interstitium, has to be considered. The interstitium is important, since it is responsible for supplying the cells with nutrients, and others. In our model, the interstitial space provides an exchange route between the cells and the plasma. Additionally, receptors on the cell surface can solely be accessed through the interstitial space.

The cellular space is the site, where drug metabolism (thus elimination) usually occurs. The cellular compartment may also be the location, where drug-target interaction takes place. The cellular volume might dominate, compared to the other described compartments. Thus, drugs may be ‘stored’
here, causing a slow decline in the overall drug concentration.
In summary, the extravascular space consists of the cellular compartment
and the interstitial compartment. The artery- and the vein, however, are not
considered as real tissues in our model. Thus, in our model they consist of
erthrocytes and plasma.

**Interconnection of the Compartments.** Drug exchange between the dif-
terent compartments within an organ is assumed to be bidirectional and can
occur via the following routes: A compound can enter or leave the erythro-
cytes from (and into) the plasma. The amount of compound, that is dissolved
in the plasma, can diffuse into the erythrocytes, or into the interstitial space
and vice versa. From the interstitial space, the drug can diffuse back into
the plasma or it can enter the cells. The cells are solely connected to the
interstitium, thus compounds can leave the cells only if they diffuse into
the interstitial space. The connectivity between the compartments is shown
conceptually in figure 2.2. The mathematical relationships are formalized in
section 4.1.2 on page 42.

It is assumed, that the bilayer membranes of the cells and the erythrocytes
form solid barriers (see figure 2.2). Diffusion through these membranes is
limited by the drug’s permeability (lipid solubility) and by the speed of fa-
cilitated transport through drug-carriers (see section 1.2.2 on page 15). The
exchange between the interstitium and the plasma, however, is not limited
by the factors mentioned above. It is assumed that the capillary membrane,
the membrane that separates the blood from the interstitium, is holey. Since
most drugs are small molecules, they should be able to flow through these
holes. Thus, the diffusion from the plasma to the interstitium is assumed to
be limited by the blood flow.
Sulfonylureas are a group of hypoglycamic agents, which have been widely used for the treatment of type II diabetes mellitus by oral administration. However, while the \textit{in vivo} and \textit{in vitro} insulinotropic actions of this important class of oral antidiabetic drugs are undisputed, the precise mechanisms underlying the diverse effects of sulfonylureas on the pancreatic beta cells remain an important focus of current research [34–36, 47–50]. Sulfonylureas have a fairly low therapeutic index and it is known that there is a remarkable inter-individual variability in the absorption rate of sulfonylureas [40]. Most sulfonylureas are weak acids with relative high lipophilicity, able to permeate cellular membranes by passive diffusion processes.

Tolbutamide is a first-generation oral sulfonylurea hypoglycaemic agent that is still frequently used for the treatment of non-insulin-dependent diabetes mellitus in many countries. It is metabolized in the liver by CYP2C9 (human hepatic cytochrome P450) to 4-hydroxytolbutamide [62]. 4-hydroxytolbutamide is oxidized by dehydrogenases (CYP2C19) to carboxytolbutamide. Both metabolites are inactive and excreted into the urine. Large inter-individual variations in tolbutamide metabolism were reported and suggested to be caused by genetic influences [27].

Besides its ability to rapidly cross phospholipid bilayer membranes [24] by passive diffusion, several saturable transport mechanisms for tolbutamide have been reported [40, 54]. Likewise the other sulfonylureas, tolbutamide stimulates insulin secretion from pancreatic \( \beta \)-cells. Its principle target is the sulfonylurea receptor, a component of the ATP-sensitive potassium channel (\( K_{\text{ATP}} \)). This channel plays a major role in controlling the \( \beta \)-cell membrane potential. At rest, the \( K_{\text{ATP}} \) channel is open and maintains the membrane potential at a hyperpolarized level that prevents insulin secretion [36].
of the $K_{ATP}$ channel by Glycose metabolism or sulfonylureas [6] causes membrane depolarization which, in turn, triggers Ca\textsuperscript{2+} influx through voltage gated Ca\textsuperscript{2+} channels [22] and thereby stimulates the exocytosis of insulin-containing secretory granules [13]. Unfortunately, tolbutamide effects on insulin secretion can not be explained by an electrically driven mechanism alone, like the one explained before. Recently, besides the effects of tolbutamide on the $K_{ATP}$ channel of the pancreatic $\beta$-cells, evidence strengthened that sulfonylureas (and tolbutamide) have intracellular, possibly $K_{ATP}$ channel independent targets in the pancreatic $\beta$-cells [13, 14, 16, 22, 36]. The proposed insulinotropic mechanisms are shown in figure 3.1. The parameters that describe the effects of tolbutamide, as proposed by J. Kirchheiner et al. [27], are summarized in table 3.1.

It has also been reported, that prolonged exposure of pancreatic islets to insulin secretologues that block $K_{ATP}$ channels induces desensitization of insulin secretion [34, 35, 47–50]. This mechanism gains importance, since poor metabolism of tolbutamide compromises the mean elimination half-life of 7h, so that tolbutamide half-life may extend to 25h [27].

It is the unbound form of tolbutamide that interacts with active sites and exerts the agent’s pharmacologic effect. Tolbutamide is highly bound in the human plasma to human serum albumin. A number of pharmaceutical agents have been demonstrated to displace tolbutamide from albumin [2]. Such displacement has been observed to cause clinically significant and life-threatening hypoglycemia in diabetes.

### 3.1 Absorption

With application to tolbutamide, the general PBPK model can be simplified, since tolbutamide undergoes only certain routes of administration and excretion.

**Routes of Administration.** For Tolbutamide, the most important routes of administration are oral administration, as described in section 1.2.1 on page 13, and intra-venous administration. Intra-venous administration can be performed by a single dose (bolus) or by a continuous administration.
Figure 3.1: Conceptual representation of insulinotropic effect of tolbutamide. Tolbutamide stimulates insulin secretion from pancreatic β-cells. Its principle target is the sulfonylurea receptor, a component of the ATP-sensitive potassium channel ($K_{ATP}$). This channel plays a major role in controlling the β-cell membrane potential. At rest, the $K_{ATP}$ channel is open and maintains the membrane potential at a hyperpolarized level that prevents insulin secretion. Closure of the $K_{ATP}$ channel by glucose metabolism or sulfonylureas causes membrane depolarization which, in turn, triggers Ca$^{2+}$ influx through voltage gated Ca$^{2+}$ channels and thereby stimulates the exocytosis of insulin-containing secretory granules. Exocytosis of insulin-containing secretory granules might be directly stimulated by tolbutamide within the cellular compartment.

In our model, the drug concentration in the vein will be increased by the amount of drug administered intra-venously. Since Tolbutamide undergoes only these two instances of drug application, the other possible routes are not considered in the PBPK model, for simplification.

**Absorption in the GI-tract.** Since inter-individual variations in the transepithelial absorption of tolbutamide have been observed, Nishimura and his colleagues [40] investigated the mechanisms for gastrointestinal absorption of tolbutamide in Caco-2 cells. They initially showed that the permeation rate of tolbutamide was 6 fold larger at pH 6, compared to pH 7.4 (5.78 ± 0.49 nmol(min×cm$^2$)$^{-1}$ compared to 0.911 ± 0.152 nmol(min×cm$^2$)$^{-1}$), which can be explained by the ionization (figure 5.3 and 3.2) of tolbutamide (see section 1.2.1 on page 13): At pH 6, 12% of the total tolbutamide content are non-ionized, whereas only 2 % are non-ionized at pH 7.4.
Besides non-saturable transport processes, Nishimura et al. [40] discovered a facilitated absorption mechanism for tolbutamide through the gut wall. Mathematically, the apical to basal (through the intestine cells, gI-tract lumen to plasma) transport has been expressed as:

\[ J_{A:B} = \frac{V_{\text{max}} C_u}{K_m + C_u} + PC_u \]

where \( J_{A:B} \) is the transepithelial flux (from the apical lumen to the basal lumen), \( V_{\text{max}} \) is the maximum velocity for the saturable transport, \( C_u \) is the concentration of unbound drug, \( K_m \) is the Michaelis-Menten parameter for the saturable transport process and \( P \) is the permeability coefficient for the non-saturable transport. Considering the pH dependency, as discussed before, we need to incorporate drug ionization, which can be obtained by rearranging the equations in section 1.2.1 on page 13:

\[ J_{A:B} = \frac{V_{\text{max}} C_{u,\text{non-ionized}}}{K_m + C_{u,\text{non-ionized}}} + PC_{u,\text{non-ionized}} \]

where \( C_{u,\text{non-ionized}} \) is the concentration of unbound, non-ionized drug. The parameters for the transepithelial transport are summarized in table 3.2. Parameters needed for the absorption model by Weiss [63], as discussed in section 1.2.1 on page 13, have been collected from the literature [27] and are listed in table 3.2.

\[ ^1 \text{Values (} V_{\text{max}} \text{and } P \text{) have to be multiplied with the surface area of the considered tissue} \]
### 3.2 Diffusion Mechanisms

The three major processes for the distribution of a compound are passive diffusion, protein binding and facilitated diffusion, as described previously. Passive diffusion is influenced by physiological factors, as well as the solubility of a compound in a lipid bilayer membrane. The parameter, which describes the permeability through cellular membranes is called the permeability coefficient ($P$). The permeability coefficient is summarized in table 4.5 for tolbutamide.

Protein binding determines the fraction of interactive drug. Furthermore, considering diffusion, it determines the distribution equilibrium between two sides of a membrane, since we assume that only unbound drug can permeate a lipid-bilayer membrane. Protein binding is determined by the amount of binding proteins and their affinity to the compound. Protein binding to tolbutamide will be described in more detail.

Some drug may be transported in- or out of certain tissues by carrier-mediated processes. For tolbutamide, facilitated diffusion processes have been reported and will be described in more detail in the following section.

#### 3.2.1 Protein Binding

Human serum albumin accounts for approximately 60% [12, 52] of the protein in extracellular fluids and serves as the primary serum transport protein. The affinity of albumin for a particular drug determines the rate at which unbound or free drug becomes available to diffuse from the circulation to sites of action and metabolism. If bound, tolbutamide is usually connected to the serum transport protein albumin within the blood plasma and interstitial space of the organs. Jakoby et al. [2] found that albumin binds tolbutamide at three distinct sites with comparable affinity (see equation 1.7). The parameters, that describe the tolbutamide-albumin binding are summarized in table 3.3. Drugs, like warfarin, clofibric acids, salicylic acids, and other sulfonylureas block the binding sites of tolbutamide to albumin, resulting in a higher fraction of unbound drug, if the worst comes to the worst, -an
<table>
<thead>
<tr>
<th>substance</th>
<th>No. of binding sites(n)</th>
<th>$K_D$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tolbutamide</td>
<td>3.0 ± 0.1</td>
<td>21 ± 9</td>
</tr>
</tbody>
</table>

Table 3.3: Binding parameters of tolbutamide to human serum albumin at 37 °C

overdose [56].

3.2.2 Facilitated Diffusion Mechanisms

Certain drugs, might be transported by saturable processes into- or out of certain tissues within the body. These processes may vary for every drug under consideration. With respect to tolbutamide, facilitated diffusion processes have been reported and quantified [40, 54]. Thus, the reported data will be discussed in more detail.

**Blood-Brain Efflux Transport.** In an attempt to determine the reason for the low brain distribution of tolbutamide, Takanaga and colleagues [54] have analyzed the tolbutamide transport processes in the brain. They described the transport of tolbutamide from the brain to the blood via an efflux transport system. They found that both, the luminal to abluminal (into the cell) and the abluminal to luminal permeabilities of tolbutamide consisted of two components; -a saturable process at lower concentrations and an apparently non-saturable process at higher concentrations. Therefore they expressed these mechanisms mathematically as

$$J = \frac{V_{\text{max}}C_u}{K_m + C_u} + DC_u,$$

where $J$ is the flux, $V_{\text{max}}$ and $K_m$ are the maximum velocity and the Michaelis-Menten parameter of the saturable process, $C_u$ is the concentration of unbound drug and $D$ is the diffusion coefficient for the non-saturable process. Since we assume that the plasma to interstitium transport is not limiting the permeation of tolbutamide (it is blood flow limited), the experiment describes the transport through the barrier between interstitium and cellular space. The corresponding parameters are shown in table 3.4².

3.3 Metabolism and Excretion

Since tolbutamide is hardly excreted before it has been metabolized, bilary excretion and excretion by the kidneys is not considered for tolbutamide in

²(mg protein)$^{-1}$ refers to the total content of protein within the tissue, since the amount of transport protein seems to linearly correlate with the total protein content.
Table 3.4: Kinetic parameters for the blood-brain efflux of tolbutamide. $i \rightarrow c$ indicates flux from the interstitium to the cellular space of the brain. $V_{\text{max}}$ and $K_m$ are the corresponding parameters for the maximum velocity and the Michaelis-Menten parameters for the saturable transport system. $D$ is the diffusion coefficient for the non-saturable transport (passive diffusion). Values were taken from Takanaga et al. [54].

<table>
<thead>
<tr>
<th></th>
<th>$i \rightarrow c$</th>
<th>$c \rightarrow i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$</td>
<td>$65.9 \pm 28.9$ [pmol(min mg protein)$^{-1}$]</td>
<td>$128 \pm 65.8$ [pmol(min mg protein)$^{-1}$]</td>
</tr>
<tr>
<td>$K_m$</td>
<td>$7.54 \pm 4.36$ [$\mu$M]</td>
<td>$5.59 \pm 4.21$ [$\mu$M]</td>
</tr>
<tr>
<td>$D$</td>
<td>$4.89 \pm 0.336$ [$\mu$l(min mg protein)$^{-1}$]</td>
<td>$4.43 \pm 0.863$ [$\mu$l(min mg protein)$^{-1}$]</td>
</tr>
</tbody>
</table>

the present study. Furthermore, metabolism of tolbutamide means elimination of active compound, since tolbutamide-metabolites are inactive. Thus, elimination has been modelled by metabolic clearance, as described in section 1.2.3 on page 21.

**Metabolism.** Tolbutamide is transformed into inactive metabolites (through phase I metabolization) by the human hepatic cytochrome P450, CYP2C9. The metabolites are excreted renally. Since the metabolization of the active source-compound means elimination of active drug, explicit modelling of excretion has been neglected. Thus, elimination has been modelled by the metabolic rate, expressed as $\text{Cl}_{\text{int}}$, as described in section 1.2.3 on page 21. The pharmacokinetics of the inactive metabolites have not been considered.

**Genetic Polymorphisms.** Tolbutamide is metabolized in the liver by CYP2C9 to 4-Hydroxytolbutamide. Apparently, several allelic variants of the CYP2C9 gene that code for enzymes with reduced activities are known in caucasians [27]. The most common variants are the allele CYP2C9*2, a 430C>T polymorphism that results in the amino acid exchange Arg144Cys, and the allele CYP2C9*3, a 1075A>C polymorphism that results in the amino acid exchange Ile359Leu. The frequencies of alleles *3 and *2 are about 0.08 and 0.13 in Caucasian populations. J. Kirchheiner et al. [27] studied the effects of these genetic polymorphisms on metabolic tolbutamide clearances. Their results are summarized in table 3.5. Corresponding *in vitro* and *in vivo* data has been reviewed by Wang et al. [62] and can be taken for comparison.
<table>
<thead>
<tr>
<th>genotype</th>
<th>weight [kg]</th>
<th>age [year]</th>
<th>no. ind.</th>
<th>$C_{l_h}$ [L/h]</th>
<th>[ml(min×kg)$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1</td>
<td>81</td>
<td>31</td>
<td>6</td>
<td>0.95</td>
<td>0.195</td>
</tr>
<tr>
<td>*1/*2</td>
<td>81.8</td>
<td>30</td>
<td>4</td>
<td>1</td>
<td>0.204</td>
</tr>
<tr>
<td>*1/*3</td>
<td>75</td>
<td>34</td>
<td>4</td>
<td>0.535</td>
<td>0.119</td>
</tr>
<tr>
<td>*2/*2</td>
<td>70</td>
<td>34</td>
<td>3</td>
<td>0.72</td>
<td>0.171</td>
</tr>
<tr>
<td>*2/*3</td>
<td>71</td>
<td>26</td>
<td>3</td>
<td>0.46</td>
<td>0.108</td>
</tr>
<tr>
<td>*3/*3</td>
<td>75</td>
<td>33</td>
<td>3</td>
<td>0.14</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Table 3.5: Measured values for oral clearance. *1,*2 and *3 indicate the different CYP2C9 alleles. Parameters were taken from Kirchheiner et al. [27]
Chapter 4

Mathematical Modelling and Parametrization

The developed physiologically based mathematical model and its parameters will be presented in the following chapter. For PBPK models, compartments are chosen to represent physiological compartments in the body. This extension towards other modelling approaches [51], makes the model more realistic and meaningful. The compartments as well as the model parameters, such as blood flow, clearance and partitioning coefficients have a physiological meaning. However, it is difficult to determine many of the physiological parameters. Therefore, the main study objective of PBPK models is to explain the pharmacokinetics of a compound, but it can be thought of applications beyond this approach.

The underlying mathematical framework of the developed PBPK model is aimed to be general, so that the model remains applicable to a multitude of different drugs and applications. Some of the parameters, which do not describe general physiological features are chosen with respect to tolbutamide pharmacokinetics.

In the following chapter, the mathematical formulation of the present PBPK model and its parameters will be presented and discussed.

4.1 Mathematical Model

A conventional model framework of 52 compartments is used. The model consists of 12 tissues with 4 compartments for each tissue. Additional compartments are within the venous blood and the arterial blood. The vein and artery consist of 2 compartments only (all together: $12 \times 4 + 2 \times 2 = 52$). The framework is conceptionally represented in figure 2.1 and in figure 2.2. With
respect to tolbutamide pharmacokinetics, the liver was considered as the only site of clearance by metabolism. Therefore other routes of elimination have not been considered in this model, but can easily be integrated, if required. In the current model, as described in the previous chapter, oral administration and intravenous administration have been considered with application to tolbutamide pharmacokinetics. The model is originally written in Matlab® code.

4.1.1 Abbreviations:

<table>
<thead>
<tr>
<th>abbreviation</th>
<th>explanation</th>
<th>abbreviation</th>
<th>explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>volume</td>
<td>C</td>
<td>concentration</td>
</tr>
<tr>
<td>Q</td>
<td>blood flow</td>
<td>D</td>
<td>diffusion coefficient</td>
</tr>
<tr>
<td>P</td>
<td>partition coefficient</td>
<td>e</td>
<td>erythrocytes</td>
</tr>
<tr>
<td>p</td>
<td>plasma</td>
<td>i</td>
<td>interstitium</td>
</tr>
<tr>
<td>c</td>
<td>cellular space</td>
<td>K</td>
<td>constant infusion rate</td>
</tr>
<tr>
<td>( f_A(t) )</td>
<td>fraction absorbed</td>
<td>( \frac{dC}{dt} )</td>
<td>conc. change over time</td>
</tr>
<tr>
<td>Cl</td>
<td>metabol. clearance</td>
<td>( C_0 )</td>
<td>initial administration</td>
</tr>
<tr>
<td>t</td>
<td>tissue/organ</td>
<td>ab</td>
<td>arterial blood</td>
</tr>
<tr>
<td>h</td>
<td>hepatic(liver)</td>
<td>( K_m )</td>
<td>Michaelis-Menten const.</td>
</tr>
<tr>
<td>vb</td>
<td>venous blood</td>
<td>l</td>
<td>lung</td>
</tr>
<tr>
<td>pan</td>
<td>pancreas</td>
<td>sp</td>
<td>spleen</td>
</tr>
<tr>
<td>glI</td>
<td>gl-tract</td>
<td>( f_u )</td>
<td>fraction unbound</td>
</tr>
<tr>
<td>( K_{oral} )</td>
<td>initial oral admin.</td>
<td>Rate_{metab.}</td>
<td>rate of metabolism</td>
</tr>
<tr>
<td>wt</td>
<td>water</td>
<td>nl</td>
<td>neutral lipids</td>
</tr>
<tr>
<td>phl</td>
<td>phospholipids</td>
<td>ex</td>
<td>extravascular (i+c)</td>
</tr>
<tr>
<td>VF</td>
<td>vol. fractions ([l/kg])</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.1.2 Generic Mass Balance Differential Equations

The following equations are aimed to be general. Thus, active transport mechanisms, which are assumed to be drug-specific, are not described here. Passive diffusion between the distinct compartments is considered explicitly, since we assume that apparently all drugs undergo the involved processes.
1. for non-eliminating tissues:

\[
V_t^e \frac{dC_t^e}{dt} = Q_t(C_{ab}^e - C_t^e) - D_t^{ep}(f_u^e C_t^e - f_u^p C_t^p) \tag{4.1}
\]

\[
V_t^p \frac{dC_t^p}{dt} = Q_t(C_{ab}^p - C_t^p) + D_t^{ep}(f_u^e C_t^e - f_u^p C_t^p)
- D_t^{pi}(f_u^p C_t^p - f_u^i C_t^i) \tag{4.2}
\]

\[
V_t^i \frac{dC_t^i}{dt} = D_t^{pi}(f_u^p C_t^p - f_u^i C_t^i) - D_t^{ic}(f_u^i C_t^i - f_u^c C_t^c) \tag{4.3}
\]

\[
V_t^c \frac{dC_t^c}{dt} = D_t^{ic}(f_u^i C_t^i - f_u^c C_t^c) \tag{4.4}
\]

2. for the eliminating tissue:

\[
V_h^e \frac{dC_h^e}{dt} = (Q_h - Q_{pan} - Q_{sp} - Q_{gl}) C_{ab}^e + Q_{pan} C_{pan}^e + Q_{sp} C_{sp}^e
+ Q_{gl} C_{gl}^e - Q_h C_h^e - D_h^{ep}(f_u^e C_h^e - f_u^p C_h^p) \tag{4.5}
\]

\[
V_h^p \frac{dC_h^p}{dt} = (Q_h - Q_{pan} - Q_{sp} - Q_{gl}) C_{ab}^p + Q_{pan} C_{pan}^p + Q_{sp} C_{sp}^p
+ Q_{gl} C_{gl}^p - Q_h C_h^p + D_h^{ep}(f_u^e C_h^e - f_u^p C_h^p)
- D_h^{pi}(f_u^p C_h^p - f_u^i C_h^i) \tag{4.6}
\]

\[
V_h^i \frac{dC_h^i}{dt} = D_h^{pi}(f_u^p C_h^p - f_u^i C_h^i) - D_h^{ic}(f_u^i C_h^i - f_u^c C_h^c) \tag{4.7}
\]

\[
V_h^c \frac{dC_h^c}{dt} = D_h^{ic}(f_u^i C_h^i - f_u^c C_h^c) - \text{Rate}_{metab.} \tag{4.8}
\]

where \( \text{Rate}_{metab.} \) is the metabolic elimination rate of the drug in the cellular space (c) of the liver (h). Our experiments suggested, that the metabolic rate grows linear at physiological concentrations (figure 4.1) with regard to tolbutamide pharmacokinetics, since the concentration of unbound drug \( C_h^c f_u^c \) at the enzyme site was less than 10 % of \( K_m \) after 500mg and 1000mg oral administration of tolbutamide. Thus, the metabolic rate (\( \text{Rate}_{metab.} \)) could be expressed as \( C_{int} C_u \), as discussed in section 1.2.3 on page 21.
3. for lung (vascular: p and e):

\[ V_e \frac{dC_e}{dt} = Q_1(C_{vb} - C_e) - D_1^{ep}(f_u^e C_e - f_u^p C_p) \]  
\[ V_p \frac{dC_p}{dt} = Q_1(C_{vb} - C_p) + D_1^{ep}(f_u^e C_e - f_u^p C_p) - D_1^{ip}(f_u^p C_p - f_i^e C_{ix}) \]  
\[ V_i \frac{dC_i}{dt} = D_1^{ic}(f_u^i C_i - f_u^e C_e) \]  
\[ V_c \frac{dC_c}{dt} = D_1^{ic}(f_u^i C_i - f_u^e C_e) \]

4. for arterial blood:

\[ V_{eab} \frac{dC_{eab}}{dt} = Q_{ab}(C_e - C_{eab}) - D_{ab}^{ep}(f_u^e C_{eab} - f_u^p C_{pab}) \]  
\[ V_{pab} \frac{dC_{pab}}{dt} = Q_{ab}(C_p - C_{pab}) + D_{ab}^{ep}(f_u^e C_{eab} - f_u^p C_{pab}) \]

5. for venous blood:

\[ V_{evb} \frac{dC_{evb}}{dt} = \sum_{t_m} (Q_{tv_m} C_{tv_m}^{evb} - D_{vb}^{ep}(f_u^e C_{evb} - f_u^p C_{pvb})) \]  
\[ V_{pvb} \frac{dC_{pvb}}{dt} = \sum_{t_m} (Q_{tv_m} C_{tv_m}^{pvb} + D_{vb}^{ep}(f_u^e C_{evb} - f_u^p C_{pvb})) + f_A(t)K_{oral} + K \]

where 't_m' stands for all tissues, whose blood disembogues into the venous blood. These tissues are: brain, heart, muscle, skeleton, skin, kidney, liver and adipose tissue.

4.2 Physiological Data

In the present model, physiological data, such as tissue volumes (V), organ surface areas (A) and blood flows (Q) are considered in order to create a more realistic and meaningful model of drug disposition. The compartment volumes influence the amount ([g]) of drug in a certain compartment, while the concentrations ([g/L]) in the considered compartments will be equilibrated by the ratio of the fractions of unbound drug (partition coefficients). Blood flows limit the drug exchange between certain organs and might as
Figure 4.1: Plot of the metabolic elimination rate of tolbutamide versus unbound tolbutamide concentration in the target compartment. At physiological tolbutamide concentrations in the target compartment (here: after a 500mg oral administration), the metabolic rate grows linear.

well limit the rate of elimination and excretion. Membrane surface areas (A) of certain organs are considered to limit permeation of drug into cellular compartments. Surface areas of the various considered organs are described in detail in section 4.3.1 together with the corresponding diffusion coefficients. Physiological parameters are assumed to be species-specific and to correlate
with the size of an organism. However, once derived, they may be used to
describe distribution processes of different compounds.

4.2.1 Volumes

Organ volumes are derived either by allometric scaling [42, 46] or by linear
scaling [44]. For allometric scaling, as implemented in the present model,
parameters like body weight [kg], gender, age [years] and body height [cm]
are needed. This method is only useful for the estimation of human body
volumes, since the equations have been derived from human data. The lin-
ear scaling method has been summarized by Poulin and Theil [44]. Poulin
and Theil [44] have applied linear scaling methods to rat and human data,
with body weight as sole input value. It has been shown, that in the major-
itity of cases, physiological data has a poor linear correlation [18]. By taking
the logarithm we retrieve good correlation. Thus, allometric scaling can
generally predict physiological parameters more precisely (see section 1.1.2 on
page 9) than linear scaling. Nevertheless, linear scaling methods have been
used in the current model, since our allometric equations resulted in unre-
liable predictions. The used body volumes, either derived by linear scaling,
or by allometric scaling are summarized in table 4.1 for comparison. The pa-
rameters to calculate extravascular volume fractions as a (linear) function of
the body weights ($V_{ex} [L/kg body weight]$) have been derived from Poulin
and Theil [44]. Since it is possible to calculate extravascular volumes with
these parameters, some conversions have to be applied in order to obtain total
organ volumes ($V [L]$) and compartment volumes ($V_e, V_p, V_i, V_c$). Conversion
can be performed applying the following equations:

$$
V_e = hct \frac{V_{ex}}{F_i + F_c} - V_{ex} \\
V_p = (1 - hct) \frac{V_{ex}}{F_i + F_c} - V_{ex} \\
V_i = \frac{V_{ex}}{F_i + F_c} F_i \\
V_c = \frac{V_{ex}}{F_i + F_c} F_c \\
V = V_e + V_p + V_i + V_c
$$

where $F_i$ and $F_c$ are the respective interstitial and cellular fractions of the
total tissue volume.

The compartmental fractions of the total organ volumes have been derived
from Kawei et al. [25]. They are assumed to be species-invariant. The used
parameters are summarized in table 4.2.

**Organ Compositions.** Tissues are composed in a certain way in order to
fulfill a determined function. Because of that, variations in the tissue com-
position between identical organs are assumed to be small. This implies that
Table 4.1: Total organ volumes. The parameters correspond to human data. $VF^{ex}$ is the fraction [L/kg body weight] of extravascular organ tissue (linear scaling). Linear scaling has been retrieved from Poulin and Theil [44] and allometric scaling has been derived from [42, 46], considering a male person of 80kg, 180cm height and an age of 25 years. $Q_{80kg}$ denotes the organ blood flow in a male, 80kg weighting human. The blood flows have been calculated from the cardiac output (equation 4.17) with parameters from table 4.4.

<table>
<thead>
<tr>
<th>organ</th>
<th>$VF^{ex}$ [l/kg]</th>
<th>$V_{lin,80kg}^{all}$ [l]</th>
<th>ref.</th>
<th>$V_{all,80kg}^{all}$ [l]</th>
<th>ref.</th>
<th>$Q_{80kg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>lung</td>
<td>0.0076</td>
<td>0.824</td>
<td>[44]</td>
<td>3.567</td>
<td>[46]</td>
<td>6.286</td>
</tr>
<tr>
<td>brain</td>
<td>0.02</td>
<td>1.778</td>
<td>[44]</td>
<td>1.350</td>
<td>[46]</td>
<td>0.138</td>
</tr>
<tr>
<td>heart</td>
<td>0.0047</td>
<td>0.509</td>
<td>[44]</td>
<td>0.349</td>
<td>[46]</td>
<td>0.337</td>
</tr>
<tr>
<td>muscle</td>
<td>0.4</td>
<td>32.85</td>
<td>[44]</td>
<td>31.93</td>
<td>[46]</td>
<td>1.914</td>
</tr>
<tr>
<td>Gl tract</td>
<td>0.0171</td>
<td>1.402</td>
<td>[44]</td>
<td>11.66</td>
<td>[46]</td>
<td>0.902</td>
</tr>
<tr>
<td>spleen</td>
<td>0.0026</td>
<td>0.289</td>
<td>[44]</td>
<td>0.214</td>
<td>[46]</td>
<td>0.138</td>
</tr>
<tr>
<td>bone</td>
<td>0.08563</td>
<td>7.143</td>
<td>[44]</td>
<td>8.003</td>
<td>[46]</td>
<td>0.840</td>
</tr>
<tr>
<td>skin</td>
<td>0.0371</td>
<td>3.025</td>
<td>[44]</td>
<td>3.075</td>
<td>[46]</td>
<td>0.399</td>
</tr>
<tr>
<td>kidney</td>
<td>0.0044</td>
<td>0.393</td>
<td>[44]</td>
<td>0.324</td>
<td>[46]</td>
<td>0.971</td>
</tr>
<tr>
<td>liver</td>
<td>0.026</td>
<td>2.350</td>
<td>[44]</td>
<td>1.529</td>
<td>[46]</td>
<td>1.205</td>
</tr>
<tr>
<td>pancreas</td>
<td>0.0016</td>
<td>0.152</td>
<td>-</td>
<td>0.115</td>
<td>[46]</td>
<td>0.070</td>
</tr>
<tr>
<td>adipose</td>
<td>0.1195</td>
<td>9.657</td>
<td>[44]</td>
<td>17.08</td>
<td>[46]</td>
<td>0.482</td>
</tr>
<tr>
<td>art. blood</td>
<td>0.0272</td>
<td>2.176</td>
<td>[44]</td>
<td>1.451</td>
<td>[46]</td>
<td>6.286</td>
</tr>
</tbody>
</table>

The inter-individual and the inter-species differences in tissue composition are small as well. However, differences between distinct organs are assumed to be large, because of their different functions in the organism. Fractions of tissue content, such as water, neutral lipids, phospholipids and protein content are assumed to organ-specific.

In the present model, organ composition data is used in order to predict the extravascular to plasma partition coefficients ($P^{ex,p}$), with the equations postulated by Poulin and Theil [43], as described later. Partition coefficients are generally assumed to be species-invariant in the current model. Organ composition data is summarized in table 4.3.

### 4.2.2 Blood Flows

In order to calculate organ blood flows, the total cardiac output has to be estimated by allometric scaling. The total cardiac output can either be calculated using the equation postulated by Brown et al. [7]:

$$co = 0.235 \times \text{bodyweight}^{0.75},$$

(4.17)
Table 4.2: Compartment fractions of organ volumes. $F_v$, $F_i$ and $F_c$ represent the respective fractions of the vascular (v), the interstitial (i) and the cellular (c) space [fractions of the total organ volumes]. The volume of the erythrocytes in each compartment can be calculated from the vascular volume ($V^v = \text{hct} \cdot V^v$).

\[
\text{co} = 2.421 \times \text{basalsurfacearea}^{1.15},
\]

resulting in a cardiac output (co) of 6.286 [l/min] for a human of 80kg, or the total cardiac output can be calculated using the equation by Price et al. [46]:

resulting in a cardiac output (co) of 5.3974 [l/min] for an 80kg human of 180cm height. In the present thesis, the equations by Brown et al. have been used in order to calculate the cardiac output.

Organ-specific blood flow rates are calculated from the total cardiac output using linear scaling. The used organ-specific fractions of cardiac output are taken from Poulin and Theil [45]. They are summarized in table 4.4. The original data is used in a rat PBPK model. We assume that those values are species-invariant.

### 4.3 Drug-Specific Data: Tolbutamide

Drug-specific data includes two classes of parameters. The first class of parameters describes features of the drug that are not influenced by physiological factors. Parameters that are determined through standardized (non-physiological) assays, in order to determine the compounds solubility belong to this class. An example is the pK-value of a drug. These ‘general’ drug-specific parameters are summarized in table 4.5 for tolbutamide. The second
<table>
<thead>
<tr>
<th>Compartment</th>
<th>water</th>
<th>n-lipids</th>
<th>p-lipids</th>
<th>remaining</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>lung</td>
<td>0.811</td>
<td>0.003</td>
<td>0.009</td>
<td>0.177</td>
<td>[44]</td>
</tr>
<tr>
<td>brain</td>
<td>0.77</td>
<td>0.051</td>
<td>0.0565</td>
<td>0.0123</td>
<td>[44]</td>
</tr>
<tr>
<td>heart</td>
<td>0.758</td>
<td>0.0115</td>
<td>0.0166</td>
<td>0.214</td>
<td>[44]</td>
</tr>
<tr>
<td>muscle</td>
<td>0.76</td>
<td>0.024</td>
<td>0.007</td>
<td>0.209</td>
<td>[44]</td>
</tr>
<tr>
<td>GI -tract</td>
<td>0.718</td>
<td>0.0487</td>
<td>0.0163</td>
<td>0.217</td>
<td>[44]</td>
</tr>
<tr>
<td>spleen</td>
<td>0.788</td>
<td>0.0201</td>
<td>0.0198</td>
<td>0.1721</td>
<td>[44]</td>
</tr>
<tr>
<td>skeleton</td>
<td>0.439</td>
<td>0.074</td>
<td>0.0011</td>
<td>0.486</td>
<td>[44]</td>
</tr>
<tr>
<td>skin</td>
<td>0.718</td>
<td>0.0284</td>
<td>0.0111</td>
<td>0.24</td>
<td>[44]</td>
</tr>
<tr>
<td>kidney</td>
<td>0.783</td>
<td>0.0207</td>
<td>0.0162</td>
<td>0.18</td>
<td>[44]</td>
</tr>
<tr>
<td>liver</td>
<td>0.751</td>
<td>0.0348</td>
<td>0.025</td>
<td>0.189</td>
<td>[44]</td>
</tr>
<tr>
<td>pancreas</td>
<td>0.75</td>
<td>0.05</td>
<td>0.019</td>
<td>0.181</td>
<td>-</td>
</tr>
<tr>
<td>adipose</td>
<td>0.18</td>
<td>0.79</td>
<td>0.002</td>
<td>0.028</td>
<td>[44]</td>
</tr>
</tbody>
</table>

Table 4.3: Tissue compositions. Parameters are given as fractions of the total tissue volume. 'N-lipids' denotes neutral lipid content and 'p-lipids' denotes phospho-lipid content. The values for the pancreas were guessed, since data could not be found in the literature. The tissue compositions are important to calculate the tissue to plasma partition coefficient $P_{t_p}$, as postulated by Poulin and Theil [43].

class of parameters is represented by data, which is influenced by drug-specific features as well as physiological factors. In the current model, parameters, such as diffusion coefficients (D), the fractions of unbound drug ($f_u$) and partition coefficients ($P_{x:y}$) belong to this class. These parameters are discussed in more detail in the following section.

### 4.3.1 Diffusion Coefficients

The diffusion coefficients (D) limit the transition between two compartments in a tissue. They are expressed as volume/time. The following drug transitions may occur: Transitions from the plasma to the erythrocytes (p→e) and vice versa, transitions from the plasma to the interstitial space and vice versa (p→i) and transitions from the interstitium to the cells and vice versa (i→c). Since the capillary membrane is rather loose, the diffusion through this membrane should not be rate-limiting. Therefore this diffusion ($D^{pe}$) is set to a high value, such that the transitions from the plasma to the interstitial space and vice versa (p→i) is only limited by the blood flow (BFL). The diffusion coefficients (D) are determined by the drug’s permeability coefficient (P) and by the membrane area (A) of the target tissue, so that:

$$P \cdot A = D$$  \hspace{1cm} (4.19)
Table 4.4: Blood flows. The organ-specific blood flows are given as fractions of the total cardiac output. The original parameters Q (original) are taken from Poulin and Theil [45]. These values have been linearly scaled (see scaling factor), so that the blood input for each compartment equals the blood output in order to ensure a closed system. The parameters that have finally been used in our model are denoted Q (internal).

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pkₐ</td>
<td>5.41</td>
</tr>
<tr>
<td>permeability P</td>
<td>3.384×10⁻⁹ cm/h</td>
</tr>
<tr>
<td>log₁₀ Pₒw</td>
<td>2.4</td>
</tr>
<tr>
<td>log₁₀ Pᵥₒw</td>
<td>2.6</td>
</tr>
<tr>
<td>therapeutic range</td>
<td>1-16.9µM</td>
</tr>
</tbody>
</table>

Table 4.5: General parameters for tolbutamide. Pₒw is the octanol:water partition coefficient of tolbutamide. Pᵥₒw is the vegetable oil : water partition coefficient. The therapeutic range of tolbutamide corresponds to the unbound plasma concentration of tolbutamide.

The corresponding parameters for tolbutamide are given in table 4.6 and table 4.5. In order to estimate the permeability coefficients of tolbutamide through the erythrocyte membranes, the surface area of the erythrocytes has to be estimated. The relative surface area of erythrocytes is around 1.5 [10⁶ cm²/l] [52]. Therefore, the absolute surface area in each tissue can be calculated using the respective volumes of erythrocytes in these tissues (calculated from Vᵣ in table 4.2, by multiplying the hematocrit, 'hct'). The cellular surface areas of the diverse tissues determine the area of total lipid bilayer membrane exposed to the interstitium. Therefore, the cellular surface area determines the speed of diffusion through this barrier (equation 4.19). Different cell types may have different shapes. Therefore, the surface areas may be disproportional between different tissues.

Kawei et al. [25] have developed a PBPK model, which uses permeability surface areas (PS) as diffusion-limiting parameters with application to the drug cyclosporineA. They further scaled their parameters from rat to human. In order to derive the respective tissue surface areas for our current model,
with application to tolbutamide, we need to calculate the permeability surface area \((PS)\) for the considered tissues in human from the original data (on rat), using the following allometric scaling function from Kawei et al. [25]:

\[
PS_{\text{i CycA}}^{\text{c CycA}, \text{rat}} = PS_{\text{i CycA}}^{\text{c CycA}, \text{rat}} (V/V_{\text{original}})^{0.67}
\]

where \(PS_{\text{i CycA}}^{\text{c CycA}, \text{rat}}\) is the original data from Kawei et al. [25] for a rat of 250g weight, and \(V_{\text{original}}\) indicates the corresponding volumes of the organs.

The corresponding surface area products for any drug can be calculated using equation 4.19, knowing the permeability of cyclosporineA \((1.11 \times 10^{-9}[\text{cm/h}]) [57]\) and of the drug in focus (tolbutamide: \(3.384 \times 10^{-9}[\text{cm/h}]\) [58].

<table>
<thead>
<tr>
<th>tissue</th>
<th>(PS_{\text{CycA}, \text{rat}}^{\text{c CycA}})</th>
<th>ref.</th>
<th>(PS_{\text{CycA}, \text{hu}}^{\text{c CycA}})</th>
<th>(A) [cm(^2)10(^{13})]</th>
<th>(PS_{\text{tolb}}^{\text{c CycA}})</th>
<th>(PS_{\text{tolb}}^{\text{p CycA}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>lung</td>
<td>BFL</td>
<td>[25]</td>
<td>BFL</td>
<td>(\gg 1)</td>
<td>BFL</td>
<td>0.493</td>
</tr>
<tr>
<td>brain</td>
<td>1000</td>
<td>[25]</td>
<td>59434</td>
<td>5.4</td>
<td>182840</td>
<td>0.406</td>
</tr>
<tr>
<td>heart</td>
<td>140</td>
<td>[25]</td>
<td>10588</td>
<td>0.96</td>
<td>32572</td>
<td>0.305</td>
</tr>
<tr>
<td>muscle</td>
<td>2300</td>
<td>[25]</td>
<td>97719</td>
<td>8.8</td>
<td>300619</td>
<td>1.951</td>
</tr>
<tr>
<td>gut</td>
<td>390</td>
<td>[25]</td>
<td>10698</td>
<td>0.97</td>
<td>32910</td>
<td>0.077</td>
</tr>
<tr>
<td>spleen</td>
<td>74</td>
<td>[25]</td>
<td>4649</td>
<td>0.42</td>
<td>14302</td>
<td>0.186</td>
</tr>
<tr>
<td>skeleton</td>
<td>390</td>
<td>[25]</td>
<td>23446</td>
<td>2.13</td>
<td>72128</td>
<td>0.669</td>
</tr>
<tr>
<td>skin</td>
<td>300</td>
<td>[25]</td>
<td>5443</td>
<td>0.49</td>
<td>16744</td>
<td>0.131</td>
</tr>
<tr>
<td>kidney</td>
<td>BFL</td>
<td>[25]</td>
<td>BFL</td>
<td>(\gg 1)</td>
<td>BFL</td>
<td>0.094</td>
</tr>
<tr>
<td>liver</td>
<td>BFL</td>
<td>[25]</td>
<td>BFL</td>
<td>(\gg 1)</td>
<td>BFL</td>
<td>0.617</td>
</tr>
<tr>
<td>pancreas</td>
<td>80</td>
<td>-</td>
<td>3692</td>
<td>0.33</td>
<td>11357</td>
<td>0.055</td>
</tr>
<tr>
<td>adipose</td>
<td>79</td>
<td>[25]</td>
<td>7896</td>
<td>0.718</td>
<td>24290</td>
<td>0.220</td>
</tr>
<tr>
<td>art. blood</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.97</td>
</tr>
<tr>
<td>ven. blood</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.94</td>
</tr>
</tbody>
</table>

Table 4.6: Permeability surface area products. These values correspond to the diffusion constants. All values for the permeability surface area products \((PS)\) are given in units [ml/h]. \(PS_{\text{CycA}, \text{rat}}^{\text{c CycA}}\) stands for the permeability surface area product of cyclosporine A in a rat of 250g (original data). The corresponding value \(PS_{\text{CycA}, \text{hu}}^{\text{c CycA}}\) indicates the permeability surface area product of cyclosporine A in a male human of 80kg (allometric scaling). \(PS_{\text{tolb}}^{\text{c CycA}}\) and \(PS_{\text{tolb}}^{\text{p CycA}}\) are the respective permeability surface area products of tolbutamide in an 80kg weighting male adult for the diffusions from the interstitium into the cell \((i\leftrightarrow c)\) and for the plasma to erythrocytes diffusion \((p\leftrightarrow e)\). BFL indicates that the corresponding diffusion process is not rate limiting, therefore it is blood flow limited (BFL). The permeability of cyclosporine A has been set to \(1.11 \times 10^{-9}[\text{cm/h}]\) [57] and the permeability of tolbutamide has been set to \(3.384 \times 10^{-9}[\text{cm/h}]\) [58].

### 4.3.2 Fractions of Unbound Drug

**Fraction of Unbound Drug in the Erythrocytes.** The fraction of unbound drug in the erythrocytes has been calculated from the blood to plasma.
partition coefficient ($P_{bl:p}$), which could be found in the literature [53] for tolbutamide:

$$P_{bl:p} = \frac{C_{bl}^{ss}}{C_{p}^{ss}} = \frac{V^{ery}C_{ery}^{ss} + V^{p}C_{p}^{ss}}{V^{bl}C_{p}^{ss}}$$

where $C_{bl}^{ss}$, $C_{p}^{ss}$ and $C_{ery}^{ss}$ are the respective concentrations as steady state conditions in the blood, plasma and erythrocytes. $V^{bl}$, $V^{p}$ and $V^{ery}$ are the respective volumes of blood, plasma and erythrocytes. $P_{bl:p}$ is the blood to plasma partition coefficient. The equation becomes:

$$P_{bl:p} = \frac{V^{bl}hctC_{ery}^{ss} + (1 - hct)V^{bl}C_{p}^{ss}}{V^{bl}C_{p}^{ss}}$$

where 'hct' is the hematocrit, defined as the fraction of solid constituents in the blood. Since the erythrocytes are by far the biggest portion of the solid constituents in the blood, we can assume that $V^{bl} \cdot hct$ equals the volume of the erythrocytes. From here we get:

$$P_{bl:p} = hct \frac{C_{ery}^{ss}}{C_{p}^{ss}} + (1 - hct).$$

Considering equation 1.9 on page 19, we get

$$P_{bl:p} = hct \frac{f_{p}^{u}}{f_{ery}^{u}} + (1 - hct),$$

where $f_{p}^{u}$ and $f_{ery}^{u}$ are the fractions of unbound drug in the plasma and the erythrocytes respectively. It follows, that:

$$P_{bl:p} - 1 + hct = hct \frac{f_{p}^{u}}{f_{ery}^{u}}$$

$$\Rightarrow f_{ery}^{u} = \frac{hct f_{p}^{u}}{P_{bl:p} - 1 + hct} \quad (4.20)$$

Hence, we need the hematocrit and the blood to plasma partition coefficient

<table>
<thead>
<tr>
<th>$P_{bl:p}$ reference</th>
<th>hct</th>
<th>$f_{ery}^{u}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>0.45</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Table 4.7: Parameters for the calculation of the fraction of unbound drug in the erythrocytes ($f_{ery}^{u}$).

($P_{bl:p}$) to calculate the fraction of unbound drug in the erythrocytes ($f_{ery}^{u}$). The necessary parameters are summarized in table 4.7 with regard to tolbutamide.
Fractions of Unbound Drug in the Plasma and Interstitium. We assume that the binding of tolbutamide to albumin solely determines the fractions of unbound drug in the plasma and interstitium, as described by equation 1.6 (general case) and equation 1.7 (tolbutamide) on page 17. Therefore the fractions of unbound drug in the interstitium and the plasma can be calculated once we know the dissociation constant ($K_D$) for the albumin-drug binding, the number of binding sites on albumin ($n$) and the corresponding concentrations of albumin in the interstitium and the plasma ($C_{alb}^i$ and $C_{alb}^p$). The corresponding parameters are summarized in table 3.3 and table 4.8.

<table>
<thead>
<tr>
<th>organ</th>
<th>$C_{alb}^i[\mu M]$</th>
<th>ref.</th>
<th>$f_u^i$</th>
<th>$f_u^c$</th>
<th>$P_{exp}$</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>lung</td>
<td>362.3 [38]</td>
<td></td>
<td>0.019</td>
<td>0.108</td>
<td>0.243</td>
<td>[53]</td>
</tr>
<tr>
<td>brain</td>
<td>145</td>
<td>-</td>
<td>0.046</td>
<td>0.219</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>heart</td>
<td>94</td>
<td>-</td>
<td>0.069</td>
<td>0.066</td>
<td>0.18</td>
<td>[53]</td>
</tr>
<tr>
<td>muscle</td>
<td>188 [11,29,38]</td>
<td></td>
<td>0.036</td>
<td>0.268</td>
<td>0.08</td>
<td>[53]</td>
</tr>
<tr>
<td>GI -tract</td>
<td>64 [29]</td>
<td></td>
<td>0.099</td>
<td>0.185</td>
<td>0.07</td>
<td>[53]</td>
</tr>
<tr>
<td>spleen</td>
<td>100</td>
<td>-</td>
<td>0.065</td>
<td>0.16</td>
<td>0.097</td>
<td>[53]</td>
</tr>
<tr>
<td>skeleton</td>
<td>145</td>
<td>-</td>
<td>0.046</td>
<td>0.146</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>skin</td>
<td>203 [29,38]</td>
<td></td>
<td>0.033</td>
<td>0.108</td>
<td>0.187</td>
<td>[53]</td>
</tr>
<tr>
<td>kidney</td>
<td>103</td>
<td>-</td>
<td>0.064</td>
<td>0.109</td>
<td>0.127</td>
<td>[53]</td>
</tr>
<tr>
<td>liver</td>
<td>22 [29]</td>
<td></td>
<td>0.241</td>
<td>0.080</td>
<td>0.13</td>
<td>[53]</td>
</tr>
<tr>
<td>pancreas</td>
<td>30 [29,38]</td>
<td></td>
<td>0.189</td>
<td>0.076</td>
<td>0.14</td>
<td>[53]</td>
</tr>
<tr>
<td>adipose</td>
<td>109 [11]</td>
<td></td>
<td>0.06</td>
<td>0.124</td>
<td>0.11</td>
<td>[53]</td>
</tr>
<tr>
<td>plasma</td>
<td>579.7 [29,38]</td>
<td></td>
<td>0.012</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.8: Albumin concentrations, fractions of unbound drug in the interstitial space ($f_u^i$) and cellular space ($f_u^c$), obtained by solving equations 1.7 and 4.21, and extravascular to plasma partition coefficients ($P_{exp} = P_{texp}$).

Fractions of Unbound Drug in the Cells. Once we know the fractions of unbound drug in the plasma and interstitium (as described above) and we have retrieved the tissue (extravascular) to plasma partition coefficients ($P_{texp}$, as explained below), we can calculate the fractions of unbound drug in the cells ($f_u^c$). The tissue to plasma partition coefficients ($P_{texp}$) are defined as follows:

$$P_{texp} = P_{exp} = \frac{1}{V_{ex}} \left( \frac{V_i C_{ss}^i + V_c C_{ss}^c}{C_{ss}^p} \right)$$


where \( P^{ex:p} \) is the extravascular to plasma partition coefficient, \( V^{ex} \), \( V^i \), \( V^c \), \( C_{ss}^i \), \( C_{ss}^c \) and \( C_{ss}^p \) are the respective volumes and concentrations at steady state conditions in the extravascular (ex), the interstitial (i), the cellular (c) and the plasma (p) compartments. Rearranging the equation, shown above, we can calculate the fractions of unbound drug in the cells \( (f^c_u) \) as a function of the extravascular to plasma partition coefficients \( (P^{ex:p}) \).

\[
P^{ex:p} = \frac{1}{V^{ex}} \left( \frac{V^i C_{ss}^i}{C_{ss}^p} + \frac{V^c C_{ss}^c}{C_{ss}^p} \right) = \frac{1}{V^{ex}} (V^i f^p_u + V^c f^p_u)
\]

from here we get:

\[
f^c_u = \frac{V_c f^p_u}{V^{ex} f^i_u - V^i f^p_u}
\]

\[
\Rightarrow f^c_u = \frac{V_c}{V^{ex} p^{ex:p} - V^i} \quad (4.21)
\]

The calculated fractions of unbound drug in the cells \( (f^c_u) \) are listed in table 4.8.

**Remarks.** One possible way to calculate the tissue to plasma partition coefficients \( (P^{t:p}) \) is to use the method developed by Poulin and Theil [43]:

- homogeneous distribution model (non-adipose):

\[
P^{t:p} = \frac{[P_{ovw}(F_{nl} + 0.3F_{phi})] + [1(F_{wt} + 0.7F_{phi})] f^p_u}{[P_{ovw}(F_{nl} + 0.3F_{phi})] + [1(F_{wt} + 0.7F_{phi})] f^p_u}
\]

- homogeneous distribution model (adipose):

\[
P^{t:p} = \frac{[D_{ovw}(F_{nl} + 0.3F_{phi})] + [1(F_{wt} + 0.7F_{phi})] f^p_u}{[D_{ovw}(F_{nl} + 0.3F_{phi})] + [1(F_{wt} + 0.7F_{phi})] 1}
\]

where \( f^t_u \) is the fraction of unbound drug in the tissue (extravascular) and \( F_{nl}, F_{wt}, F_{phi} \) are the respective fractions of neutral lipids, water and phospholipids. Poulin and Theil hereby suggested that the concentration of binding proteins in the tissue equalizes half of the concentration of binding proteins in the plasma. With respect to tolbutamide pharmacokinetics this means that:

\[
C_{alb}^t = 0.5C_{alb}^p
\]
where \( C_{alb}^t \) and \( C_{alb}^p \) are the respective concentrations of albumin in the tissue \((t)\) and plasma \((p)\).

\[
\Rightarrow f_u^t = \frac{1}{1 + 0.5nK_aC_{alb}^p}
\]

Unfortunately, physiologically reasonable values for the tissue to plasma partition coefficient could not be recovered for tolbutamide, using realistic parameters (table 4.5). Realistically, the steady state plasma concentration of tolbutamide should be higher than the concentration in the cells due to the high plasma binding of tolbutamide. The respective partition coefficients \( P_{ery:p} \) and \( P_{c:p} \) are defined as (see also equation 1.9):

\[
P_{ery:p} = \frac{f_u^p}{f_u^{ery}} = \frac{C_{ss}^{ery}}{C_{ss}^p}
\]

and

\[
P_{c:p} = \frac{f_u^c}{f_u^c} = \frac{C_{ss}^c}{C_{ss}^p}
\]

where \( f_u^p \), \( f_u^{ery} \) and \( f_u^c \) are the respective fractions of unbound drug in the plasma, the erythrocytes and the cells and \( C_{ss}^p \), \( C_{ss}^{ery} \) and \( C_{ss}^c \) are the corresponding concentrations at steady state. From here, it is quite obvious that the erythrocyte to plasma \( (P_{ery:p}) \), as well as the cell to plasma partition coefficient \((P_{c:p})\) should be less than 1 for tolbutamide. A comparison between the partition coefficients, derived using the above mentioned equations [43] and by using the partition coefficients measured by Sugita et al. [53] is shown in figure 4.2. The comparison shown in figure 4.2 shows that the method developed by Poulin and Theil [43] seems to be ill-conditioned to predict the tissue to plasma partition coefficients \((P_{t:p})\) for tolbutamide, though it might be a good predictor for other drugs. Because of these results, tissue to plasma partition coefficients \((P_{t:p})\), see table 4.8 by Sugita et al. [53] have been used in our simulations and led to reasonable results.
Figure 4.2: Comparison of partition coefficients. The tissue to plasma ($P_{t:p}$) partition coefficients were derived by solving the equations given by Poulin and Theil [43] with parameters from table 4.3 and 4.8. The tissue to plasma partition coefficients ($P_{t:p}$) from Sugita et al. [53] could directly be taken from published data. Cellular to plasma partition coefficients ($P_{c:p}$) were calculated from the fractions of unbound drug in the plasma and the cells ($f_{p}^{u}/f_{c}^{u}$). For better illustration, a cut-off at the y-axis value of 3 has been applied.
Chapter 5

Numerics

The main objective of the current PBPK model is to predict and analyze drug distribution. Thereby, the main goal is to develop a mathematical framework, which describes the mechanisms involved in the drug distribution processes, that have been considered to be most important. Reproduction of experimental data is not a primary goal of the current model and will just be performed by 'eye norm' and not by means of statistical analysis. However, detailed comparison to experimental data will be future work on this model, when the primary goal of the model development has been shifted towards model enhancement.

The current model, however, offers a wide range of possible study goals. Besides predicting drug distribution in one organism, different settings can be simulated by altering the corresponding parameters in the present model. Genetic polymorphisms in the metabolizing CYP-enzymes can be simulated by readjusting the corresponding values which describe the liver metabolism. Alterations in physiological factors, such as body sizes can be performed by changing the corresponding physiological parameters. Diseases may influence a multitude of physiological factors. An example of an illness-induced complication may be a kidney damage, through temporary or chronic diseases. The effects of the kidney damage may be an increased expulsion of albumin. Therefore, albumin levels in the body will be decreased. In the current model this setting can be simulated by adjusting the corresponding data on albumin concentrations.

Comparing drug disposition in different species requires close reflection of species differences. As discussed earlier in section 1.1.1, both, general physiological factors and factors that influence ADME+effect of a drug have to be adjusted. As an example, differences in metabolic enzyme activity towards tolbutamide may occur. These differences, however, do not scale with the overall physiology of the compared species.
In the following chapter, simulations showing some of the abilities of the current PBPK model will be demonstrated and discussed.

5.1 Concentration-Time Profiles in Human

The concentration-time profiles of tolbutamide in lung, brain, heart, muscle, gut, spleen, skeleton, skin, kidney, liver, pancreas and adipose tissue following an oral administration of 500mg tolbutamide to a male human (80kg) are shown in figure 5.1. An intrinsic clearance of $C_{\text{int}} = 1.905 \ [\text{L/min}]$ was used. The value for the intrinsic clearance $C_{\text{int}}$ is set, so that the half-life of tolbutamide corresponds to the measured data [27] of $t_{1/2} = 6.6 \ [\text{h}]$. Hereby,
we calculated the half-life from the predicted concentration-time profiles using the following equation [29]:

\[
    t_{1/2} = \frac{0.693(t_2 - t_1)}{\ln(C_{t1}/C_{t2})},
\]

where \( t_2 \) and \( t_1 \) are distinct time points. The chosen time points correspond to a strictly logarithmic concentration-time decline.

The distribution phase in the erythrocytes is noticeably larger than in the other compartments (blue line in figure 5.1). Since the erythrocytes’ drug concentration is dependent on the ease of molecules from the plasma into (and out of) the erythrocytes, the reason for the slow achievement of a distribution equilibrium is due to the fact that distribution processes between the erythrocytes and the plasma (\( D^{ep} \)) are quite slow compared to the distribution processes between the other compartments (see table 4.6).

For almost all tissues, the highest drug concentrations appear in the plasma compartment, followed by the erythrocyte compartment. However, in the lung, the interstitial drug concentration is higher than the concentration within the erythrocytes, which can be explained by the high albumin concentration within the interstitial space of the lung (see table 4.8). Since the ability of a drug to permeate through a bilayer membrane is heavily constricted when the drug is bound to a macromolecule like albumin, the overall concentration in a compartment will be increased by the concentration of bound drug. Higher albumin concentrations will result in a higher concentration of bound drug in the compartment under consideration. Thus, in the present example, more drug is bound in the interstitial space of the lung, than within the erythrocytes.

Within the liver and the pancreas, unlike the other tissues, the interstitial drug concentrations are relatively small compared to the cellular drug concentrations. This circumstance has the following indications in the model: Albumin concentrations (see table 4.8) are relatively small within these compartments. If the albumin concentration is remarkably smaller within the interstitial space (\( f_u^i \uparrow \)), compared to other tissues, while the extravascular to plasma partition coefficient (\( P^{exp} \)) has a similar magnitude (compared with other tissues), the low albumin concentration in the interstitial compartment will lead to a relatively high cellular drug binding (\( f_u^c \downarrow \), see equation 4.21) in our model.

In the present simulation, as illustrated in figure 5.1, 500mg of tolbutamide have been administered to a male (80kg) human with ‘normal’ metabolic activity. Our results suggested that tolbutamide is therapeutically active after 32 minutes (based on the parameters given by Takanaga et al. [54] for the therapeutic range, as summarized in table 4.5) and falls below therapeutic
Figure 5.2: Concentration of unbound tolbutamide in human (80kg) plasma, following a 500mg oral administration. The red line indicates the therapeutic range [54] of tolbutamide as a measurement of unbound plasma concentration. Tolbutamide is therapeutically active after 32 minutes and falls below therapeutic concentration range after 257 minutes.

Concentrations of unbound drug in the venous plasma and the corresponding therapeutic range are shown in figure 5.2. It is worth mentioning that, under ‘normal’ circumstances, a toxic tolbutamide concentration [54] (see table 4.5) cannot be reached after administering an oral dose of either 500mg or 1000mg tolbutamide (sold pill sizes) to a male human of 80kg (data not shown). However, if the dose is greatly increased (> 6g), toxic concentrations may be obtained nonetheless (data not shown).

Tolbutamide seems to be a relatively safe drug. However, due to the strong binding to albumin, this may change dramatically if tolbutamide is displaced from albumin, resulting in a multiple-fold increase in the concentration of unbound tolbutamide. Examples may be multi-drug treatments, such as warfarin and tolbutamide co-administrations.

The tolbutamide absorption in the gI-tract was simulated using the equation 1.3, as postulated by M. Weiss [63] with parameters from table 3.2. The amount of absorbed drug ($f_A(t)K_0^{oral}$) versus time is shown in figure 5.3.
After 3 hours, tolbutamide absorption hardly occurs.

![Tolbutamide absorption graph]

Figure 5.3: Absorption in the GI-tract following an oral administration of 500mg tolbutamide, using the absorption model postulated by M. Weiss [63] with parameters from table 3.2.

### 5.2 Polymorphisms in Human

Hereditary variabilities in the tolbutamide metabolizing enzymes, CYP2C9, as major modifiers of drug disposition and response have been reported previously [27,37]. Therefore, the reported data has been included into our physiologically based pharmacokinetic model in order to simulate these variabilities. The results are shown in figure 5.4. Concentration-time profiles are based on an oral tolbutamide administration of 500mg to male humans of 80kg body weight. Our results are compared with the experimental data and show good agreement, based on visual comparison. The previously reported data, summarized in table 3.5, has been linearly scaled, since individual physiological data (e.g. weights) from the previous study [27] is not available and the inter-individual variations in body weights are very small (see table 3.5). We hereby assume that the hereditary variability in the tolbutamide metabolizing enzymes is the sole factor, besides varieties in body weight, involved in the diverse observed pharmacokinetics. The metabolic clearances
Figure 5.4: Genetic Polymorphisms. Panel A shows clinical data on tolbutamide pharmacokinetics of various genotypes [27]. Panel B shows concentration-time profiles of the corresponding genotypes, simulated by the current physiologically based pharmacokinetic model with respective clearance parameters, summarized in table 5.1. The solid red line in panel B indicates the minimum therapeutic plasma concentration of tolbutamide, based on data, summarized in table 4.5.

are calculated following the parameters summarized in table 5.1. As shown
Table 5.1: Used scaling parameters to estimate metabolic clearances in genetic polymorphisms of tolbutamide metabolizing enzymes (CYP2C9).

<table>
<thead>
<tr>
<th>gen. variant</th>
<th>*1/*1</th>
<th>*1/*2</th>
<th>*1/*3</th>
<th>*2/*3</th>
<th>*3/*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>fraction of max. Cl</td>
<td>0.955</td>
<td>1</td>
<td>0.583</td>
<td>0.838</td>
<td>0.529</td>
</tr>
</tbody>
</table>

in figure 5.4, hereditary variability in the metabolizing capability may cause unintended drug response: Whereas a drug response for the major genotype (*1/*1 -alleles) may be expected within a relatively small time window (32-257 min), poor metabolizers (e.g. *3/*3) may respond to tolbutamide even up to 1610 min after oral administration of 500 mg compound. The delayed elimination may result in either toxic overdosing due to a prolonged exposure to high drug concentrations, or it could cause unexpected implications based on drug-drug interactions. Therefore, it is advisable to consider hereditary variability in metabolizing enzymes of a drug, especially if the drug is metabolized by only one CYP-isoform, as in the case of tolbutamide.

### 5.3 Illness Induced Complications

Some diseases may effect the pharmacokinetics of a drug. If the kidney is affected, the outcome might be a sufficiently higher expulsion of albumin. Since albumin binds tolbutamide within the plasma and the interstitium of the organs, illness induced decrease of albumin concentration have been simulated with the present PBPK model. As for the previous simulations, 500 mg tolbutamide have been orally administered to an 80 kg human. The various outcomes of decreased albumin concentration (50% and 20% of normal albumin levels) are shown in figure 5.5 and table 5.2. With respect to tolbutamide pharmacokinetics, decrease in albumin concentration has the following effects: The concentration of unbound tolbutamide will be increased.

<table>
<thead>
<tr>
<th>parameter</th>
<th>control</th>
<th>50% Albumin</th>
<th>20% Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$</td>
<td>6.6 [h]</td>
<td>4.26 [h]</td>
<td>2.95 [h]</td>
</tr>
<tr>
<td>Cl$_h$</td>
<td>100%</td>
<td>197%</td>
<td>477%</td>
</tr>
<tr>
<td>active</td>
<td>32-257 [min]</td>
<td>24-338 [min]</td>
<td>18-353 [min]</td>
</tr>
</tbody>
</table>

Table 5.2: Derived parameters for illness induced albumin decrease. In this simulation 500 mg tolbutamide have been orally administered to an 80 kg male human. $t_{1/2}$ is the half-life of tolbutamide in hours. Cl$_h$ is the hepatic clearance, calculated using the 'well stirred' model [21, 41]. 'Active' denotes the time interval, when the concentration of unbound tolbutamide is above the value for the minimum therapeutic concentration, summarized in table 4.5.
Figure 5.5: Effects of albumin decrease on tolbutamide pharmacokinetics. The dotted line represents tolbutamide concentrations at 20% albumin levels (of normal) whereas the dashed-dotted line represents tolbutamide concentrations at 50% albumin levels. The solid line represents concentrations of tolbutamide at control conditions. Panel A shows concentrations of unbound tolbutamide in the venous plasma. The red line represent the therapeutic window. Panel B shows total tolbutamide concentrations within the venous plasma.

Therefore a bigger amount of tolbutamide will reach its target compartment, probably resulting in a more intense pharmacological response, compared to normal albumin levels. Also, a bigger amount of tolbutamide enters the liver, where it is eliminated through metabolism, resulting in a higher elimination rate (see $t_{1/2}$ values in table 5.2) The increased elimination process, therefore works opposing to the intensity of the pharmacological response.

## 5.4 Inter-Species Comparisons

Since the present physiologically based pharmacokinetic model is aimed to be general, it is interesting to evaluate if the present model is transferable to other species. Therefore, we compare the drug disposition of tolbutamide in two different species (human and rat). First of all, it is important to figure out which species-differences to account for and which factors to consider to be species-invariant. There may be obvious differences, like the sizes of the individuals. Besides this, scrutinizing differences in metabolic activity may be valuable. Chiou et al. [8,9] compared species-specific metabolic clearances.
of a multitude of drugs, including tolbutamide, and scaled the unbound drug clearances allometrically. Their results indicated that unbound drug clearances per basal surface area between rat and human varied by a factor of 2.3, indicating that the human liver enzymes metabolize tolbutamide more efficiently. These results are supported by a study by Easterbrook [10], who compared apparent intrinsic clearance data, based on in vitro assays for rat and human cell lines. Besides this, plasma binding of tolbutamide in rat has been reported to be sufficiently smaller than in human [8]. These considerations have been included into the apparent physiologically based pharmacokinetic model. The corresponding parameters are summarized in table 5.3.

Table 5.3: Parameters used for inter-species comparison. $K_D$ is the dissociation constant for the albumin binding, $f_u^p$ is the fraction of unbound drug in the plasma. $Cl_{int}$ is the intrinsic clearance and $Cl_u/BSA$ is the intrinsic clearance per square meter basal surface area (BSA).

<table>
<thead>
<tr>
<th>parameter</th>
<th>$K_D \mu M$</th>
<th>$n$</th>
<th>$f_u^p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>60</td>
<td>3</td>
<td>0.03335</td>
</tr>
<tr>
<td>human</td>
<td>21</td>
<td>3</td>
<td>0.011931</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>parameter</th>
<th>$Cl_{int}$ [l/min]</th>
<th>$Cl_u/BSA$ [l/(min×m^2)]</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>0.0095</td>
<td>0.46698</td>
<td>-</td>
</tr>
<tr>
<td>human</td>
<td>1.905</td>
<td>1.9634</td>
<td>-</td>
</tr>
</tbody>
</table>

It is assumed that the relative albumin concentrations are species-invariant. Furthermore, partly along with the previous assumption, it is assumed that the experimental extravascular to plasma partition coefficients ($P_{exp}$) are species-invariant as well.

Since the rat is a standardly used test animal, many experimentally determined parameters are available (besides the above mentioned, these are tissue volumes and compositions) and included into the current model in order to achieve an interpretable result. Physiological data on organ volumes have been taken from Poulin and Theil [44]. Blood flows have been allometrically scaled, based on the equations by Brown et al. [7].

In the present simulations, drug disposition between a human of 80kg and a rat of 250mg, following an intravenous administration of 80mg/kg were compared. The results of the simulations on species differences are shown in figure 5.6. The rat shows higher tolbutamide elimination rates compared to the human, which is in good agreement with experimental concentration-time data [53], based on 'eye norm' (figure 5.6). In agreement with the above mentioned considerations, the apparent metabolic capacity to eliminate tolbutamide is sufficiently lower in the rat than the in human. However, in both species, the metabolic clearance is not saturated. Based on our assumptions,
Figure 5.6: Inter-species comparison. Panel A shows the simulated concentration-time profiles of human and rat after a bolus intravenous injection of 80 mg/kg tolbutamide. Panel B shows the experimental (dots) and simulated data (solid line) obtained by Sugita et al. [53] for a bolus injection of 80 mg/kg tolbutamide in rats. Panel C shows our simulated results with a scaled x- and y-axis, for comparison.

It is the lower albumin binding to tolbutamide that increases the elimination of tolbutamide in the rat. If we assume that the albumin concentrations are roughly species invariant, rat albumin must have a lower affinity to tolbutamide, compared to human serum albumin.
Chapter 6

Discussion and Outlook

The aim of this thesis is to establish a generic physiologically based pharmacokinetic (PBPK) model to predict drug disposition. The underlying mathematical framework needs to particularly consider drug transfer kinetics between different physiological compartments, e.g. interstitial and intracellular spaces, in order to fully characterize their delivery to biological targets. Through the comprehension of general physiologically based mechanisms involved in the diverse drug transfer kinetics, a mathematical framework will prove to be, or not to be, a useful tool when interpreting local and systemic data and when building bridges between in vitro and in vivo data. However, parametrization has a major influence on the goodness of fit, when comparing simulation results with experimental data in order to validate the model. In the following chapter, the model will be discussed with respect to its parametrization, abilities and limitations.

6.1 Achievements

The anatomical data on organ connections via blood flow, integrated into the present PBPK model, as represented in figure 2.1, is well documented [1,52] and thus out of discussion. However, at this level of model building, simplifications in organ topology are applied in the current study. Thereby, based on other whole-body multi-compartment models [19, 25, 32, 45, 53, 55], an organ topology, that considers the most important tissues involved in the ADME+effect (Absorption, Distribution, Metabolism, Excretion and effect) of a drug has been used. The organ’s substructure, however, is not that well defined. Rough approximations had to be applied here. It is not guaranteed that it is always possible to draw the lines between the extravascular constituents (interstitial and cellular space) and between the vascular and the
extravascular compartments. Furthermore, it is a rough approximation to assume that substances are uniformly distributed within these compartments. The cell, for example, is known not to be a uniform lumen. Here, a non-uniform distribution could be expected. This issue gains importance, when analyzing the distribution of basic drugs. These drugs might be concentrated within the lysozomes, in the cells, via ‘ion-trapping’ processes. The cytoplasmatic concentration, however, might be low. The resulting the intra-cellular distribution is not uniform in this case.

Furthermore, since biological membranes might noticeably differ in their composition and thereby their lipophilicity, diffusion might not scale with the membrane surface area of a particular tissue. However, taking these uncertainties into account, the studies with tolbutamide showed that the presented PBPK model is able to characterize drug transfer kinetics and to satisfactory predict drug distribution with experimentally verified parameters as shown in figure 5.1 and discussed in section 5.1. Thus, it can be assumed, that the generic mathematical framework somewhat provides a stable mathematical characterization of the processes involved in drug disposition. The ability of the current model to predict concentration-time profiles may be of use in therapeutics (response-time) when drug response data is included.

**Genetic Polymorphisms.** Adverse drug reactions and ineffective drug treatment are responsible for a large health care burden. Considerable variability in drug response makes the prediction of the individual reaction difficult. In the last years, genetic polymorphisms in drug metabolizing enzymes have been a focus of the research [27, 37]. The polymorphisms in the cytochrome P450 enzyme system have been investigated most extensively. Most drugs are metabolized through these enzymes (an extensive list of the allelic variants can be seen at: [http://www.imm.ki.se/CYPalleles/](http://www.imm.ki.se/CYPalleles/)).

Knowing the importance of these recent findings, polymorphic drug response is studied with the current PBPK model. Thereby, experimental data for tolbutamide metabolism has been used [27] to perform simulations. The generic PBPK model is able to simulate genetic polymorphisms, based on the data by Kirchheiner et al. [27] and produces results that are in good agreement with the experimentally determined results, as shown in figure 5.4 in section 5.2.

Our results show that the current PBPK model might be useful in performing risk analysis. Since the concentration-time course after tolbutamide administration varied heavily between the carriers of the different allelic variants, cases can be thought of, in which experiments on different individual drug response might be life threatening and thus not realizable. Just to give an example, a similar study as the one performed by Kirchheiner et al. [27] with
application to warfarin would not be recommendable.
Warfarin is a frequently used long-term anticoagulant. It is metabolized through the same enzymes as tolbutamide, CYP2C9. Therefore, it can be assumed, that genetic polymorphisms will have a similar effect on the warfarin metabolism. As seen in our simulations on genetic polymorphisms, poor metabolizers may be exposed to a therapeutic drug concentration for a multiple-fold time compared to ‘normal’ metabolizers. In the case of warfarin, this will cause a loss of blood coagulation, lead to uncontrolled bleeding and probably death. In these cases computational models, like the current one, would be applied in order to evaluate safety of drug treatment in human.

**Illness Induced Complications.** Diseases may have an influence on drug disposition. In this thesis we analyzed the influence of illness induced decrease in albumin concentration on tolbutamide pharmacokinetics. The results are shown in figure 5.5 and table 5.5. With application to tolbutamide pharmacokinetics, alteration in albumin concentration does not have a dramatic effect. Two effects are working against each other. Higher concentrations of unbound tolbutamide may cause a more intense pharmacological response, whereas on the other hand, higher concentrations of unbound tolbutamide result in faster elimination rates. Therefore, even if a toxic concentration of unbound tolbutamide is achieved in the target compartment, the relatively higher elimination rate will make sure, that the target will not be exposed to the sufficiently higher unbound tolbutamide concentration for too long (considering the simulation settings).

The reasons for the adverse tolbutamide pharmacokinetics are twofold: Tolbutamide metabolism in the liver is not saturated at ‘normal’ conditions (concentration of unbound tolbutamide is less than 10 % of $K_m$ after oral administrations of 500mg and 1000mg tolbutamide to a male human of 80kg). On the other hand, since albumin concentrations are relatively high, alterations in albumin concentration have little effects, compared to plasma binding proteins, whose concentrations are sufficiently lower at ‘normal’ conditions.

Effects may be more dramatic if we consider drugs, that are bound to glycoproteins (mainly basic drugs), since glycoproteins are less frequent than albumin. If a drug’s elimination is almost saturated at ‘normal’ conditions, an increase in the concentration of unbound drug may have toxic effects. Therefore, the current PBPK model can help to analyze the discussed risk factors and help to readjust dose regimen.

**General Applicability.** Knowing that the developed model works for the human parameter set (with respect to tolbutamide pharmacokinetics), it demands to test its performance based on different parameter sets, like for
different drugs, individuals or species. In order to test its transferability, we compared the distribution of tolbutamide in human and rat, as shown in figure 5.6 in section 5.4. Plausible results could be attained for the distribution of tolbutamide in rats. The study revealed that tolbutamide is eliminated faster in rats than in humans. Nonetheless, the metabolic efficiency to transform tolbutamide is higher in humans, as proposed by Chiou and Easterbrook [8–10]. Plasma protein binding to tolbutamide in both species varies [8]. A sufficiently lower fraction of bound tolbutamide, together with the assumption that tolbutamide metabolism is not saturated at studied concentrations, could explain the coincidence that the elimination rate of tolbutamide is higher in the rat than in the human, based on confirmed parameters. The question remains on whether the sufficiently lower plasma binding to tolbutamide is due to a lower concentration of binding proteins (albumin) or if the reason is a lower affinity for the albumin-tolbutamide binding in rat. Albumin is a transport protein for fatty acids. Thus, the primary function of albumin in our organism in not the transport of tolbutamide. A sufficiently lower concentration of albumin in rats would have the consequence that rats would have a lower ability to transport fatty acids, which includes hormones or substances from nutrition. This seems quite unlikely, since the underlying processes (fatty acid transportation by albumin) have been developed early in evolutionary development and are assumed to be quite conserved. On the other hand, small divergences in albumin appearance (like for any other protein) are assumed to occur, along with the evolutionary divergence in the substances, which are naturally transported by albumin (co-evolution). Since tolbutamide exposure is an artificial situation, a different affinity to tolbutamide would not have any impact on the homeostasis of the rat organism. Thus, we conjecture that the affinity of albumin to tolbutamide varies between human and rat.

The results of this study indicate that the developed mathematical framework is applicable to different parameter sets, being as diverse as two species are. The results further show that including physiological data and experimental data into the present physiologically based pharmacokinetic model produces reasonable results, which allow interpretation beyond distribution processes.

6.2 Limitations of the Current Model

As for all mathematical models, parametrization of the model remains critical. The present model heavily relies on a multitude of measured parameters.
Especially the drug-specific parameters have to be addressed, since (exclusively) physiological parameters, once derived, will be applicable in many different simulations. The current model, however, can be simplified in terms of the required input parameters and improved along with drug development, as soon as more experimental parameters are available to specify the drug’s pharmacokinetics. A more elegant way, however, would be the use of novel input functions, that sufficiently predict required parameters at an early stage in drug discovery, without the need of model simplification. The predicted parameters could be used in order to rank candidate compounds towards their ADME properties, prior to animal testing, resulting in the optimization of the drug in focus. The predicted parameters can easily be replaced, if experimental data becomes available. Currently, the PBPK model relies on the following drug-specific input parameters:

- elimination:
  - clearance parameters

- protein binding:
  - fraction unbound \( f_u \)
  - blood to plasma partition coefficient \( P_{b:p} \)
  - extravascular to plasma partition coefficients \( P_{ex:p} \)

- diffusion:
  - permeability coefficient

- oral absorption:
  - mean absorption time (MAT)
  - normalized variance of MAT (CV)
  - bio-availability (F)

The parameters needed to describe the oral absorption processes and the protein binding within the cellular space \( P_{ex:p} \) are not attainable in an early drug discovery stage through \textit{in vitro} experiments. Poulin and Theil developed a method to predict the extravascular to plasma partition coefficient from \textit{in vitro} data. Unfortunately, this method showed bad correlation for tolbutamide.

Since parameters like the mean absorption time and the bio-availability of a drug will not be available during early drug development, explicit models of the gastro-intestinal processes \[3, 26, 64\] could sufficiently simulate drug absorption processes with a little set of drug-specific input parameters like the solubility, permeability and the pK-value of the drug. These parameters can be derived by \textit{in vitro} experiments.
Inclusion of these input models into the current model, will enable the user to perform ADME drug optimization in parallel to lead optimization due to activity assays.

6.3 Outlook

At the present stage, the model can be used to predict time-integrated drug distribution. Concentration-time profiles are important in order to predict drug effects. In order to fully describe the processes following administration, we might need to simulate drug effects and also drug-drug interactions. Thereby we might get pharmacology, therapeutics and pharmacokinetics together in one model.

**Drug-Drug Interactions.** The next step in model development will be the simulation of drug distribution of two and more drugs at the same time. Within our model, drug-drug interactions can be simulated, if the necessary parameters have been taken together.

Drug-drug interactions play a major role in adjusting drug dosing. Since many people die of toxic drug co-administration each year, drug-drug interactions are also important to consider, in order to make the right decision of which drug to (co-)administer. Drug-drug interactions are assumed to effect saturable processes. Thus, they may influence transport processes and protein binding and therefore alter drug distribution. Inhibition of drug metabolism might occur as well, changing drug clearance. When drug-target binding is influenced, drug efficiency will be altered. Saturable processes can be approximated using Michaelis-Menten kinetics. If drug-drug interactions occur, the parameters that describe the saturable mechanisms will be modified ($V_{\text{max}}$ and $K_m$).

<table>
<thead>
<tr>
<th>type of inhibition</th>
<th>$V_{\text{app}}$</th>
<th>$K_{\text{app}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>$V_{\text{max}}$</td>
<td>$K_m$</td>
</tr>
<tr>
<td>competitive</td>
<td>$V_{\text{max}}$</td>
<td>$\alpha K_m$</td>
</tr>
<tr>
<td>un-competitive</td>
<td>$V_{\text{max}}/\alpha'$</td>
<td>$K_m/\alpha'$</td>
</tr>
<tr>
<td>mixed</td>
<td>$V_{\text{max}}/\alpha'$</td>
<td>$\alpha K_m/\alpha'$</td>
</tr>
</tbody>
</table>

Table 6.1: $V_{\text{app}}$ and $K_{\text{app}}$ are the apparent values for $V_{\text{max}}$ and $K_m$. Values for $\alpha$ and $\alpha'$: $\alpha = 1 + \frac{[I]}{K_{D}}$, where $[I]$ is the concentration of the inhibiting substance and $K_D$ is the dissociation constant for the inhibitor-protein binding. $\alpha' = 1 + \frac{[I]}{K_{D}'}$, where $K_D'$ is the dissociation constant for the drug-protein-inhibitor-complex [61].
Two drugs may either compete for a certain ADME process (competitive inhibition), or the ADME of the effected drug will be varied by changing the enzyme’s ability to facilitate a mechanism in focus. The later is called uncompetitive inhibition. Mixed inhibition means, that both processes (competitive and uncompetitive inhibition) are involved. The effects of inhibition on saturable processes, obeying Michaelis-Menten kinetics, are summarized in table 6.1.

For tolbutamide, drug-drug interactions, effecting all of the above mentioned mechanisms, have been reported [2,35,36,40,53,56,62]. Tolbutamide interactions with well studied drugs might be easiest to model, since, as mentioned before, parametrization is a critical point in modelling drug disposition. Warfarin is a well studied drug. It is known that it interferes into the tolbutamide distribution (and vice versa) by displacing tolbutamide from albumin [2] by competitive inhibition. Furthermore both drugs are metabolized by the same enzymes (CYP2C9).

Hereby, the next extension to the current model will be the inclusion of drug-drug interactions with application to tolbutamide-warfarin interactions.

**Drug Effects.** Drugs are designed to take an effect on the homeostasis of an organism. The processes involved, however, might be of gordian quality. The various processes involved in the homeostasis of an organism and their modification through drug exposure are rarely understood for most drugs. Regulatory networks in the organism might quickly adapt to drug exposure and level out drug effects. It is quite interesting to see hereby, how much luck is (still) involved in the developing of a save and efficient drug.

In the case of tolbutamide, we know that sufficient administration leads to insulin secretion (mechanisms shown in figure 3.1). The precise effects, however, are just partly understood. As discussed before in section 1.2.5, it is still a subject of recent investigation [13–16, 22, 36, 39] to find out whether tolbutamide objects intracellular targets, besides the well known electrical effects on $K_{\text{ATP}}$-channels of the pancreatic $\beta$-cells [6]. Besides this, desensitization has been reported previously [34, 35, 47–50]. Since the direct effects of tolbutamide will cause an increase in insulin secretion, the various networks of glucose, glucagon and insulin interplay have to be modelled in order to approximately describe tolbutamide effects on the homeostasis of an organism.
6.4 Closing Remarks

Wherever the future in science will take us, nowadays it is unimaginable to come along without computation. Computational tools have become an indispensable tool, accelerating our learning processes. By implementing past discovery, the present model aims to provoke and direct new discovery. Concerning this, I leave it open to the readers to decide themselves: "To err is human. To really foul things up requires computers." (Anonymous) Discovery, of which I think is the most thrilling experience, needs questions to be asked and almost "all questions are good questions". Nonetheless, let’s listen to what Picasso said: "computers are useless [, since] they can only give you answers." -Not useless, but Picasso was partly right: It is still our responsibility to come up with the right questions.

Will we ever know the universe? -It doesn’t matter, says Douglas Adams: "There is a theory which states that if ever anyone discovers exactly what the Universe is for and why it is here, it will instantly disappear and be replaced by something even more bizarre and inexplicable. There is another theory which states that this has already happened." As Scientists, we can be happy; there will be much more to do: "Nothing is rich but the inexhaustible wealth of nature. She shows us only surfaces, but she is a million fathoms deep." (Ralph Waldo Emerson) Also, progress is relative: "We trained hard, -but it seemed that every time we were beginning to form up into teams we were reorganized. I was to learn later in life that we tend to meet any new situation by reorganizing, and what a wonderful method it can be for creating the illusion of progress while actually producing confusion, inefficiency, and demoralization." (Petronius Arbiter, 210 B.C.)

In order to do so, let’s keep up the good work and "don’t worry about the world coming to an end today. It’s already tomorrow in Australia." (Charles Schultz).
Bibliography


