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Pharmacokinetics of Sulfadiazine in Pigs

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Abstract

A mathematical model describing absorption, metabolism and excretion of sulfadiazine in pigs is developed in order to predict the concentration course of sulfadiazine and metabolites in pig urine after oral administration. First order kinetics is assumed for all processes. Distribution processes are found to be negligible for this aim, and are therefore not considered. Parametrization with maximum and minimum rate constants derived from literature yields qualitatively comparable results at a daily temporal resolution.

The model is compared to available experimental data whose suitability for pharmacokinetic purposes is limited so that a quantitative analysis is impossible. Qualitative comparison of the data with the model scenarios shows good accordance for one pig. For the other pigs, the model conforms to the data at the end and possibly at the beginning of the measurement period, but shows large discrepancies in the intermediate period. After exclusion of other hypotheses, intratubular crystallization of sulfadiazine and its acetyl metabolite is found to be the most probable explanation. The design of an experiment delivering more suitable pharmacokinetic data is outlined.

Chapter 1

Introduction

Veterinary medicines are frequently used in animal husbandry. They may enter the environment via different pathways, especially by fertilizing with liquid manure. Concentrations of antibiotics in soils ranging from some μg/kg to g/kg have been detected recently [1, 2]. Environmental risk assessment of veterinary medicines is therefore of great interest. Furthermore, most of these pharmaceuticals are "old substances" that were not subject to an official environmental risk assessment before registration, contrarily to substances registered since 1998 [3]. Hence, still too little is known about fate and effect of these substances in soil: Transport, binding and degradation processes in soil are not sufficiently understood. Additionally, it has yet to be investigated whether long term exposure of low concentrations of pharmaceuticals causes antimicrobial resistance, and whether the presence of manure affects this process. Another very important point is that additionally to the parent compound, fate and effect of metabolites have to be studied, including transformation processes during storage. Though the parent compound is generally more potent than its metabolites, they may still be significantly active. The real environmental effects may therefore be underestimated if only the parent compound is considered [4]. These questions are currently investigated in the DFG (Deutsche Forschungsgemeinschaft) project "Veterinary Medicines in Soils" for the antibiotic sulfadiazine, which has "a high potential of entering the environment" [4]. In the project, manure from pigs medicated orally with sulfadiazine is collected in a feeding experiment and used for various laboratory and controlled field experiments.

For a profound understanding of the processes occuring in manure and soil, it is fundamental to understand the preliminary processes. Uptake, metabolism, and excretion of a pharmaceutical in the organism determine the total drug concentration in manure as well as the relative concentrations of parent compound and metabolites. It is thus essential to get a deeper insight into the mechanisms involved in these processes and to get a picture of their variability.

Therefore, it is the aim of this diplom thesis to describe the basic pharmacokinetic processes of pharmaceuticals in the organism, and more specifically of sulfadiazine in pigs. Based on this, a pharmacokinetic model suitable for prediction of the temporal concentrations of sulfadiazine and its main metabolites in pig manure after oral administration shall be developed. The model will be calibrated with existing literature data and compared with data from the feeding experiment.

The work is structured as follows: After a brief characterization of sulfadiazine in Chapter 2,

Chapter 3 presents the relevant processes governing pharmacokinetics in general, and pharmacokinetics of sulfadiazine in pigs in particular. This also includes the introduction of basic pharmacokinetic variables and of the most important pharmacokinetic models. Based on this knowledge, a pharmacokinetic model for sulfadiazine and metabolites in pigs is constructed in Chapter 4. The model is analyzed, and parameterized with maximum and minimum rate constants derived from literature values. In Chapter 5, the model is evaluated with data of the DFG project, which comprises considerations about data restrictions and hypotheses about observed disagreements. Chapter 6 summarizes the results, resumes the open questions and outlines the design of an experiment suitable for their investigation.

Chapter 2

Sulfadiazine

Sulfadiazine or 2-sulfanilamidopyrimidine (SDZ, Figure 2.1) is an antibiotic belonging to the group of sulfonamides, the derivatives of sulfanilamide (Figure 2.2). The nomenclature of sulfadiazine obeys to the following rules: The nitrogen at the sulfonamido group is called N_1, and the nitrogen at the para-amino group is denoted N_4 [5]. The heterocyclic moiety is called pyrimidine ring and is numbered counter-clockwise so that the positions of the two nitrogen atoms obtain the numbers 1 and 3 [6]. The CAS of SDZ is 68-35-9.

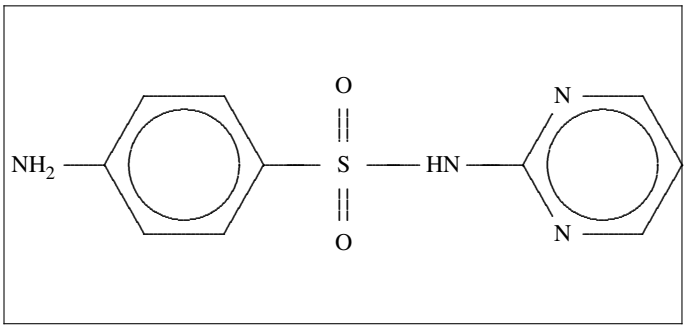

Figure 2.1: Chemical structure of sulfadiazine

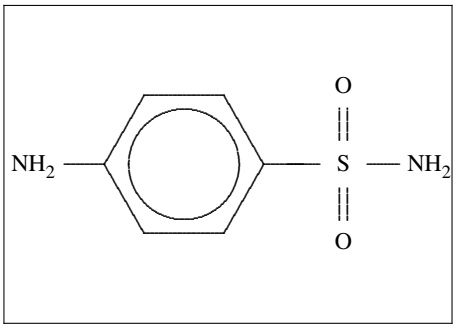

Figure 2.2: Chemical structure of sulfanilamide

The antibacterial activity of the sulfonamides was discovered in the early 1930s and "initiated a new era in the treatment of infections" [5]. Sulfonamides are bacteriostatic pharmaceuticals, i.e. they inhibit bacterial growth. As structural analogues of para-aminobenzoic acid (PABA), they act as an alternative substrate in the synthesis of folic acid. They are competitive inhibitors of dihydropteroate synthetase, which is the enzyme responsible for the incorporation of PABA into dihydropteroic acid, the direct precursor of folic acid [7, 8, 9, 10]. The decisive group for the pharmacological activity is the free amino group in para-position [11].

Sulfonamides are widely used in veterinary medicine, especially in poultry, pig and calf livestock [12]. The annual consumption of sulfonamides was 94 t in the UK in 2000 (22 % of the total consumption of antibiotics in veterinary medicine), 14 t in Weser-Ems in 1997 (21 %), 2.5 t (16 %) in Mecklenburg-Vorpommern in 2001, and 139 t (22 %) in France in 1980. On this background, a consumption of only 78 t in the EU in 1999 appears to be unrealistically low (data from different sources cited in Thiele-Bruhn [2]).

Sulfonamides can be classified based on their pharmacokinetics. Campbell [10] differentiates between absorption velocity and elimination velocity, and classifies SDZ as a sulfonamide that is both rapidly absorbed and eliminated in animals. Vree and Hekster [5] categorize SDZ as a medium long-acting sulfonamide based on its elimination half-life in humans. This difference in classification can be explained by the different half-lives of SDZ in different species: Half-lives in most animal species are between 3 and 10 h, whereas in humans, half-lives are significantly longer, ranging from 10 to 24 h [8].

Physicochemical Properties Among the physicochemical substance properties, the pK_a value plays a major role. It specifies to what extent a substance occurs in ionized or nonionized form at a certain pH. It is defined as the negative decadic logarithm of the acidic dissociation constant:

$$pK_a = -\log K_a \tag{2.1}$$

where

$$K_a = \frac{[A^-][H_3O^+]}{[AH]} \tag{2.2}$$

This term is derived from the law of mass action which states that

$$\frac{[A^-][H_3O^+]}{[AH][H_2O]} = const \tag{2.3}$$

As $[H_2O]$ remains almost constant during the reaction, it can be included in K_a. The pK_b-value of a base can be calculated analogously. It is normally given as the pK_a-value of its conjugated acid, these values being related by

$$pK_b = pK_W - pK_a \tag{2.4}$$

where pK_W is the negative decadic logarithm of the ion product of water, $pK_W = 14$. The dissociation of an acid at a given pH can be calculated by the Henderson-Hasselbach equation

$$\log \frac{[A^-]}{[AH]} = pH - pK_a \tag{2.5}$$

It follows that

$$\Phi = \frac{1}{1 + 10^{a(pH - pK_a)}} \tag{2.6}$$

where Φ is the fraction of neutral molecules, and $a = 1$ for acids and -1 for bases [13]. Consequently, 50 % of a substance are ionized at $pH = pK_a$, whereas 9 % of an acid and 91 % of a base are ionized at $pH = pK_a - 1$.

SDZ has two dissociation constants: The pK_{a1} is 2.14 [14] and refers to the protonation of the NH_2-group [12]. The pK_{a2} is 6.4 [5] and refers to the deprotonation of the sulfonamido group [12]. For this study, only the pK_{a2} is important because the pK_{a1} has no significant influence on the dissociation behavior at the relevant pH range in the body (see Chapter 3).

Dissociation also affects the partitioning behavior of a substance between two phases. The K_{OW} (octanol-water partition coefficient) is a measure for the partitioning equilibrium between organic lipids and water. It is defined as

$$K_{OW} = \frac{C_O}{C_W} \tag{2.7}$$

where C_O and C_W are the equilibrium concentrations [mg/l] of the substance in octanol and water, respectively. Octanol serves as a surrogate for organic lipids.

The K_{OW} refers to the partitioning of the neutral species only. For dissociating substances an apparent K_{OW} is observed depending on the actual pH. A pH correction of the K_{OW} can be made via the fractions of neutral molecules:

$$K_{OW}(pH_2) = K_{OW}(pH_1) \cdot \frac{\Phi(pH_2)}{\Phi(pH_1)} \tag{2.8}$$

where $\Phi(pH)$ is the fraction of undissociated molecules at a given pH (cf. [13]).

SDZ has a relatively small K_{OW} of 0.9 at 35°C and pH 4.24 where it is almost completely undissociated. Temperature variations only have a small influence on the K_{OW}: The K_{OW} at 20°C and pH 4.24 is 0.8 [14]. Some physicochemical properties of sulfadiazine are resumed in Table 2.1.

Table 2.1: Physicochemical properties of sulfadiazine [5, 14]

molar mass [g/mol]	250.28
pK_{a1}	2.14
pK_{a2}	6.40
K_{OW} (35°C, pH 4.24)	0.9

Literature reports about water solubility of SDZ are contradictory. While "Clarke's Isolation and Identification of Drugs" qualifies SDZ as "practically insoluble in water" [15], the substance data sheet in Winckler and Grafe [3] states a water solubility of 130 mg/l at 37 °C, and Krüger-Thiemer and Bünger [16] even report a solubility of 678 mg/l at pH 7 and 37 °C. The solubility of 950 mg/l in the work of Vree and Hekster [5] refers to a lower temperature (25 °C), but it is doubtful whether this higher solubility can be contributed to the temperature difference alone. As a result, it can be stated that SDZ is moderately water soluble, but literature sources are contradictory with respect to the exact amount. The aqueous solubility of SDZ at different temperatures and pH values according to different sources is resumed in Table 2.2.

Table 2.2: Aqueous solubility of sulfadiazine at different temperatures and pH values according to different sources

pH	temp. [°C]	solubility [mg/l]	source
5.5	25	265	[5]
7	25	950	[5]
5	37	127	[16]
6	37	177	[16]
7	37	130	[3]
7	37	678	[16]
8	37	5694	[16]

Chapter 3

The Fate of Pharmaceuticals in the Organism: Basics of Pharmacokinetics

This chapter gives a short review about the main processes affecting pharmaceuticals in the organism, especially sulfadiazine in pigs, by tracing the way of a drug after administration. After some general remarks, the main processes absorption, distribution, metabolism and excretion are described. This also includes the definition of principal pharmacokinetic parameters describing these processes, and the presentation of common modeling approaches. More detailed information about the involved processes is given in pharmacology and toxicology textbooks (e.g. [17] - [24]).

3.1 General Remarks

Pharmacokinetics can be defined as the processes determining the temporal changes of the concentration of drugs in the biophase [22]. Consequently, a fundamental parameter is the concentration of the drug in blood plasma, defined by the plasma concentration-time curve C(t) [mg/l]. A resulting parameter is the area under the concentration-time curve, AUC [mg h/l] [18],

$$AUC = \int_0^\infty C(t)\,dt \tag{3.1}$$

The fact that pharmacokinetics focuses on the concentration of the drug, which in many cases is the only pharmacologically active compound, also affects the notion of elimination. Contrarily to the intuitive definition, elimination in pharmacokinetics not only encompasses excretion of the substance, but the sum of excretion and metabolism processes, i.e. the sum of all processes reducing the drug concentration [24].

Though the main processes described below are similar in most mammals, pharmacokinetic parameters often vary considerably between different species. Elimination half-life for the sulfonamide sulfadoxine, for instance, is 6 - 9 h in pigs and 170 - 200 h in humans [8]. Metabolic pathways also differ very much between species. In most animal species, N_4-acetylation is the main metabolic pathway of sulfonamides, whereas dogs are unable to acetylate sulfonamides at

the N_4-position [25]. Lin and Lu [26] state "that extrapolation of drug metabolism from animals to humans is very difficult, if not impossible, both in the qualitative and quantitative aspects". The same holds for extrapolation between different animal species. Therefore, extreme caution is necessary when drawing conclusions from data for other than the considered species, though it may be unavoidable if no other data are available.

3.2 Absorption

The following sections trace the way of a drug through the body from administration until excretion. The most common drug administration forms are intravenous (IV) and oral (PO) administration. After intravenous administration, a drug is fully and immediately present in the circulatory system. In contrast, after oral administration, the drug must first be absorbed, which includes loss processes as well as a certain delay.

Oral administration is mostly carried out in solid form, and the drug must thus be dissoluted before it can be absorbed. This step frequently controls the rate of drug absorption. After dissolution, the substance is absorbed from the gastro-intestinal tract into blood. This step involves the passage of a biological membrane, which is a very important process because it occurs not only during absorption but at any of the pharmacokinetically relevant processes absorption, distribution, metabolism and excretion [19]. The cell membrane consists of a bimolecular layer of polar lipids whose hydrocarbon chains are directed inwards to form a continuous lipophilic phase and whose hydrophilic heads are directed outwards. Single lipid molecules can move sidewards, rendering the membrane relatively impermeable toward strongly polar molecules [19]. Passive diffusion is by far the major mechanism for passage of pharmaceuticals across membranes [20]. Therefore, pharmaceuticals can be better absorbed if they are non-ionized [19], thus the pH in the intestine and the pK_a of the substance affect the absorption rate. Small, water-soluble substances (molecular weight less than 200 Da [19]) can also pass the cell membrane by filtering through aqueous pores [20]. For SDZ with a molecular weight of 250.28 Da [5], this mechanism is most likely not important.

In the stomach, absorption does not play an important role, except for ruminants, because the residence time in the stomach is limited [21] and the surface of the stomach is much smaller than the surface of the small intestine [19]. Absorption mainly takes place in the small intestine where it is faster than in the stomach even if the substance is mainly ionized in the intestine and non-ionized in the stomach [19].

Acids and bases are well absorbed if the pK_a-value is beyond 2.5 for acids, and below 8.5 for bases [21]. Sulfadiazine is rapidly and almost completely absorbed in humans [27] and in pigs (see bioavailability data below). This might be explained by some simple data and calculations: The pH of the intestinal contents is about 6.6, but the effective pH in the microenvironment of the membrane is 5.3 in the human small intestine [18]. The pH of pig blood plasma is 7.4 (e.g. [28]), kept constant by the renal buffering system [18]. The relevant pK_a-value of SDZ is 6.4 [5]. If we assume equal concentrations of the nonionized form of SDZ in the aqueous solutions of plasma and small intestine, and if we further assume that the pH in the microenvironment of the membrane in humans is the same as in pigs, we can calculate the concentration ratio of total

SDZ in intestine and in plasma. The fraction of the neutral molecules of SDZ in the intestine, Φ_I, is

$$\Phi_I = \frac{1}{1+10^{pH-pK_a}} = \frac{1}{1+10^{5.3-6.4}} = 0.93 \tag{3.2}$$

Analogously, the fraction of the neutral molecules of SDZ in plasma, Φ_P, is

$$\Phi_P = \frac{1}{1+10^{7.4-6.4}} = 0.091 \tag{3.3}$$

The concentration of total SDZ in the intestine, $C_{tot,I}$ [mg/l] can be written as

$$C_{tot,I} = \frac{C_{N,I}}{\Phi_I} \tag{3.4}$$

where $C_{N,I}$ [mg/l] is the concentration of the neutral species in the intestine. Analogously, the concentration of total SDZ in plasma, $C_{tot,P}$ [mg/l] can be expressed as

$$C_{tot,P} = \frac{C_{N,P}}{\Phi_P} \tag{3.5}$$

where $C_{N,P}$ [mg/l] is the concentration of the neutral species in plasma. As we have assumed $C_{N,P} = C_{N,I}$, we obtain

$$\frac{C_{tot,I}}{C_{tot,P}} = \frac{\Phi_P}{\Phi_I} = \frac{0.091}{0.93} \tag{3.6}$$

Thus, the concentration ratio of total SDZ between intestine and plasma in this assumed equilibrium is about 1:10.

Of course, this result has to be viewed with a lot of caution, not only because of the established analogy between pigs and humans. In addition, elimination and distribution processes in the body are not considered in this approach. Despite these restrictions, the calculation corroborates the assumption of almost complete absorption and also gives a possible explanation.

Retention time of digesta in the small intestine, where absorption mainly takes place, is relatively short compared to retention time in the large intestine. Kirchgeßner et al. [29] determined retention times of digesta in the small and large intestine of adult sows whose fodder contained different amounts of wheat bran. Mean retention times in the small intestine were 10 - 19 h, whereas the mean retention times in the large intestine were 39 - 63 h. As the average volumetric capacity of the small intestine in pigs is 9.2 l, and that of the large intestine is 10.3 l [30], the longer retention time in the large intestine is not caused by a larger volumetric capacity, but most likely by digesta volume reduction due to absorption.

After absorption, the substance enters the portal circulation and is transported to the liver [20] where a certain fraction is metabolized (see Section 3.4). This metabolism before the systemic availability of the drug is called “first pass effect” [23]. The fraction of a drug entering the systemic circulation intact is called bioavailable fraction [20]. Bioavailability is defined as

$$F = \frac{AUC_{PO}}{AUC_{IV}} \tag{3.7}$$

where F is the bioavailability [%], and AUC_{PO} and AUC_{IV} [mg h/l] are the areas under the plasma concentration-time curve after oral and intravenous administration, respectively [20].
Hence, bioavailability is decreased both by incomplete absorption and by first pass metabolism. While this is a reasonable combination for pharmacokinetic purposes, it is inadequate for the purpose of this study, because in contrast to the non-absorbed fraction, the metabolized fraction enters the circulatory system, so that more than the bioavailable fraction is absorbed. Fortunately, the bioavailability of sulfadiazine in pigs is high, ranging from 85 % [31] to 90.3 % [32][1], so that despite the unknown extent of the first pass effect it can be stated that SDZ is well absorbed in pigs.
Absorption is usually modeled as a first-order-kinetic, for example in the models of Baert et al. [33] and Garwacki et al. [34] for SDZ pharmacokinetics in pigs. Besides a first order absorption, they assume immediate distribution in the body and first order elimination. This leads to the following model:

$$C_p(t) = -Ae^{-k_{ab}t} + Be^{-\beta t} \tag{3.8}$$

where $C_p(t)$ [mg/l] is the concentration in the plasma, k_{ab} and β [h^{-1}] are the absorption and the elimination rate constants, and A and B [mg/l] are the regression coefficients. For intravenous administration, the absorption term disappears and we get the classical model of exponential decay:

$$C_p(t) = C_0 \cdot e^{-\beta t} \tag{3.9}$$

where C_0 [mg/l] is the initial concentration in plasma.
The rapid absorption of SDZ is reflected in the absorption rate constants reported in literature, ranging from 0.36 h^{-1} to 1.5 h^{-1} [32, 34]. This corresponds to absorption half-lives between 0.25 h and 2.2 h.
Though generally rapid absorption is observed, there may be exceptions: In a study of Søli et al. [35] about sulfadiazine/trimethoprim combined preparations given orally to pigs, one pig out of twelve showed unusual plasma concentration profiles for both drugs and was therefore excluded. The time of maximal plasma concentration (t_{max}) was 5 - 6 times higher than that of the other pigs, which corresponds to a t_{max} of about 18 h for SDZ. This points out the large individual variability in pharmacokinetics. Søli et al. do not speculate about the reasons, but it is obvious that the delayed maximum in plasma concentrations is a result of delayed absorption. Though nothing was reported about the absorbed amount, it can be assumed that absorption of this pig was incomplete.

3.3 Transport and Distribution

Transport of pharmaceuticals between organs is carried out by the blood stream, so that the circulatory system is the essential transport system in pharmacokinetics. Transport time between organs is normally negligibly small compared to elimination and distribution processes. Distribution within the vascular system is also fast, so that the substance may be considered to

[1]The value of 106 % reported by Baert et al. [33] is obviously not realistic. It may be the result of a slower elimination in the PO experiment than in the IV experiment due to individual differences.

be homogeneously distributed within this system [36]. As the vessels are rather porous, small substances can easily pass this barrier so that the plasma space and the interstitial space may be considered as one compartment from the kinetic point of view [22].
While distribution between plasma and interstitial space is very fast, distribution to other spaces, for example the intracellular space, is often considerably slower. An extension of the model described above (Equations 3.8 and 3.9) may be necessary to cover this situation. The most common model is the two compartment open model. It assumes a central compartment in which input (via intravenous injection or absorption) and elimination take place, and a peripheral compartment. The central compartment is often denoted "plasma" and the peripheral compartment "tissues". "Plasma" stands for blood and all tissue systems in which equilibrium is rapidly attained (e.g. well perfused organs such as heart, liver or kidney), while "tissue" stands for any compartment to which distribution is relatively slow [20]. This model leads to the following equation for the plasma concentration in the central compartment after intravenous administration:

$$C_p(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \tag{3.10}$$

where A and B [mg/l] are regression coefficients, and α and β [h^{-1}] are the rate constants for distribution and elimination, respectively. Distribution processes are usually much faster than elimination processes [18]. Therefore, the curve consists of two parts, a rapid decrease mainly determinated by α and a slow decrease determinated by β. Luther [32] and Friis et al. [37] modeled the pharmacokinetics of SDZ in pigs as a two compartment model. Friis et al. report $\alpha = 5.9\ h^{-1}$ and $\beta = 0.27\ h^{-1}$ for 60 - 75 day-old pigs [37].
A very suggestive measure for the elimination velocity is the half-life in the elimination phase, $t_{1/2,\beta}$ [h], defined as

$$t_{1/2,\beta} = \frac{ln(2)}{\beta} \tag{3.11}$$

where β [h^{-1}] is the elimination rate constant in any of the described models. Reported elimination half-lives for SDZ in pigs range from 2.4 h [32] to 8 h [9], indicating that SDZ is rapidly eliminated in pigs.

The Volume of Distribution The volume of distribution (Vd) is a parameter describing the extent of distribution of a substance in a body. It can be defined as "the volume of fluid that would be required to contain the amount of drug in the body if it were uniformly distributed at a concentration equal to that in plasma" [20]. In mathematical terms, this means

$$Vd = \frac{m_B}{C_p} \tag{3.12}$$

where m_B is the mass of the drug in the body [mg] and C_p is the concentration of the substance in the blood plasma [mg/l]. For a better comparability, Vd is often normalized to body weight and then given in l/kg. It does not correspond to any physiological volume, but reflects the distribution of the substance from plasma into tissues, with larger Vd values indicating higher amounts in the tissues.
The definition of Vd assumes very fast distribution between plasma and tissues so that the

system is immediately in equilibrium. If distribution is slower, Vd becomes time dependent. In this case, we have to consider several forms of Vd, corresponding to different time points and administration forms. The Vd used in this work is the volume of distribution during the elimination phase Vd_β, also known as Vd_{area} or Vd_z. It can be calculated as

$$Vd_\beta = \frac{D \cdot F}{AUC \cdot \beta} \tag{3.13}$$

where D [mg] is the administered dose and β [h^{-1}] is the elimination rate constant.
Reported Vd_β values range from 0.55 l/kg [33] to 0.83 l/kg [6]. As these values correspond well to the water content in the organism (0.6 l/kg [23]), they might indicate that SDZ distributes into the total body water. However, such conclusions are often incorrect in the presence of fast renal excretion [21].

Binding Processes Polar substances can be bound to proteins in plasma, mostly by reversal binding of the ionized form of the pharmaceutical on ionized groups of the plasma proteins [21]. Acids predominantly bind to albumin, whereas bases preferably bind to AGP [19]. Bound pharmaceuticals can neither leave the vessels nor can they be glomerularly filtrated (see Section 3.5) or metabolized [23]. The extent of plasma protein binding (PPB) is determined by the protein concentration in blood [20]. At high doses, saturation of the potential binding sites is possible [21], but in the therapeutical dose range, PPB is linear for most pharmaceuticals [36]. Nouws et al. [6] measured a PPB of SDZ in pigs of 28.1 %, the PPB of AC was 33.3 %. This is a rather low value compared to the PPB of methylated sulfonamides such as sulfamerazine (47.7 % [6]), sulfadimidine (74.0 % [6]), sulfafurazole (80 % in humans [5]) and sulfaethidole (95 % in humans [5]).
Pharmaceuticals can also be bound to tissues. As in the case of PPB, saturation of the possible binding sites does not occur in the therapeutical dose range, so that a linear relationship can be assumed. The higher intracellular pH can result in different concentrations in plasma and tissue [36].

3.4 Metabolism

Pharmaceuticals in the organism can only be renally or biliarily excreted if they are sufficiently water soluble. Efficiency of excretion increases with increasing polarity, so the essential aim of metabolism is to increase the polarity of a substance. This process often results in a decrease or loss of toxicity and of pharmacological activity. In common pharmacokinetics, metabolized substances are considered as eliminated, since the focus of common pharmacokinetics is on the plasma concentration of the parent compound. Besides, metabolites are often rapidly excreted.
In general, metabolism is an enzymatic process. It "can be either limited by the rate of presentation to the organs of transformation, or limited by the capacity of the enzymatic system involved in the biotransformation." [20]. The enzymes catalyzing the transformation are mainly present in the liver, but also to a smaller extent in other tissues [18].

Enzyme-catalyzed metabolic reactions often follow a Michaelis-Menten kinetics. The velocity v [mol/h] of this type of reaction is defined by the Michaelis-Menten equation

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]} \tag{3.14}$$

where [S] is the substrate concentration [mol/l], V_{max} is the maximum velocity [mol/h], and K_m [mol/l] is the Michaelis constant for the given substrate-enzyme system [18]. The substrate concentration is the drug concentration at the site of biotransformation. If metabolism takes place in well perfused organs, such as the liver, this concentration is proportional to the concentration in blood plasma [18].
Many drugs occupy only a small fraction of the available metabolic sites when administered in the therapeutical dose range, so that [S] is negligibly small compared to K_m. In this case, [S] can be eliminated from the denominator and Equation 3.14 simplifies to a first order kinetics,

$$v = \frac{V_{max}}{K_m} \cdot [S] \tag{3.15}$$

The principal processes to be considered for metabolism of SDZ in pigs are acetylation and hydroxilation [6, 32, 38, 39]. In the following, these two processes will be regarded in more detail.

Acetylation Acetylation takes place in two steps: It involves formation of acetyl coenzyme A followed by a nucleophilic attack of the amino-containing compound on the acetylated enzyme. The reaction takes place in liver, spleen, lungs and intestinal mucosa [20]. The chemical structure of the acetyl metabolite of SDZ, N_4-acetyl-sulfadiazine, (denoted as AC in the following) is given in Figure 3.1.

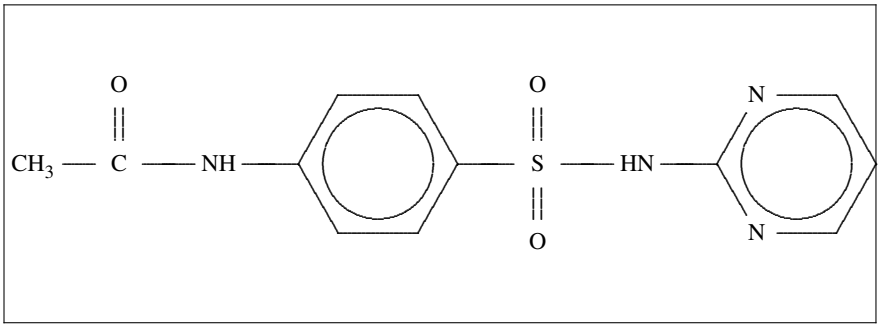

Figure 3.1: Chemical structure of N_4-acetyl-sulfadiazine

AC has a higher molar mass than SDZ (292.3 g/mol), its relevant pK_a is lower than that of SDZ (5.86) [5]. Acetylation increases the water solubility of SDZ [5]. Aqueous solubility of AC at different pH and temperatures is resumed in Table 3.1. Just like for SDZ (see Chapter 2), it is doubtful whether the reported solubilities can be trusted: The difference in solubility at pH 7 between the data of Vree et al. [5] and that of Krüger-Thiemer and Bünger [16] cannot solely be

explained by the temperature difference. Furthermore, it is unlikely that increasing temperature decreases the solubility of SDZ whereas it increases the solubility of AC (cf. Table 2.2).

It is improbable that speciation of SDZ affects acetylation reactions: The pH of blood as well as the intracellular pH are kept constant by buffering systems, and even if there was a pH variation, the dissociation of SDZ in the relevant pH range would take place at the sulfonamido group, while the acetylation takes place at the aromatic NH_2 group.

In humans, slow and fast acetylation behavior of SDZ could be observed, depending on the individual genetic phenotype [5]. In pigs, the studies of Nouws et al. [6], Shimoda et al. [39] and Mengelers et al. [40] could not distinguish obvious acetylator phenotypes.

Acetylated metabolites of sulfonamides can also be deacetylated in the organism. It is not known whether acetylation and deacetylation reactions proceed via the same or via two different enzymic systems [25]. Deacetylation is of minor importance for SDZ in pigs, but it affects significantly the pharmacokinetics of other sulfonamides, such as sulfamonomethoxine and sulfamethazine [39].

Table 3.1: Solubility of AC at different temperatures and pH values

pH	temp. [°C]	solubility [mg/l]	source
5.5	25	411	[5]
7	25	1620	[5]
5	37	198	[16]
6	37	416	[16]
7	37	2595	[16]
8	37	24400	[16]

Hydroxilation Hydroxilation is catalyzed by the cytochrome P 450 (CYP) system mainly in the liver but to a smaller extent also in the intestine, the skin and other organs. The reaction requires NADPH and oxygen [21]:

$$RH + NADPH + H^+ + O_2 \overset{CYP}{\rightarrow} ROH + NADP^+ + H_2O \tag{3.16}$$

Hydroxilation of SDZ can occur either at the 4- or 5-position, depending on the animal species [25]. In pigs, only 4-hydroxy-SDZ (in the following abbreviated as OH) [6, 38] and 4,6-dihydroxy-sulfadiazine [41] have been detected. The chemical structure of OH is presented in Figure 3.2.

Much less is known about OH than about AC because acetylation is the main elimination pathway in many species and synthesis of OH is difficult [25]. Except the molar mass (266.28 g/mol [5]) no physicochemical properties of OH are reported in the literature.

Just like acetylation, hydroxilation at the pyrimidine ring is not likely to be affected by speciation. In contrast to acetylation, hydroxilation is a monodirectional process [25].

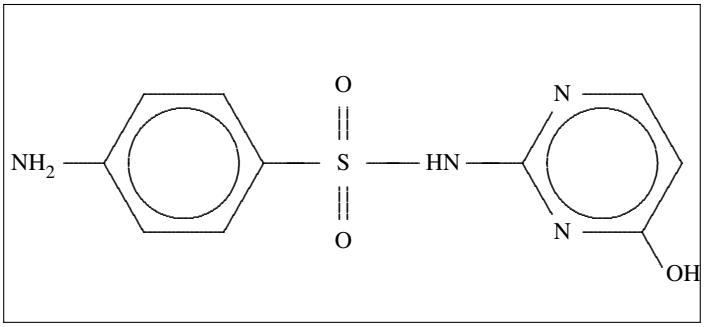

Figure 3.2: Chemical structure of 4-hydroxy-sulfadiazine

Urinary Recoveries of SDZ and its Metabolites Urinary recovery is a natural and easily accessible measure for metabolism of substances that are almost completely renally excreted, such as SDZ. Literature data for urinary recoveries of SDZ and its main metabolites in pigs are summarized in Table 3.2[2]. Obviously, there are large differences between data from the differ-

Table 3.2: Urinary recovery of SDZ and its main metabolites in pigs. diOH = 4,6-dihydroxy-SDZ, x = not analyzed, - ? = probably not detected (see text and footnote), time = collection period. Distinctive features of studies from the same source are given in "remarks". The column "total" gives the total urinary recovery, i.e. the sum of SDZ and all analyzed metabolites, in % of the administered dose. Luther [32] also detected small amounts of a metabolite named "spot#2" and of a "polar metabolite".

source	remarks	time [h]	total [%]	SDZ	AC	OH	diOH
				[% of administered dose]			
[6]		120	94.2	29.4	41.4	23.4	x
[41]		168	45	21.2	18	- ?	5.8
[32]	IV		82.8	44.4	33.5	x	x
[32]	PO		75.7	40.1	33.8	x	x
[39]		56	55.6	35.5	20.0	x	x
				[% of recovered dose]			
[38]	1 day old	3	11	≈ 30	> 50	≈ 1	x
[38]	1 week old	3	20	≈ 30	> 50	≈ 1	x
[38]	60-75 days old	3	56	> 50	≈ 20	≈ 20	x

ent sources. Such a finding is not unexpected, since the metabolic behavior of a substance in animals depends on many different factors such as species differences, dosage, age, gender, genetic predisposition, nutrition, environmental factors and deseases [42]. This makes predictions of concentration profiles very difficult. Brown [20] even notes "that it is virtually impossible

[2]Though it is not explicitely mentioned in the data, it is natural that these percentages are mol rather than gram based, because otherwise recoveries of more than 100 % would be possible. In any case, the difference between mol and gram based urinary recoveries would not be large, as the molar masses of SDZ, AC and OH do not differ much.

to quantitatively predict the relative concentrations of metabolites that will be produced after administration of a drug in any given species."

To a large extent, species variations in metabolism can be explained with differences in rates of similar metabolic reactions [18]. A significant gender-dependence of metabolism in pigs could not be detected for sulfamethazine (Figure 3.3), a substance structurally similar to SDZ [43]. On the other hand, age seems to play a decisive role in SDZ metabolism: In 1 week old piglets,

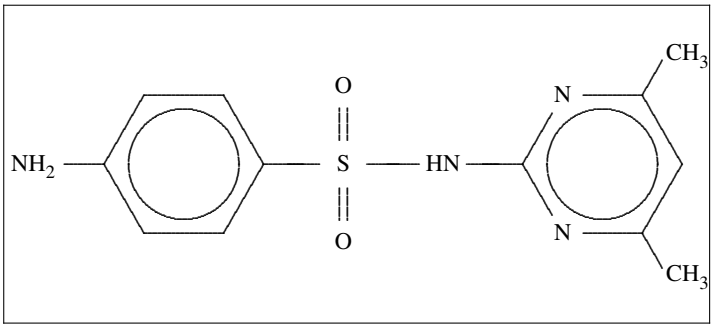

Figure 3.3: Chemical structure of sulfamethazine (sulfadimidine)

the urinary recovery of OH was only about 1 % [38], compared to approximately 20 % in 60-75 day-old piglets, indicating that this metabolic pathway is not yet established in newborn pigs.

The influence of age might also explain the surprising results of the study of Vree et al. ([41], see Table 3.2) on a three-month old piglet. If in fact the 4-hydroxy-metabolite has not been detected in the urine[3], the recollected amounts of SDZ and detected metabolites during one week are surprisingly low (45 % of the administered dose). This could be explained either by a yet unknown metabolic pathway, by measurement errors, or by an unusually slow excretion. Another surprising feature of this study is the detection of 4,6-dihydroxysulfadiazine, a metabolite mentioned in no other work about SDZ in pigs. It must be pointed out, though, that this study by Vree et al. [41] is only a pilot study on one pig, and does not give many details, nor does it present possible explanations for the unusual findings.

Pharmacological Activity of the Metabolites The pharmacological activity of the sulfonamides is the result of the free amino group in para-position [11]. Therefore, the N_4-acetyl metabolite has no antibacterial effect [44] unless reconverted into the parent sulfonamide, whereas a metabolite hydroxilated at the N_1 substituent still shows bacteriostatical activity [5, 11]. The extent of this bacteriostatical activity has not been quantified for the 4-hydroxy metabolite. Though, the bacteriostatical activity of the 5-hydroxy metabolite of SDZ, found e.g. in the urine of rhesus monkeys, is only 2.5 % of the activity of SDZ [44].

[3]The formulation in the article suggests this, but the possibility that OH has simply not been analyzed cannot be fully excluded.

3.5 Excretion

Clearance In the context of excretion processes, the notion of clearance is very important and shall therefore be defined here before considering the excretion processes in detail. The clearance measures the capability of the body to eliminate a substance. Clearance may be defined as the (hypothetical) volume of plasma that is cleared of the substance per minute by a specific process. The renal clearance (Cl_R [ml/min]) is a parameter allowing to calculate the renal elimination velocity (i.e. the velocity of excretion via the kidneys) of a substance at a given plasma concentration. It is defined as

$$Cl_R = \frac{C_U \cdot Q_U}{C_P} \tag{3.17}$$

where C_U is the concentration of the substance in urine [mg/ml], Q_U is the urine flow rate [ml/min] and C_P is the concentration in plasma [mg/ml]. C_U and C_P only refer to the concentrations of the parent compound, not of the metabolites, which have separate clearance values. One can also calculate the renal clearance of the unbound drug, in which case the term C_P is replaced by $C_P \cdot (1 - f_B)$, where f_B is the fraction of pharmaceutical that is bound to plasma proteins.
Analogously to the renal clearance, the body clearance Cl_B, denoting the sum of all clearance processes, can be defined as

$$Cl_B = \frac{m/t}{C_P} \tag{3.18}$$

where m/t is the mass of the substance eliminated per unit time [mg/min] and C_P is the concentration in plasma [mg/ml] [24]. Since elimination is the sum of all excretion processes and metabolism, the renal clearance is not the same as the body clearance even for substances that are completely renally excreted. The body clearance can rather be viewed as

$$Cl_B = Cl_R + Cl_{NR} \tag{3.19}$$

where Cl_{NR} is the nonrenal clearance of the drug, consisting of metabolic clearance, biliary clearance and others [20]. Body clearance and elimination half-life ($t_{1/2,\beta}$) are related by

$$t_{1/2,\beta} = \frac{ln(2) \cdot 50}{3} \cdot \frac{Vd_\beta}{Cl_B} \tag{3.20}$$

(cf. [20]).
Just like the volume of distribution, the clearance is often normalized to body weight for better comparability, and then given in ml/min/kg body weight.

Renal Excretion Among the different excretion pathways, renal excretion is the final route of elimination for sulfonamides and their N_4- and hydroxy metabolites. In humans, at least 90 % of a sulfonamide dose are renally excreted [5]. The delay between oral administration and first urinary excretion is small: SDZ can be detected in human urine 30 minutes after oral administration [9]. Urine production in pigs is high, as well as feces production: Adult pigs

excrete 2 - 6 l urine [45] and 0.5 - 3 kg feces, depending on the diet [30].

Three mechanisms are important for renal excretion: Glomerular filtration, tubular secretion and tubular reabsorption. The process of plasma filtration during kidney passage is called glomerular filtration. About 20 % of the plasma volume passing the kidneys are glomerularly filtrated. Molecules with a molecular mass of > 60000 Da cannot pass the membrane (these are e.g. plasma proteins or pharmaceuticals bound to plasma proteins) and remain in plasma, while small molecules (molecular mass < 15000 Da) can freely pass the filter [24]. The resulting fluid is called primary urine.

The clearance of the exogenous substance inulin is a measure for the glomerular filtration rate since inulin is completely glomerularly filtrated, but neither reabsorbed nor tubularly secreted (see below). Apparently, there are large differences of glomerulary filtration rates in pigs: Gyrd-Hansen [46] reports an inulin clearance of 2.1 (1.8-2.5) ml/min/kg, whereas Dalgaard-Mikkelsen and Poulsen ([47], cited according to [25]) report inulin clearances of approximately 4 ml/min/kg. A close approximation of the glomerular excretion rate can also be obtained by determining the clearance of creatinine, an endogenous substance that is almost completely renally excreted [25]. The renal clearance of creatinine in pigs is 3.25 ml/min/kg [48].

Another renal excretion mechanism is tubular secretion, which is an active transport from blood into renal tubules. This mechanism only takes place for certain substances, e.g. glucuronic acid conjugates [20]. Plasma protein binding does not affect tubular secretion [21]. The carrier capacity is limited so that above a certain plasma concentration, a constant amount is excreted due to saturation [20]. Active tubular transport can also occur from primary urine into plasma, but this transport mechanism is primarily important for endogenous substances rather than for pharmaceuticals [19]. The reference drug used for quantifying tubular secretion is p-aminohippuric acid [25].

Tubular secretion is the predominant excretion process for the acetyl and the hydroxy metabolite of SDZ in pigs, whereas it could not be demonstrated for SDZ itself. This additional excretion mechanism is a reason why the renal clearance values of these metabolites are about 10 times higher than that of SDZ: Nouws et al. [6] report clearances of the total drug of 0.70 ml/min/kg for SDZ, 7.9 ml/min/kg for AC and 8.8 ml/min/kg for OH[4]. These values can be converted into excretion rate constants by dividing through the volume of distribution (cf. [36]). The Vd_β reported by Nouws et al. [6] is 0.83 l/kg. Assuming that the Vd_β is the same for SDZ and its metabolites, this leads to excretion rate constants of 0.051 h^{-1} for SDZ, 0.57 h^{-1} for AC, and 0.64 h^{-1} for OH. As the renal clearance of SDZ is much lower than the inulin or creatinine clearance, there must exist a loss process after filtration. This process is reabsorption.

Reabsorption occurs during passage through renal tubules, where lipophilic substances are reabsorbed into plasma. This is why excretion of lipophilic substances is much less effective than excretion of polar substances [21], and why the excretion of N_4-acetyl and hydroxy metabolites of sulfonamides is not affected by urine pH changes [49]. Lipid solubility, pK_a of the substance and pH of primary urine determine the extent of reabsorption [18, 25]. Pig urine may be acid or alkaline, depending on the diet [18]. Reported urinary pH values of SDZ medicated pigs are

[4]Clearances for SDZ and AC have been derived from the clearance of the unbound drug by multiplying with the fraction of unbound drug reported in the same publication (see page 18).

between 5.4 [37] and 8.15 [6].
Sulfadiazine reabsorption in humans with urine pH 5 - 8 is much less pH dependent than reabsorption of sulfonamides with pK_a between 5 and 6 [25]. This seems surprising, as the pK_a of SDZ is almost in the middle of the considered pH range. However, the pH in the tubuli is not necessarily the same as in urine. It has been shown that the pH of urine in the human urinary bladder is 0.7 smaller than that of voided urine [50]; pH difference in the nephron, where secretion and reabsorption processes take place, may even be larger.
An interesting feature about excretion of SDZ and metabolites was observed by Vockel et al. [51]. They medicated 12 pigs with a sulfadiazine/trimethoprim combination during 2 weeks, and measured the urine concentrations in intervals of 2 weeks, starting 1 day after the last medication. One day after the last medication, the mean concentration of SDZ was about 17 mg/l, the mean concentration of AC was about 12 mg/l. 14 days later, the mean concentrations of SDZ and AC were both still about 1.6 mg/l, and after 10 weeks, SDZ and AC were still detectable in urine, though only in the range of 0.17 to 59 μg/l for SDZ, and 1.4 to 282 μg/l for AC. This result was also surprising for the authors of the study, as they expected that due to the rapid excretion of sulfonamides, these substances would not be detectable after some weeks.
Another interesting and unexplained result of this study is the decrease of the mean urine concentration ratio SDZ/AC (Table 3.3), though observed at very low concentrations. It is not reproducable with the common models if we assume that the acetyl metabolite is excreted faster than SDZ, which is in accordance with all other literature studies (e.g. [6, 25, 39]).

Table 3.3: Urine concentration ratio of SDZ to AC after medication [51]

Time after medication [weeks]	Mean ratio SDZ/AC
0	1.48
2	0.78
4	0.37
6	0.35
8	0.29
10	0.25

Other Excretion Pathways Among the excretion pathways other than renal excretion, excretion via salivary, sweat and mammary glands has no quantitative importance [22]. Excretion via the lungs is the decisive pathway for some volatile substances [22, 20].
For certain compounds, biliary excretion is an important elimination pathway. Biliarily excreted substances are excreted from liver into bile and subsequently absorbed into intestine. From there, they are excreted via the feces, or reabsorbed into plasma and excreted in urine [19]. The importance of this excretion route is primarily dependent on the specific substance and partly also on the animal species [20]. Compound properties facilitating excretion in bile are the presence of polar groups and a molecular weight greater than 300 - 500 Da, depending

on the species. A drug can obtain these properties by conjugation with glucuronic acid [20]. Animal species dependency of biliary excretion mainly affects "the minimum molecular weight for extensive biliary excretion of polar compounds" [20]. Poor biliary excretors are e.g. guinea pigs [20]. Polar substances with a molecular weight greater than 500 Da are predominantly biliarily excreted in all species [20].

3.6 Pharmacokinetic Parameters of Sulfadiazine in Pigs: A Compilation

As a summary of this chapter, Table 3.4 resumes minimal and maximal values reported in literature for principal pharmacokinetic parameters of SDZ in pigs. The unrealistic bioavailability of 106 % reported by Baert et al. [33] was ignored in this table. The results of Friis et al. [37] for 1 day old and 1 week old pigs were not considered either.
The volume of distribution and the bioavailability appear to show little variations, but this may also be due to the fact that these parameters have only been reported in two publications. With the exception of $t_{1/2,a}$, the other parameters vary by a factor of about 2 - 3. Given the large spectrum of variables affecting pharmacokinetics, these variations seem acceptable. However, it has to be taken into account that most studies report mean values so that individual differences are leveled off.

Table 3.4: Important pharmacokinetic parameters of SDZ in pigs: Minimum and maximum values reported in literature. t_{max} is the time of maximum plasma concentration after oral administration.

	min	max	source
$t_{1/2,\beta}$ [h]	2.4	8	[32, 9]
$t_{1/2,a}$ [h]	0.25	2.2	[32, 34]
F [%]	85	90	[31, 32]
t_{max} [h]	2.2	4.3	[33, 31]
Vd_{β} [l/kg]	0.55	0.83	[33, 6]
Cl_R [ml/min/kg]	0.78	2.3	[39, 33]

Chapter 4

Model Development and Analysis

In this chapter, a simple model for the pharmacokinetics of sulfadiazine will be developed and analyzed. In contrast to the common pharmacokinetic models, this model has the aim to describe metabolism and excretion processes separately in order to estimate the amount of sulfadiazine and its main metabolites in urine and feces. Common models combine metabolism and excretion to elimination processes, whereas a model of Shimoda et al. [39] considers acetylation, deacetylation and excretion separately, but after intravenous administration. As in most pharmacokinetic models, we assume all processes to follow first order kinetics. The resulting system is therefore linear. Though widely used, the assumption of linearity of all processes is not a trivial one. As we have seen in Chapter 3, there can be many reasons for nonlinearity. It is mostly the result of saturation of metabolism, plasma protein binding or tubular secretion [19]. By assuming linearity of the processes, we therefore implicitly assume that none of these processes is saturated for the examined dose. This is justified, as there is no evidence in literature for such a saturation and since, on the contrary, models with first order absorption and elimination have been found to model SDZ pharmacokinetics in pigs appropriately [31, 32, 33, 34, 39]. In the next sections, I will construct the model step by step, considering the processes presented in Chapter 3, and then proceed to its detailed analysis. Calculations were performed with the computer algebra system Mathematica 4.

4.1 Absorption

The first process to be modeled is absorption of the substance from intestine into blood. A spatially differentiated model based on concentration gradients would surely be appropriate, but the volume of the intestinal content is not known and most probably variable, so that a concentration based approach is not possible. Spatial differentiation would greatly increase complexity and aggravate the mathematical treatment. This is not justified given that the absorption process has no importance for the relative concentrations of sulfadiazine and its metabolites in this linear model (see Section 4.4). Furthermore, it will be shown below (see Section 4.5) that absorption is a relatively fast process for SDZ, compared to excretion and metabolism.
As sulfadiazine and its metabolites do not have the same molar mass, a mol based model is chosen, since no mass correction coefficients are then required. As mentioned above, we assume

absorption to be a first order process:

$$\frac{dm_{SI}(t)}{dt} = -k_{ab} \cdot m_{SI}(t) \tag{4.1}$$

where $m_{SI}(t)$ [mol] is the mass of sulfadiazine in the intestine, and k_{ab} [h^{-1}] is the absorption rate constant. The non-absorbed mass is excreted via the feces, which is assumed to be an independent pathway and is therefore not considered in this simple model. Consequently, the initial value of this model is the total absorbed mass of drug m_{abs} [mol], $m_{SI}(0) = m_{abs}$. m_{abs} can be determined in two ways: It can be estimated via the bioavailability, which is inaccurate because it does not take into account the first pass effect. It can also be obtained as the difference between administered mass and mass recovered in the feces if there is neither relevant metabolism in the intestine nor relevant biliary excretion of the substance. In case of SDZ, these considerations are secondary as SDZ is normally almost completely absorbed.
This approach makes several simplifications: Firstly, it assumes that the whole amount of the drug is immediately present in the intestine or, at least, that absorption mechanisms in the intestine are quantitatively comparable to mechanisms in the stomach. Secondly, it supposes that the drug is immediately present in absorbable form, which is not necessarily the case. Thirdly, it assumes that there is a constant fraction of drug that is not absorbed. This does not take into account that the absorbed fraction may depend to a large extent on the residence time in the small intestine if absorption is incomplete [23]. However, as SDZ is almost completely absorbed, this argument can be neglected in the present work. Finally, absorption takes place by diffusion through a lipid membrane and thus depends on concentration gradients, which is not considered in this approach.
Nevertheless, the approach of mass based first order absorption is widely used in the literature (e.g. [17, 33, 34, 52]) and thus seems to approximate the kinetics sufficiently well in spite of its simplifications. Furthermore, the absorption process is not the most important one, hence any more complex function would unnecessarily complicate the system.

4.2 Transport and Distribution

The next question to address is how to model the body compartment. A very detailed model is proposed by Sweeney et al. [53]. They modeled the kinetics of sulfamethazine (see Figure 3.3), a substance structurally similar to sulfadiazine, in pigs by using a multi-compartment-model with the central compartment plasma and peripheral compartments fat, kidney, liver, lung, muscle, spleen and miscellaneous. This multi-compartment pharmacokinetic model is a simplified PBPK (physiologically based pharmacokinetic) model not differentiating between arterial and venous blood. Figure 4.1 shows the structure of this model.
The model equations are not explicitly given, but the reported rate constants most likely refer to a concentration based model. A simplified version of the model equations is presented below. Rate constants for the flow between the central compartment and the peripheral compartments were fitted to experimentally determined data. Elimination and absorption rate constants are 0.30 h^{-1} and 0.33 h^{-1}, respectively. Uptake rate constants from the central into the peripheral

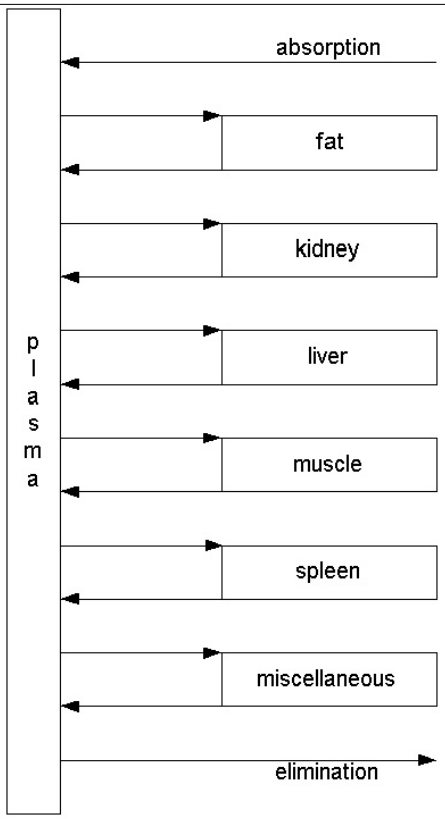

Figure 4.1: System diagram of the model of Sweeney et al. Figure adapted from Sweeney et al. [53], and created with Microsoft Visio 2000

compartments are higher than elimination and absorption rate constants (0.41 h^{-1} to 3.2 h^{-1}). Rate constants for uptake from the peripheral into the central compartment are all much higher than elimination and absorption rate constant (2.1 h^{-1} to 4.5 h^{-1}). Obviously, we can assume that distribution processes are fast compared to absorption and elimination and can be neglected at least in a model focussing on elimination processes.

In order to corroborate the assumption of rapid exchange between the compartments, we have a closer look at a simplified version of the model. We assume that there are only two compartments, the central compartment (1) and an arbitrary peripheral compartment (2), with transfer rate constants k_{12} and k_{21}. As a further simplification, we assume that there is no absorption and no elimination. We measure the distribution velocity by calculating the distribution half-life time $t_{\frac{1}{2}d}$, defined as

$$C_1(t_{\frac{1}{2}d}) = \frac{1}{2}(C_1(t_0) + C_1^*) \tag{4.2}$$

where $C_1(t)$ is the concentration in compartment 1 [mg/l], and C_1^* [mg/l] is the equilibrium concentration of compartment 1.

Reconstructing the model of Sweeney et al. in the simplified version as described above, we

obtain

$$\frac{dC_1(t)}{dt} = \frac{V_2}{V_1}k_{21} \cdot C_2(t) - k_{12} \cdot C_1(t) \tag{4.3}$$

$$\frac{dC_2(t)}{dt} = -k_{21} \cdot C_2(t) + \frac{V_1}{V_2}k_{12} \cdot C_1(t) \tag{4.4}$$

where $C_i(t)$ [mg/l] is the concentration of the substance in compartment i, V_i [l] is the volume (of distribution) of compartment i, and k_{ij} [h^{-1}] is the transfer rate constant from compartment i to compartment j.

The $t_{\frac{1}{2}d}$ of this model is $\frac{ln(2)}{k_{12}+k_{21}}$ h, independent of the initial conditions and the volumes. With the rate constants given by Sweeney et al., $k_{12}+k_{21}$ is between 2.6 and 5.6 h^{-1} for the different compartments. It follows that for the simplified model considered here, the $t_{\frac{1}{2}d}$ is between 0.12 h and 0.27 h. This is very fast, compared to elimination and absorption half-lives of 2.3 h and 2.1 h, respectively (calculated as $\frac{ln(2)}{k}$). As sulfamethazine and sulfadiazine are structurally similar substances, it is likely that this finding also applies to sulfadiazine.

This is all the more probable since the rapidness of the distribution processes compared to elimination and absorption is in accordance with common pharmacokinetic knowledge: As mentioned in Section 3.3, the α-phase (distribution phase) is much faster than the β-phase (elimination phase) in the usual two-compartment model. These general results are confirmed for SDZ by Friis et al. [37]: They studied the pharmacokinetics of SDZ in young pigs (1 - 3, 8 and 60 - 75 days old) after intravenous administration using a two compartment model as described in Equation 3.10. For the distribution rate constant α, they report values of 10.68, 17.52 and 5.88 h^{-1}, respectively. This corresponds to half-lives of 5.0, 3.0 and 7.7 min, respectively. The elimination rate constants were 0.095, 0.132 and 0.267 h^{-1} with corresponding eliminiation half-lives of 7.4, 5.4 and 2.6 h.

Given the fast distribution processes and the emphasis of this study being on elimination rather than on distribution processes, a detailed model like that of Sweeney et al. (or an even more sophisticated PBPK model) appears inappropriate for the purpose of this study. Instead, we even neglect distribution processes and assume the body to be one compartment. With this assumption, we do not exclude concentration differences between the different phases in the body, but only assume constant equilibrium between these phases.

As mentioned in Section 3.3, the plasma protein binding is linear for most pharmaceuticals in the therapeutical dose range. Therefore, we may consider the plasma protein binding as included in the rate constants for metabolism and SDZ excretion. This is all the more plausible since the plasma protein binding of SDZ is rather low compared to other sulfonamides.

4.3 Metabolism and Excretion

We can now in a first step combine excretion and metabolism to elimination. Together with Equation 4.1, we obtain the following differential equation system for the mass of sulfadiazine

in the body compartment:

$$\frac{dm_{SI}(t)}{dt} = -k_{ab} \cdot m_{SI}(t) \tag{4.5}$$

$$\frac{dm_{SB}(t)}{dt} = -\beta \cdot m_{SB}(t) + k_{ab} \cdot m_{SI}(t) \tag{4.6}$$

where $m_{SB}(t)$ [mol] is the mass of sulfadiazine in the body (except the intestine) and β [h^{-1}] is the elimination constant[1]. Solving the system of differential equations for $m_{SI}(0) = m_{abs}$ and $m_{SB}(0) = 0$, we obtain the following function for $m_{SB}(t)$:

$$m_{SB}(t) = m_{abs} \cdot \frac{k_{ab}}{k_{ab} - \beta}(e^{-\beta t} - e^{-k_{ab}t}) \tag{4.7}$$

This is equivalent to the equation given by Wagner [17]. We now consider metabolism and excretion separately, so that

$$\beta = k_{exS} + k_{met_1} + k_{met_2} + ... + k_{met_n} \tag{4.8}$$

where k_{exS} [h^{-1}] is the excretion rate constant of sulfadiazine, and k_{met_i} [h^{-1}] are the metabolism rate constants for the different metabolites. In this model, we assume that no other metabolites than met_1, ..., met_n are excreted. In case of sulfadiazine, we consider the most important metabolites, N_4-acetyl-sulfadiazine (AC) and 4-hydroxy-sulfadiazine (OH). This appears reasonable taking into account that Nouws et al. [6] recovered 94.2 % of the administered dose as SDZ, AC or OH. Resuming, we obtain the following system of differential equations:

$$\frac{dm_{SI}(t)}{dt} = -k_{ab} \cdot m_{SI}(t) \tag{4.9}$$

$$\frac{dm_{SB}(t)}{dt} = -(k_{exS} + k_{ac} + k_{oh}) \cdot m_{SB}(t) + k_{ab} \cdot m_{SI}(t) \tag{4.10}$$

$$\frac{dm_{AB}(t)}{dt} = k_{ac} \cdot m_{SB}(t) - k_{exA} \cdot m_{AB}(t) \tag{4.11}$$

$$\frac{dm_{OB}(t)}{dt} = k_{oh} \cdot m_{SB}(t) - k_{exO} \cdot m_{OB}(t) \tag{4.12}$$

where $m_{AB}(t)$ [mol] is the mass of AC in the body, $m_{OB}(t)$ [mol] is the mass of OH in the body, k_{ac} [h^{-1}] is the acetylation rate constant, k_{oh} [h^{-1}] is the hydroxilation rate constant, k_{exA} [h^{-1}] is the AC excretion rate constant, and k_{exO} [h^{-1}] is the OH excretion rate constant. In the following, this model is referred to as model **1**. A similar model (without absorption) has been developed by Krüger-Thiemer and Bünger [16].

Model **1** can be extended by taking into account deacetylation. In case of sulfadiazine, deacetylation is of minor importance, but it plays a major role for other sulfonamides, such as sulfa-

[1]Plasma concentration can easily be calculated from $m_{SB}(t)$: $C_{SB}(t) = \frac{m_{SB}(t) \cdot M_S}{Vd}$, where $C_{SB}(t)$ [g/l] is the concentration of sulfadiazine in the plasma, M_S [g/mol] is the molar mass of sulfadiazine, and Vd [l] is the volume of distribution of sulfadiazine. Therefore, formulation in terms of concentration instead of mass does not affect the rate constants.

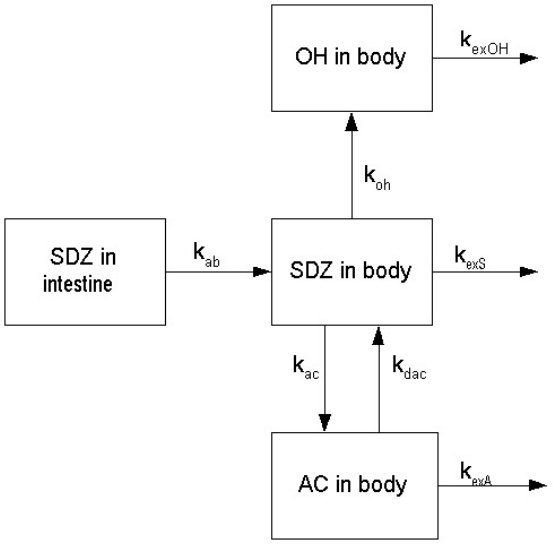

Figure 4.2: System diagram of model **1** and **2**. Figure created with Microsoft Visio 2000

monomethoxine or sulfadimidine [39]. Analogously to the model of Shimoda et al. [39], we assume the same volume of distribution for SDZ and AC, and a first order deacetylation rate constant k_{dac} [h^{-1}]: .

$$\frac{dm_{SI}(t)}{dt} = -k_{ab} \cdot m_{SI}(t) \tag{4.13}$$

$$\frac{dm_{SB}(t)}{dt} = -(k_{exS} + k_{oh} + k_{ac}) \cdot m_{SB}(t) + k_{dac} \cdot m_{AB}(t) + k_{ab} \cdot m_{SI}(t) \tag{4.14}$$

$$\frac{dm_{AB}(t)}{dt} = k_{ac} \cdot m_{SB}(t) - (k_{exA} + k_{dac}) \cdot m_{AB}(t) \tag{4.15}$$

$$\frac{dm_{OB}(t)}{dt} = k_{oh} \cdot m_{SB}(t) - k_{exO} \cdot m_{OB}(t) \tag{4.16}$$

In the following, this model is referred to as model **2**. Model **1** is a special case of model **2**, with $k_{dac} = 0$. The model structure of the two models is illustrated in Figure 4.2. For a complete mass balance, the amount of SDZ and metabolites in cumulated urine should also be considered. Appropriate equations can easily be added and will be presented in the next section.

A similar model was developed by Vree et al. [25]. Shimoda et al. [39] developed a deacetylation model for SDZ in pigs, but after intravenous administration. However, both models do not take into account hydroxilation. Furthermore, they both give concentrations in mg/l, but do not mention correction factors for the different molar masses of SDZ and AC. If in fact this point was not taken into account, the models are erroneous.

With initial conditions $m_{SI}(0) = m_{abs}$, $m_{SB}(0) = 0$, $m_{AB}(0) = 0$, $m_{OB} = 0$ and the abbreviation $\beta = k_{exS} + k_{ac} + k_{oh}$ (see Equation 4.8), the equations of model **1** lead to the following functions:

$$m_{SI}(t) = m_{abs} \cdot e^{-k_{ab}t} \tag{4.17}$$

$$m_{SB}(t) = \frac{m_{abs} \cdot k_{ab}}{k_{ab} - \beta} \cdot (e^{-\beta t} - e^{-k_{ab}t}) \tag{4.18}$$

$$m_{AB}(t) = m_{abs} k_{ab} k_{ac} \cdot \left(\frac{e^{-\beta t}}{(k_{ab} - \beta)(k_{exA} - \beta)} + \frac{e^{-k_{ab}t}}{(k_{ab} - k_{exA})(k_{ab} - \beta)} - \frac{e^{-k_{exA}t}}{(k_{ab} - k_{exA})(k_{exA} - \beta)}\right) \tag{4.19}$$

$$m_{OB}(t) = m_{abs} k_{ab} k_{oh} \cdot \left(\frac{e^{-\beta t}}{(k_{ab} - \beta)(k_{exO} - \beta)} + \frac{e^{-k_{ab}t}}{(k_{ab} - k_{exO})(k_{ab} - \beta)} - \frac{e^{-k_{exO}t}}{(k_{ab} - k_{exO})(k_{exO} - \beta)}\right) \tag{4.20}$$

As the solution of model **2** is much more complicated and this work will primarily rely on model **1**, the equations for model **2** will not be given here, but in Appendix A.

Multiple Administration The approach described above considers the pharmacokinetics after single administration. Extension for multiple administration can easily be attained since both models are linear. Therefore, kinetics after multiple administration can be described as a superposition of single administration kinetics, the kinetics after multiple administration $g(t)$ can thus be written as

$$g(t) = \sum_{t_i \leqslant t} f(t - t_i, D(t_i)) \tag{4.21}$$

where t_i [h] are the administration time points, $f(t, D)$ [mol] is the kinetics for single administration of a dose D [mol], and $D(t_i)$ [mol] is the dose administered at time t_i.

Urine Concentrations The model described above calculates masses in the body, while the aim is to describe urine concentrations. This can be achieved if we make two assumptions: Firstly, we assume that there is no relevant delay between elimination from blood and excretion in urine. This seems a plausible simplification, given that SDZ can be detected in urine 30 minutes after oral administration [9], and that 48 % of the administered dose have been recollected in the urine of young pigs 4 h after (intravenous) administration [37]. We also assume a constant urine flow Q_U [l/h]. Per unit time, the flow of SDZ out of the system, $Q_{SDZ}(t)$ [mol/h], is then

$$Q_{SDZ}(t) = m_{SB}(t) \cdot k_{exS} \tag{4.22}$$

Thus, the concentration of SDZ in urine $C_{SDZ,U}(t)$ [mol/l] is

$$C_{SDZ,U}(t) = \frac{Q_{SDZ}(t)}{Q_U} = \frac{m_{SB}(t) \cdot k_{exS}}{Q_U} \tag{4.23}$$

Analogously, the concentrations of AC and OH in urine, $C_{AC,U}(t)$ and $C_{OH,U}(t)$ [mol/l], can be calculated as

$$C_{AC,U}(t) = \frac{Q_{AC}(t)}{Q_U} = \frac{m_{AB}(t) \cdot k_{exA}}{Q_U} \tag{4.24}$$

and

$$C_{OH,U}(t) = \frac{Q_{OH}(t)}{Q_U} = \frac{m_{OB}(t) \cdot k_{exO}}{Q_U} \tag{4.25}$$

Mean concentrations of certain time intervals can be calculated as

$$\int_{t_1}^{t_2} \frac{C_U(t)}{t_2 - t_1} dt \tag{4.26}$$

where $C_U(t)$ is the urine concentration curve of the respective substance [mol/l], and $[t_1, t_2]$ [h] is the considered time interval.

4.4 Mathematical Model Analysis

Before calculating some scenarios, I will outline basic properties of the two models (or, more precisely, of model **2** and its special case model **1**). Both models are systems of linear differential equations. The functions resulting of systems of linear differential equations are also linear, therefore a modification of the input by the factor a will modify the output by the same factor a. Here, we have m_{abs} as input, and the statement can easily be verified by looking at the model equations. Thus, it is very simple to predict the effects of changes of the dose or of the absorption capability. A feature of model **1** that is immediately evident from the model structure is that the excretion rate constants of the metabolites only affect the mass of the metabolite itself, but not the mass of SDZ and the other metabolite. Analogously, $m_{SB}(t)$ and $m_{AB}(t)$ in model **2** do not depend on k_{exO}. Some interesting statements can be made about the model behavior for $t \to \infty$: It is obvious from the model structure that $m_{SI}(t)$, $m_{SB}(t)$, $m_{AB}(t)$ and $m_{OB}(t)$ converge to 0 for positive rate constants and $t \to \infty$ in both models. For the AUCs [2] in model **2**, we get

$$\begin{aligned} AUC_{SDZ} &= \int_0^\infty m_{SB}(t)\, dt \\ &= \frac{m_{abs}(k_{dac} + k_{exA})}{k_{ac}k_{exA} + (k_{dac} + k_{exA})(k_{exS} + k_{oh})} \end{aligned} \tag{4.27}$$

$$\begin{aligned} AUC_{AC} &= \int_0^\infty m_{AB}(t)\, dt \\ &= \frac{m_{abs}k_{ac}}{k_{ac}k_{exA} + (k_{dac} + k_{exA})(k_{exS} + k_{oh})} \end{aligned} \tag{4.28}$$

$$\begin{aligned} AUC_{OH} &= \int_0^\infty m_{OB}(t)\, dt \\ &= \frac{(k_{dac} + k_{exA})k_{oh}m_{abs}}{k_{exO}(k_{ac}k_{exA} + (k_{dac} + k_{exA})(k_{exS} + k_{oh}))} \end{aligned} \tag{4.29}$$

[2] I will continue using the term AUC for the area under the mass-time curve. Strictly speaking, its definition refers to the concentration-time curve, but the two curves can be easily transferred into each other.

For model **1**, this reduces to

$$AUC_{SDZ} = \frac{m_{abs}}{\beta} \tag{4.30}$$

$$AUC_{AC} = \frac{m_{abs} \cdot k_{ac}}{k_{exA} \cdot \beta} \tag{4.31}$$

$$AUC_{OH} = \frac{m_{abs} \cdot k_{oh}}{k_{exO} \cdot \beta} \tag{4.32}$$

In order to make the complete mass balance of the model, we can add three differential equations describing the change of the (cumulated) mass of SDZ, AC and OH in urine, $m_{SU}(t)$, $m_{AU}(t)$ and $m_{OU}(t)$ [mol]:

$$\frac{dm_{SU}(t)}{dt} = k_{exS} \cdot m_{SB}(t) \tag{4.33}$$

$$\frac{dm_{AU}(t)}{dt} = k_{exA} \cdot m_{AB}(t) \tag{4.34}$$

$$\frac{dm_{OU}(t)}{dt} = k_{exO} \cdot m_{OB}(t) \tag{4.35}$$

This leads to the following equations:

$$m_{SU}(t) = \int_0^t k_{exS} \cdot m_{SB}(t)\,dt = k_{exS} \int_0^t m_{SB}(t)\,dt \tag{4.36}$$

$$m_{AU}(t) = \int_0^t k_{exA} \cdot m_{AB}(t)\,dt = k_{exA} \int_0^t m_{AB}(t)\,dt \tag{4.37}$$

$$m_{OU}(t) = \int_0^t k_{exO} \cdot m_{OB}(t)\,dt = k_{exO} \int_0^t m_{OB}(t)\,dt \tag{4.38}$$

We can now calculate the total urinary recoveries for SDZ, AC and OH, SU_{tot}, AU_{tot} and OU_{tot} [mol] in model **2**:

$$\begin{aligned} SU_{tot} &:= \lim_{t\to\infty} m_{SU}(t) = k_{exS} \cdot AUC_{SDZ} \\ &= \frac{m_{abs} \cdot k_{exS} \cdot (k_{dac} + k_{exA})}{k_{ac}k_{exA} + (k_{dac} + k_{exA})(k_{exS} + k_{oh})} \end{aligned} \tag{4.39}$$

$$\begin{aligned} AU_{tot} &:= \lim_{t\to\infty} m_{AU}(t) = k_{exA} \cdot AUC_{AC} \\ &= \frac{m_{abs} \cdot k_{exA} \cdot k_{ac}}{k_{ac}k_{exA} + (k_{dac} + k_{exA})(k_{exS} + k_{oh})} \end{aligned} \tag{4.40}$$

$$\begin{aligned} OU_{tot} &:= \lim_{t\to\infty} m_{OU}(t) = k_{exO} \cdot AUC_{OH} \\ &= \frac{(k_{dac} + k_{exA})k_{oh}m_{abs}}{k_{ac}k_{exA} + (k_{dac} + k_{exA})(k_{exS} + k_{oh})} \end{aligned} \tag{4.41}$$

For model **1**, this reduces to

$$SU_{tot} = \frac{m_{abs} \cdot k_{exS}}{\beta} \tag{4.42}$$

$$AU_{tot} = \frac{m_{abs} \cdot k_{ac}}{\beta} \tag{4.43}$$

$$OU_{tot} = \frac{m_{abs} \cdot k_{oh}}{\beta} \tag{4.44}$$

We can observe that neither the urinary recovery nor the AUC depend on the absorption rate constant in both models. An interesting notice can be made for model **1**: Calculating the ratio between SU_{tot} and AU_{tot}, we get

$$\frac{SU_{tot}}{AU_{tot}} = \frac{k_{exS}}{k_{ac}} \tag{4.45}$$

Analogously, we have

$$\frac{SU_{tot}}{OU_{tot}} = \frac{k_{exS}}{k_{oh}} \tag{4.46}$$

This simple relationship which can also be derived from the model structure can be quite useful, e.g. for inverse modeling of the acetylation, hydroxilation and excretion rate constant when the elimination rate constant β and the total urinary recoveries, SU_{tot}, AU_{tot} and OU_{tot}, are given. As we have $\beta = k_{exS} + k_{ac} + k_{oh}$, we can determine the three variables out of the three equations. This is consistent with the work of Krüger-Thiemer and Bünger [16] who found the same result for a similar model.

4.5 Model Scenarios

We will now choose an appropriate model structure for modeling of SDZ pharmacokinetics in pigs, and parameterize it with rate constants derived from literature values. As these literature values show considerable variations, we choose minimum and maximum values with respect to the excretion velocity, and discuss the obtained model results.

Model Choice Shimoda et al. [39] have shown that deacetylation is of minor importance for pharmacokinetics of SDZ in pigs. If their model, corrected for the different molar masses of SDZ and AC but maintaining the rate constants, is run without deacetylation, the amount of total excreted AC changes from 29.1 % to 29.9 %, elimination half-life of SDZ changes from 2.45 h to 2.44 h (own calculations). As these are unsignificant variations, we first choose the structure of model **1**, which also facilitates parametrization. In a second step, we show that deacetylation does not largely affect the model results for SDZ.

Model Parametrization In the following, the model is parameterized with literature values for minimal and maximal excretion velocity, in order to assess the possible variations. Absorption rate constants k_{ab} are between 0.36 h^{-1} [34] and 1.5 h^{-1} [33]. Reported elimination half-lives range from 8 h [9] to 2.4 h [32], the resulting elimination rate constants β are 0.087 h^{-1}

and 0.29 h^{-1}. Though these rate constants refer to a concentration based model, this model can easily be transfered into a mass based model without affecting β by multiplying with Vd. As described in Section 4.4, β can be used to determine the rate constants for excretion, acetylation and hydroxilation of SDZ in a model without deacetylation if the total urinary recoveries for SDZ, AC and OH are known. We have

$$k_{exS} + k_{ac} + k_{oh} = \beta \tag{4.47}$$

$$k_{exS} : k_{ac} : k_{oh} = SU_{tot} : AU_{tot} : OU_{tot} \tag{4.48}$$

Few data are available about recovered OH in pig urine. Neglecting the results for newborn pigs [38], only two values have been reported: 25 % [6] [3] and about 20 % [38] of the recovered dose. For the model parametrization, we choose a constant value of 25 %, since the values do not differ much and 25 % is the precisely reported value.
The remaining 75 % are now distributed according to the ratio of excreted SDZ and AC which varies considerably in literature. The highest reported ratio is more than 50 % SDZ to about 20 % AC [38]. We regard the 20 % AC as an exact amount and thus obtain a SDZ recovery of 55 %, as we have assumed that only SDZ, AC and OH are excreted (see Section 4.3).
Since acetylation and hydroxilation are no immediate excretion processes, total excretion is slightly slowed down if contribution of k_{exS} to β is small. Therefore, the high SDZ : AC ratio will contribute to the fast excretion scenario. In this scenario, we thus have

$$k_{exS} + k_{ac} + k_{oh} = 0.29\, h^{-1} \tag{4.49}$$

$$k_{exS} : k_{ac} : k_{oh} = 55 : 20 : 25 \tag{4.50}$$

Consequently, we get $k_{exS} = 0.16\, h^{-1}$, $k_{ac} = 0.058\, h^{-1}$, and $k_{oh} = 0.072\, h^{-1}$.
The lowest reported SDZ : AC ratio is 29 % SDZ to 41 % AC [6]. Keeping the fraction of OH constant (25 %), this corresponds to a total ratio of 31 % to 44 %. The lowest elimination rate constant is 0.087 h^{-1}, thus we obtain

$$k_{exS} + k_{ac} + k_{oh} = 0.087\, h^{-1} \tag{4.51}$$

$$k_{exS} : k_{ac} : k_{oh} = 31 : 44 : 25 \tag{4.52}$$

and therewith $k_{exS} = 0.027\, h^{-1}$, $k_{ac} = 0.038\, h^{-1}$, and $k_{oh} = 0.022\, h^{-1}$ for the slow excretion scenario.
For the determination of the excretion rate constants of the metabolites, we assume just like Shimoda et al. [39] that SDZ and its metabolites have the same volume of distribution. In Section 3.5, we have transformed renal clearance values from the data of Nouws et al. [6] into excretion rate constants under this assumption, yielding $k_{exA} = 0.57\, h^{-1}$ and $k_{exO} = 0.64\, h^{-1}$. Another renal clearance value for AC has been determined by Shimoda et al. [39] and can analogously be converted into $k_{exA} = 0.48\, h^{-1}$, using the Vd_{β} reported in the same publication. Shimoda et al. also determined rate constants for an acetylation-deacetylation model. The AC excretion rate constant in this model is surprisingly high ($k_{exA} = 0.73\, h^{-1}$) compared to the

[3] Reported as 23 % of the administered dose

rate constant derived from the renal clearance. This may be partly due to the fact that their concentration based model does possibly not correct for the different molar masses of SDZ and AC. However, both values shall be taken into account as the minimum and the maximum value reported in literature. As the corresponding excretion rate constants for OH are not known, we make the following assumption: AC and OH being excreted by the same processes, the ratio of their excretion rate constants is equal, corresponding to the ratio of $\frac{0.57}{0.64}$ derived from Nouws et al. ([6], see above). Using this assumption, we obtain $k_{exO} = 0.54\ h^{-1}$ for the slow and $k_{exO} = 0.82\ h^{-1}$ for the fast excretion scenario. Minimum and maximum rate constants for the two scenarios are summarized in Table 4.1. This parametrization covers the whole range of

Table 4.1: Rate constants for the slow and fast excretion scenario. All parameters are given in h^{-1}.

	slow	fast
k_{ab}	0.36	1.51
k_{exS}	0.027	0.16
k_{ac}	0.038	0.058
k_{oh}	0.022	0.072
k_{exA}	0.48	0.73
k_{exO}	0.54	0.82

reported elimination velocities and of SDZ excretion rate constants. In order to assess the range of possible values for k_{ac}, we interchange the elimination rate constants in Equations 4.49 and 4.51. Solving the two systems, we obtain $k_{ac} = 0.017\ h^{-1}$ as minimum and $k_{ac} = 0.13\ h^{-1}$ as maximum value.
The daily urine volume and the absorbed dose have linear influence on the concentration and thus cannot induce qualitative changes. Furthermore, it is easily possible to convert a given scenario into a scenario with different urine volumes or absorbed doses. Therefore, we arbitrarily assume a daily urine volume of 1.8 l and a completely absorbed dose of 1440 mg (5.75 mmol) administered after 0, 24, 48, and 72 h. A further motivation for this choice will be given in Section 5.2.1.

Model Results In the fast excretion scenario (Figure 4.3), the substance is almost completely excreted after 24 h. It is well visible that after absorption of the substance, the concentration decrease of both SDZ and metabolites is exponential, as was expected from the model structure. Maximum SDZ concentrations in urine are approximately 2000 mg/l [4]. If the scenario parameters are modified so that the ratio SDZ : AC corresponds to the ratio of of 31 : 44 used in the slow excretion scenario, we obtain maximum excreted concentrations of more than 1400 mg/l AC and about 1200 mg/l SDZ.
In the slow excretion scenario (Figure 4.4), about 81 % of the dose are excreted on the administration day so that the remaining 19 % can accumulate in the body during the application

[4] Model results are given as gram based concentrations for a better conceivability.

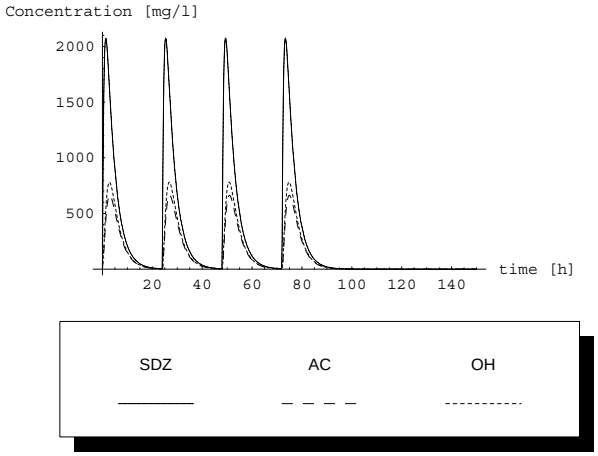

Figure 4.3: Concentrations of SDZ and main metabolites in urine according to the fast excretion scenario. Figure created with Mathematica 4.0.

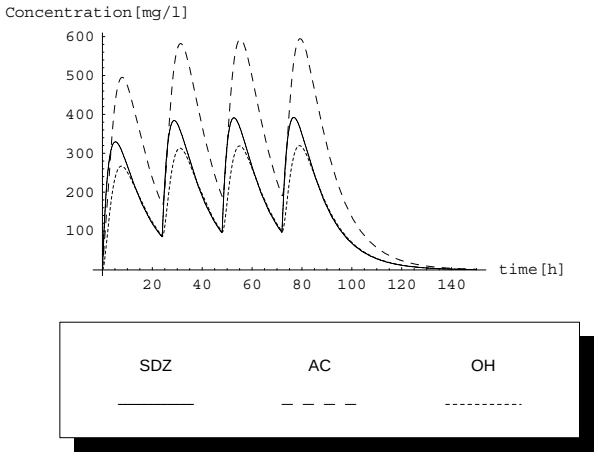

Figure 4.4: Concentrations of SDZ and main metabolites in urine according to the slow excretion scenario. Figure created with Mathematica 4.0.

period. Therefore, the mean daily concentrations increase slightly on days 1 - 4, and then decrease exponentially. About 95 % of the totally administered dose are excreted 96 h after the first administration. After 150 h, concentrations have almost approached zero. On the other hand, the maximum concentrations are much lower than in the fast excretion scenario. Nevertheless, both scenarios are qualitatively comparable, with equally high or slightly increasing mean daily concentrations on the first four days and a very fast concentration decrease shortly after the last administration. The principal qualitative difference in the two scenarios is the proportion between SDZ and the two metabolites, which is the result of a different proportion of k_{exS}, k_{ac} and k_{oh}.

Inclusion of deacetylation into the model would normally require sophisticated adjustments of the parameters in order to maintain the same acetylation-deacetylation ratio as in Shimoda et al. [39] and to obtain the same total urinary recoveries. These adjustments would mainly consist in lowering the deacetylation rate constant and increasing the acetylation rate constant, since the acetylation rate constant reported by Shimoda et al. is much higher than the acetylation rate constant in the slow excretion scenario. As the maximum impact, i.e. the maximum delaying potential, of deacetylation shall be assessed, we simply add the deacetylation rate constant of 0.030 h^{-1} reported by Shimoda et al. [39] to the slow excretion scenario, so that the model structure now corresponds to model **2**.

Table 4.2 exemplifies that the variations induced by inclusion of deacetylation are negligible, all the more since the true influence of deacetylation is likely to be even lower. The structure of model **1** is therefore sufficient for modeling the pharmacokinetics of SDZ. However, Shimoda et al. [39] show that deacetylation cannot be neglected for other sulfonamides such as sulfamonomethoxine and sulfadimidine.

Table 4.2: Variations induced by inclusion of deacetylation in the slow and fast excretion scenario

	slow excretion		fast excretion	
	$k_{dac} = 0$	$k_{dac} = 0.030$	$k_{dac} = 0$	$k_{dac} = 0.030$
$t_{1/2,\beta}$ [h]	8.0	8.3	2.39	2.42
SU_{tot}/AU_{tot}	0.72	0.76	2.76	2.87

It is obvious that in both the slow and the fast excretion scenario, k_{ab}, k_{exA}, and k_{exO} are one order of magnitude bigger than k_{exS}, k_{ac} and k_{oh}. The latter therefore determine the kinetics of the system, contribution of the former to the kinetics is secondary as long as they remain in this order of magnitude. Consequently, a further simplification of the system could be achieved in two ways: One could assume that the metabolites are excreted immediately after metabolism and therewith redundantize the excretion rate constants of the metabolites and the state variables m_{AB} and m_{OB}; and one could neglect absorption and thus assume the immediate presence of the drug in the bloodstream, as in the case of intravenous administration. This simplification is naturally only possible if high temporal resolution of the data is not required.

Chapter 5

Available Experimental Data

In order to test the developed model approach, evaluation with an independent data set is necessary. However, such data are scarce and the few existing data are hard to obtain because they are often confidential. Vockel et al. [51] report time dependent concentrations of SDZ and AC in pig urine, but the temporal resolution of 2-week-intervals is much too low. In this chapter, data from the feeding experiments in the DFG project "Veterinary Medicines in Soils" are used as no other data are available. Since it will be shown that they are hardly suitable for a detailed model evaluation, the slow excretion scenario is used as a reference scenario for a system analysis with the aim of a better understanding of the processes present in the experimental data. The figures presented in this chapter were created with gnuplot 4.0. The corresponding data are presented in Appendix B.

5.1 Experimental Setup

Three parallel feeding experiments with two piglets each were carried out by Bayer CropScience AG, Monheim, and Bayer HealthCare AG, Leverkusen. Two piglets (in the following denoted ^{14}C-SDZ pigs) were fed ^{14}C-labelled sulfadiazine, two were medicated with ^{12}C-SDZ (in the following denoted ^{12}C-SDZ pigs) and two were not medicated. The priority objective of the experiments was to collect manure for subsequent chemical and biological experiments, and not to generate pharmacokinetic data.

^{14}C-SDZ Experiment The ^{14}C-SDZ experiment was carried out by Bayer CropScience AG. The piglets, approximately 8 weeks old, were kept separately in metabolism cages. They were fed with specific food for pig breeding twice a day, with water ad libitum, and received ^{14}C-labelled sulfadiazine orally in gelatin capsules on 4 consecutive days in a dose of 30 mg/kg each day. SDZ was labelled at the 2-pyrimidine position [54], and merged with ^{12}C-SDZ in the ratio of 1:39. The specific radioactivity was 220 KBq/mg for the ^{14}C/ ^{12}C-SDZ mixture. Animal weights and daily SDZ doses are given in Table 5.1. Mixed feces of both pigs were collected as the residues on a grid situated below the slatted floor. Mixed urine was collected in a collection vessel under the grid [55]. Samples of the cumulated urine and feces of each day were taken on the 4 application days and the 6 subsequent days [54]. Volumes of the daily urine

and feces were not determined, though this was requested. After sampling, the remaining urine and feces were merged, and stored at room temperature. Total manure mass after 10 days was 22.5 kg [56].

Table 5.1: Animal weights and daily SDZ doses. Data from Köster [57]

	pig 1	pig 2
weight at first application [kg]	26	22
weight at slaughter [kg]	30	25
daily dose [mg]	780	660

^{12}C-SDZ and Control Experiments In the ^{12}C-SDZ and control experiments which were carried out by Bayer HealthCare AG, two pigs each were held in comparable cages and received the same food as the ^{14}C-SDZ pigs. Two of them were medicated with ^{12}C-SDZ, and two of them were not medicated [58]. Just like the ^{14}C-SDZ pigs, the ^{12}C-SDZ pigs received 30 mg/kg SDZ orally in gelatin capsules on 4 consecutive days. Their weight was not reported (though it was obviously measured), no absolute doses can therefore be determined. Urine and feces of these two pigs were not collected separately, because no grid was below the slatted floor in their cage. In contrast to the ^{14}C-SDZ pigs, samples of the cumulated daily manure of the ^{12}C-SDZ medicated and of the unmedicated pigs were collected individually. They were taken during the 4 application days and the 10 subsequent days. After sampling, the remaining manure of the two medicated pigs was merged, and stored cooled. Total manure mass after 10 days was 25 kg for the ^{12}C-SDZ pigs and 31 kg for the control pigs.

Sample Analysis Sample analysis was performed by the INFU (Institut für Umweltforschung, Institute of Environmental Research, University of Dortmund) by means of LC-MS-MS and radioactivity quantification. pH was measured for all samples of SDZ medicated pigs and for the samples of one unmedicated pig. Manure was subject to an extraction procedure in order to separate the liquid constituents which will be denoted supernatant in the following. This supernatant, amounting to about 95 % of the total mass, is not the same as urine, since it also contains the liquid constituents of the feces, and visibly has a different composition [56]. Concentrations of SDZ and AC in the collected matter were determined for all samples of medicated pigs. Metabolites other than AC could only be analyzed by means of radioactivity quantification and were therefore not determined in the ^{12}C-SDZ manure (supernatant). Daily radioactivity concentrations in urine and feces, and radioactivity concentration in total manure were determined for the ^{14}C-SDZ samples. All data presented in this chapter have been reported by Lamshöft [56], unless otherwise noted. Table 5.2 resumes the experimental setup and the reported variables of the three experiments.

Restrictions caused by the Experimental Setup The experimental setup described above restricts a pharmacokinetic analysis and the model evaluation in several ways:

Table 5.2: Experimental setup and variables reported in the three experiments and in subsequent analysis

	^{14}C-SDZ	^{12}C-SDZ	unmedicated
collected matter	urine and feces	manure	manure
individual samples	no	yes	yes
sampling period	10 days	14 days	14 days
daily pH	determined	determined	determined (1 pig)
absolute dose	determined	not reported	-
total manure mass	22.5 kg	25 kg (10 days)	31 kg (10 days)
daily manure mass	not determined	not determined	not determined
indiv. manure mass	not determined	not determined	not determined
manure storage	room temp.	cooled	not reported
analysis method	radioact. quant.	LC-MS-MS	-
SDZ concentration	determined	determined	not determined
AC concentration	determined	determined	not determined
other metabolites	determined	not determined	not determined

The results of the ^{14}C-SDZ and the ^{12}C-SDZ experiment are hardly comparable. Primarily, the ^{14}C-SDZ data are given as concentrations in urine and feces, while the ^{12}C-SDZ data consist of concentrations in manure (supernatant), without any information about urine and feces weights. In addition, the ^{14}C-SDZ samples were taken as mixed samples of both ^{14}C-SDZ pigs, whereas individual samples were taken for the ^{12}C-SDZ pigs. As manure volumes lack for the individual pigs, the individual samples cannot be converted into mixed samples.

The fact that the ^{14}C-SDZ data are mixed samples of two pigs also complicates the interpretation since the concentrations may differ considerably for both pigs. Despite this possibility, the only way to deal with the situation is to assume the same concentration course for both pigs as any other assumption would be arbitrary.

Furthermore, the measured concentrations in total manure are not informative: Since the ^{14}C-SDZ manure was stored at room temperature, AC could almost completely deacetylize. In addition, the ^{12}C-SDZ manure is the mixed manure of both pigs, whereas individual daily samples were taken. Therefore, concentrations in total manure cannot be considered in this work. For completeness, data are given in Appendix B.

Besides, parametrization of a pharmacokinetic model is hampered if data for the blood plasma concentration are lacking. Plasma concentration is the principal pharmacokinetic variable, since plasma is the central compartment in which absorption occurs and from which metabolism and excretion take place. The lack of plasma concentrations therefore greatly increases the incertainty, because relevant processes can only be assessed indirectly, and model parametrization has to rely to a great extent on literature values.

A further fact deteriorating the suitability for a pharmacokinetic analysis is the low temporal resolution of the data. As the highest reported elimination half-life of SDZ in pigs is 8 h [9],

it is likely that there are pharmacokinetic processes occuring within hours rather than days[1]. These processes cannot be characterized with average daily samples.
Data analysis is also hampered by an observation of Köster who performed the ^{14}C-SDZ experiment [55]: He reports that the pigs ate their feces. After 3 or 4 days, he also observed that the pigs had urinated into their drinking vessels and could therefore drink their urine. The extent of this uptake is unknown.
The values for concentration in feces are another limitation of the data: Since the samples were taken as residues on the grid, some of the feces samples had contact to urine, while others had not. Lamshöft, who performed the concentration measurements, is therefore convinced that the feces concentrations are just random numbers [56].
The most striking restriction of the data is the lack of daily urine, feces and manure masses. This is highly unfortunate, as it makes the calculation of a mass balance impossible. For the ^{12}C-SDZ medicated pigs, even more reasons inhibit the calculation of a mass balance: Only the total manure mass for both pigs together was reported, while concentrations were measured individually. Additionally, the absolute doses of administered SDZ were not reported and the concentration of metabolites other than AC could not be determined.
Summarizing, it can be stated that it is hardly possible to parameterize a mass balance model with these data. We may however try to gain qualitative insights into the processes governing the kinetics by comparing the experimental data with the developed scenarios. In the following, I will therefore analyze the data qualitatively, verifying my argumentation with some calculations based on plausible assumptions.

5.2 Results and Discussion

In this section, the experimental data are presented and discussed, with emphasis on the ^{14}C-SDZ data because there is more information about these data than about the ^{12}C-SDZ data. This concerns particularly separation of urine and feces, activities in total manure and in daily urine and feces samples, absolute doses and determination of more metabolites. Furthermore, the concentration course of the ^{14}C-SDZ data is more pronounced and much more unexpected.

pH Before discussing the concentration course, the pH values in the urine of the ^{14}C-SDZ pigs and in the manure of the ^{12}C-SDZ pigs and the control pig (Figure 5.1) shall be considered. The most surprising feature of these data is the urinary pH of the ^{14}C-SDZ pigs, which is about 7 on the first three days and about 9 on the remaining days. Since the pH of the other pigs mostly ranges between 8 and 9, the normal value for the ^{14}C-SDZ pigs is probably 9. However, this is not certain, since the pH in urine is compared with the pH in manure. In any case, the sharp pH increase after 3 days is exceptional. No evident cause for this phenomenon could be found[2]. At first view, it seems as if the SDZ administration could be the reason for the low pH, but this can be excluded: A potential influence of SDZ would affect the pH on the day after administration. The pH 4 days after first administration would therefore still be affected

[1] The evident assumption that all processes principally occur within hours is valid for literature experiments and for the calculated scenarios, but does not conform to the given data, as will be explicated in more detail in the next

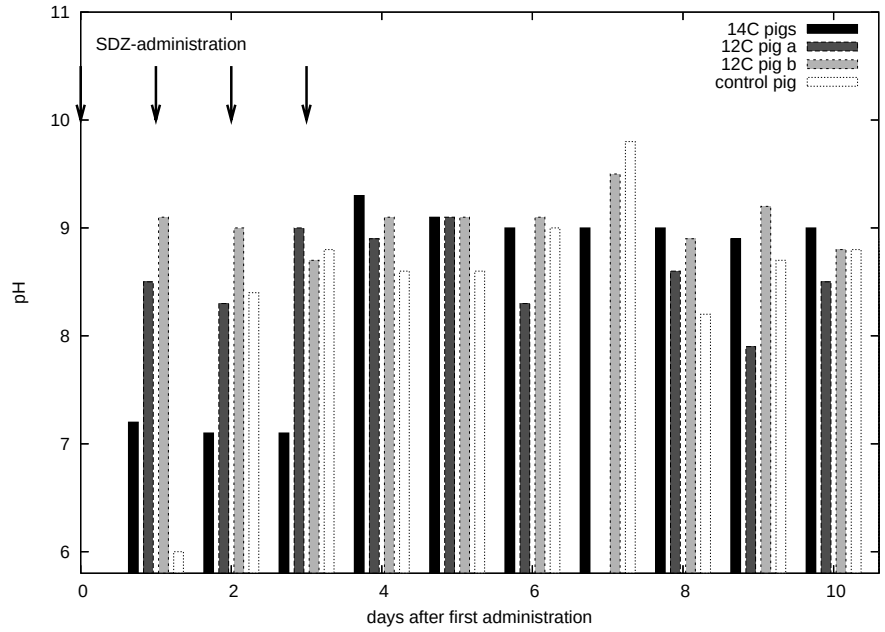

Figure 5.1: pH of urine of the ^{14}C-SDZ pigs and manure of the ^{12}C-SDZ pigs and one control pig on days 1-10. pH of the ^{12}C-SDZ pig **a** was not determined on day 7, because its manure was pasty.

by the dose administered on day 3. Urinary pH is mainly determined by the diet, but according to Köster [55], there was no change in diet during the experiment, nor were additional drugs applied.
A potential hypothesis is that the pigs had not enough time to familiarize with the new environment so that the pH on the first days could be the result of stress, but there is no evidence for this theory. It also appears unclear why the familiarization should occur abruptly and synchronously for the two ^{14}C-SDZ pigs. Another hypothesis is that the pH was altered by the uptake of urine and feces.
pH of the ^{12}C-SDZ pig **a** could not be determined on day 7 because the manure was pasty. This may be an indication for varying urine volumes.

5.2.1 Comparison of Experimental Data and Model Results

^{14}C-SDZ Data

Two main metabolites were detected in urine, AC and a hydroxy metabolite. It has not yet been analyzed whether hydroxylation occured at the 4- or at the 5-position, but given the literature findings, the metabolite is most likely 4-hydroxy-SDZ [56]. Besides these two metabolites, up to 5 % of the daily concentration were present as N_4-acetyl-hydroxy-sulfadiazine (ACOH). As in the case of the hydroxy metabolite, hydroxylation most likely occured at the 4-position.

section.

[2]Similarly, there is no explanation for the pH of 6 in the manure of the unmedicated control pig on day 1.

Furthermore, traces of formylsulfadiazine and N-glucuronide-sulfadiazine were detected [56]. ACOH, formylsulfadiazine and N-glucuronide-sulfadiazine have not yet been reported in literature about sulfadiazine in pigs.
The activity concentration of total recollected manure was 40.5 KBq/ml. Daily activity concentrations in urine and feces are given in Figure 5.2. The data corroborate the assumption

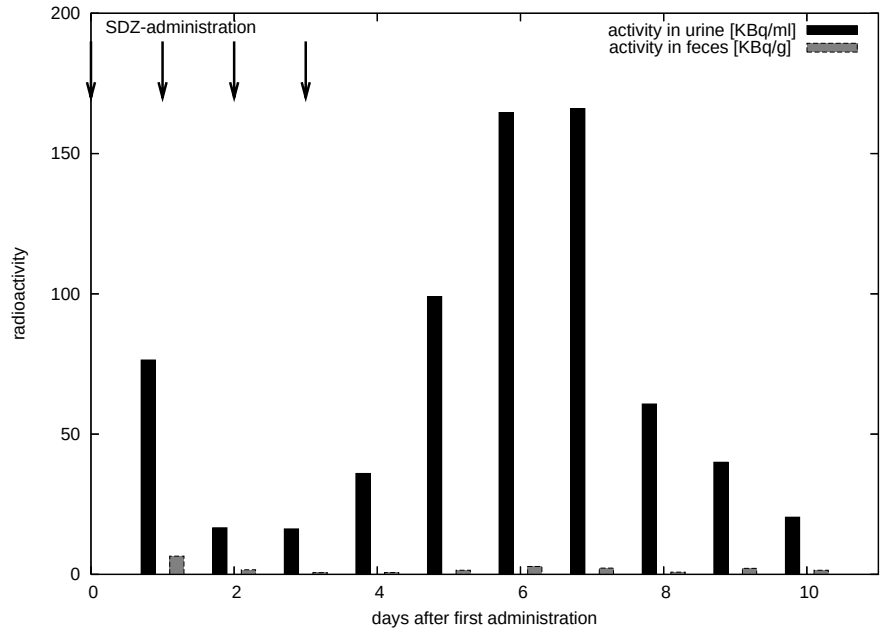

Figure 5.2: Radioactivity in urine and feces after administration of ^{14}C-SDZ

of complete absorption, as very few activity was recollected in feces. Lamshöft [56] is even convinced that the measured activity is mainly caused by the contact to urine and that almost no SDZ and metabolites are present in feces. This is all the more probable since the highest concentration in feces was reached on the first day, whereas Kirchgessner et al. determined a retention time of digesta in the intestinal tract of 78 h [29]. As almost complete absorption is also reported in literature, I will adopt this assumption for the ^{14}C-SDZ data. However, the interpretation of the data for the ^{12}C-SDZ pig **a** will show that this supposition may not always be valid.
The rapid decrease of the urine concentrations starting on day 7 suggests that almost all SDZ was excreted during the experiment. This is consistent to the official statement that only 1.6 % of the administered dose remained in the pigs after 10 days [57], and to literature reports (e.g. [34]). Calculation of a mass balance is hampered by the fact that no urine volumes were determined. A possible way to deal with this situation is to assume equal volumes for each day, and to calculate the mass balance under this assumption. However, it can be shown that this assumption does not hold, at least for the ^{14}C-SDZ data. Under the assumption of equal volumes, we can calculate the concentration in total urine as the mean of the daily concentrations, which is 69.6 KBq/ml. The measured activity concentration in total manure (supernatant) is 40.5 KBq/ml. A rough calculation demonstrates that this difference cannot solely be due to the fact that concentration in urine is compared to concentration in manure: The water content of pig feces is between

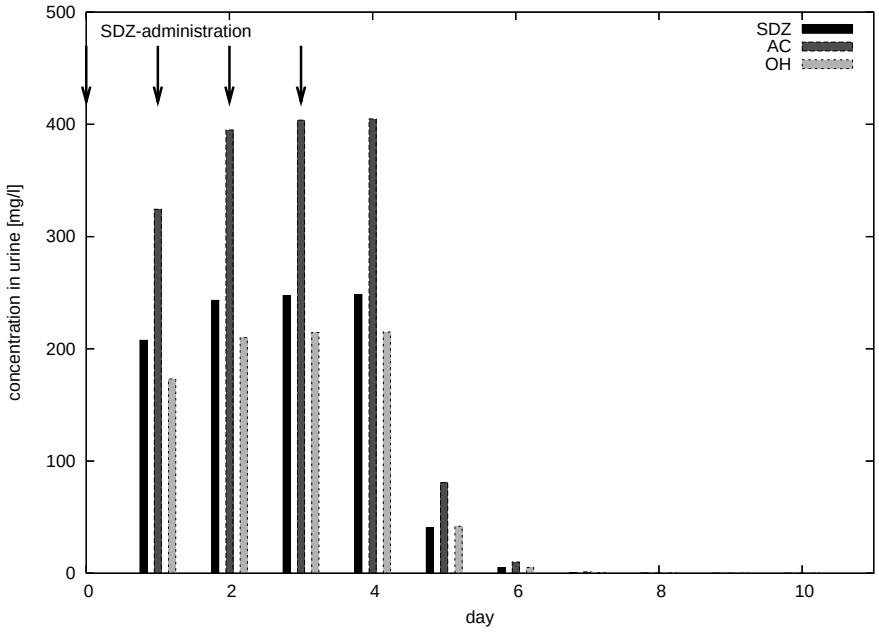

Figure 5.3: Daily urinary concentrations of SDZ and metabolites in the slow excretion scenario

55 % and 75 % [30]. The dry matter of the manure was about 5 % [56], so that the contribution of the feces to the liquid phase of manure was probably not more than 15 %. If we assume that no activity was present in 15 % of the supernatant, the concentration in the remaining 85 % (corresponding to the urine concentration) is still only 47.6 KBq/ml. Consequently, there must have been large variations in the urine volumes. This conforms to the inofficial and uncertain information that the urine volume decreased continously during the experiment, and to the fact that the ^{14}C-SDZ pigs produced much less manure than the unmedicated pigs.

The mean daily concentrations of SDZ and metabolites shall now be compared to the results of the slow excretion scenario. This is possible since the input of both scenarios developed in Section 4.5 was chosen so that it corresponds to the input of the ^{14}C-SDZ experiment. 22.5 kg of manure were collected, thus it seems plausible to assume that it consisted of about 18 l urine, or 1.8 l/day, for the two pigs. Just like in the scenario, the daily administered dose for the two pigs was about 1440 mg for the two pigs (see Table 5.1). Obviously, a comparison of the experimental data to the slow excretion scenario is more interesting because the kinetics governing the experimental data is slow (see Figure 5.2). Daily concentrations in the slow excretion scenario were calculated according to Equation 4.26 and are presented in Figure 5.3. Concentrations of ^{14}C-SDZ and its metabolites in urine determined by radioactivity quantification are given in Figure 5.4.

Figure 5.3 points out that based on the developed model, the concentrations of SDZ and metabolites in the experiments were expected to be high and slightly increasing during the first 4 days, exponentially decreasing on day 5 and 6, and negligibly small on the remaining days. SDZ, AC and OH were expected to occur in roughly equal concentrations.

In fact, the determined relative concentrations of SDZ and metabolites are in the range of reported literature values. In contrast, the concentration course does not seem to meet the expec-

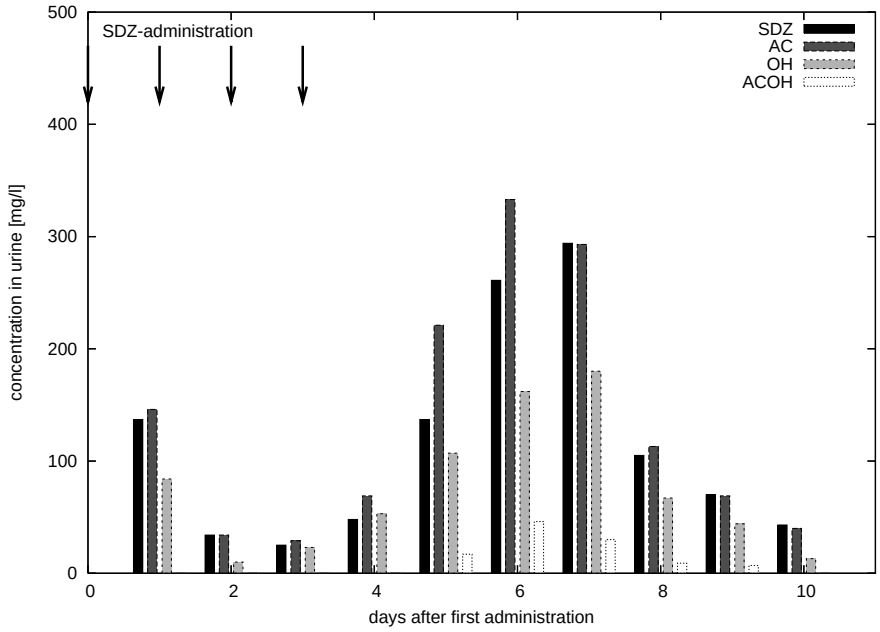

Figure 5.4: Concentrations of ^{14}C-SDZ and metabolites in urine determined by radioactivity quantification

tations at all. However, further investigation reveals that there is evidence that it corresponds to the model at the end and possibly at the beginning of the measurement period:
The concentrations after the last peak (day 7 - 10) resemble an exponential decrease. Actually, regression of the data for the last phase to an exponential function (Table 5.3) yields good coefficients of determination not only for the ^{14}C-SDZ but also for the ^{12}C-SDZ data (see below)[3]. Though a single good regression of four data points to an exponential function is not very significant, the fact that the final phase of each data set can be fitted is strong evidence for the hypothesis of an exponential decrease. Furthermore, the decay constants for SDZ excretion (0.026 - 0.083 h^{-1}) are similar to the reported SDZ excretion rate constants (0.027 - 0.16 h^{-1}, see Section 3.6). Decay constants for the metabolites have to be compared to the metabolism rate constants, as metabolism occurs much slower than metabolite excretion and therefore is the process governing the kinetics. They are within the parameter range determined in Section 4.5 (0.017 - 0.13 h^{-1} for k_{ac}, 0.022 - 0.072 h^{-1} for k_{oh}). The only reported metabolism rate constant ($k_{ac} = 0.077\ h^{-1}$ [39]) also fits in. If indeed the decrease follows first order kinetics in the final phase, the experimental data in this phase are in accordance to the model developed in Chapter 4.
Pharmacokinetics at the beginning of the measurement period is difficult to judge. The model predicts an exponential concentration decrease during the day, and a sharp increase after the next application, followed by a new exponential decrease until the next application. As the characteristic pharmacokinetic behavior happens within one day, the resolution of the given

[3]Naturally, this approach is only reasonable under the assumption of approximately equal urine volumes in the final phase. Furthermore, the regression of the SDZ concentrations of pig **a** is questionable if we assume excretion in feces on day 8 (see below).

Table 5.3: Coefficients of determination and decay constant λ for regression of the last phase of the concentration data to an exponential function. The regression was carried out with Microsoft Excel 2002.

		r^2			$\lambda\ [h^{-1}]$		
	day	SDZ	AC	OH	SDZ	AC	OH
^{12}C, pig **a**	8 - 11	0.962	0.987	-	0.071	0.046	-
^{12}C, pig **b**	8 - 11	0.961	0.983	-	0.083	0.092	-
^{14}C	7 - 10	0.949	0.974	0.971	0.026	0.027	0.034

data is too low for a detailed examination of the model. However, the model predicts that most of the substance will be excreted one day after administration. In the following, we will check whether the experimental data conform to this prediction.

We first calculate the urine volume leading to the activity concentration on the first day if the whole dose is excreted in urine. We denote this volume as V_{max} [l], because it is the upper bound for the excretion volume on the first day. We have

$$V_{max} = \frac{D}{C} \tag{5.1}$$

where D is the dose [KBq] and C is the concentration in urine [KBq/ml]. The dose for the two pigs on the first day is 780 mg + 660 mg = 1440 mg, or $1440\,mg \cdot 220\,KBq/mg = 316.8\,MBq$. Activity concentration in urine on the first day is 76.3 MBq/l, thus V_{max} is $\frac{316.8\,MBq}{76.3\,MBq/l} = 4.15\,l$. The average excreted manure volume per day is $\frac{22.5\,l}{10\,d} = 2.25\,l/d$[4]. Manure consisted of about 95 % liquid constituents [56]. If we assume that the feces contained 65 % water, which is the average value reported in literature [30], urine contributed about 86 % (1.9 l) to the daily manure volume. Supposing that this urine volume was excreted each day, we obtain an excretion of 46 % of the given dose on the first day. However, we have the information that volumes decreased continously. Therefore, it is likely that the volume excreted on the first day was much higher than the average excreted volume. Taking the average daily manure production of the unmedicated pigs (3.1 kg manure, corresponding to 2.7 l urine) as a reference value for the excretion on the first day, the excreted dose would be about 65 %. This is considerably less than the 81 % in the slow excretion scenario. However, the value of 65 % can be attained if the slow excretion scenario is parameterized with an elimination rate of 0.054 h^{-1} instead of 0.087 h^{-1}, which is still an imaginable variation.

Thus, the ^{14}C-SDZ data conform to the developed model at the end and possibly at the beginning of the experiment. This is obviously not the case for the intermediate period, except if we assume enormous variations in the manure volume (this hypothesis is examined in more detail in the next subsection). The most remarkable feature for this period is not the fact that elimination is slower than predicted. This could be modeled with smaller excretion or metabolism rate constants, which could be due to race differences or renal impairment.

[4]The manure density is close to 1 kg/l [56].

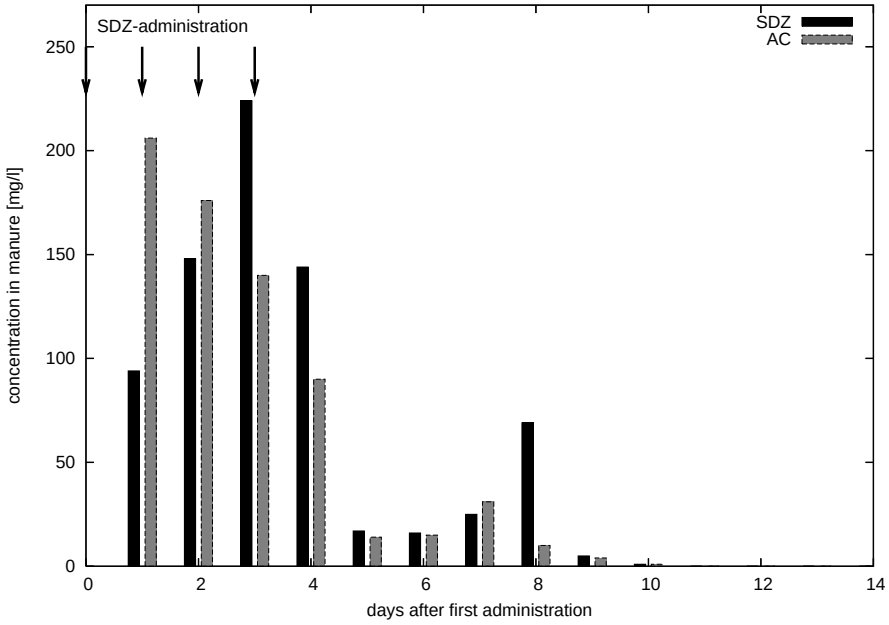

Figure 5.5: Concentrations of ^{12}C-SDZ and AC in manure of pig **a** determined by LC-MS-MS

Essentially, the experimental data seem to violate a principal assumption not only of this model, but of pharmacokinetics in general: The excreted amount of SDZ does not seem to depend on the concentration in blood plasma. Plasma concentrations are most probably high and increasing during the first 4 days, while they decrease monotonously during the rest of the experiment. In contrast, concentrations in urine are very low on days 2 - 4, when they should have been highest, whereas they are extremely high on days 6 and 7, when they should have been rapidly decreasing. The measured concentrations would indicate administration on days 1, 5, 6 and 7 rather than on days 1 - 4.
This phenomenon can neither be modeled with any of the common pharmacokinetic models nor with the model developed in the preceding chapter, as they are all based on the assumption that higher plasma concentrations lead to greater or equal excretion. If we assume that no fundamental errors occured during sampling and analysis, explanation can only be based on missing data or unconsidered processes. Subsections 5.2.2 and 5.2.3 present some hypotheses for this behavior.

^{12}C-SDZ Data

The concentration course of pig **a** (Figure 5.5) corresponds well to the expectations: High concentrations on the first four days are followed by low concentrations on the remaining days, with the exception of day 8. 13 days after first administration, concentrations in urine were lower than 0.1 mg/l, so that it can be assumed that SDZ was completely excreted.
Only two features of these data differ from the expectations: Firstly, relative concentrations of SDZ and AC vary considerably during the first days. This cannot be explained with the fixed rate constants in the model. However, the model can help to assess the rate constant variations

required to explain the observed behavior. Except the extraordinarily high ratio SDZ/AC on day 8 which will be considered below, the highest ratio on the first 10 days is 1.56 on day 3, the lowest ratio is 0.46 on day 1. Equation 4.45 states that $\frac{SU_{tot}}{AU_{tot}} = \frac{k_{exS}}{k_{ac}}$. As excretion of the administered doses is probably almost complete on day 1 and 3, and since excretion velocity of AC is very fast in any case, SU_{tot}/AU_{tot} can be replaced with the ratio of SDZ/AC recovered on day 1 or 3. It follows that a variability of both k_{exS} and k_{ac} by a factor of 2 (or one of these rate constants varying by a factor of 4) would be sufficient to model the measured variations. This is imaginable given the large spectrum of factors affecting metabolism and excretion.

Secondly, the SDZ peak on day 8 is astonishing at first view. However, it can plausibly be explained by incomplete absorption on day 4 and subsequent excretion of SDZ in feces. There are several arguments corroborating this theory: Concentrations on day 4 are lower than on the preceding days. Additionally, the delay of 96 - 120 h between uptake and excretion corresponds roughly to the retention time of 78 h observed by Kirchgessner et al. [29]. The longer retention time may e.g. be caused by a diet containing less fibres. Finally, there is an untypically large discrepancy between SDZ and AC concentrations on day 8: While SDZ and AC concentrations on all other days from day 5 - 10 are roughly equal, SDZ concentration on day 8 is more than six times larger than AC concentration. This is very strong evidence for incomplete absorption followed by excretion in feces, as unabsorbed substances are mostly excreted unchanged. The observed absorption behavior may be similar to that of the pig excluded from the study of Søli et al. [35] (see Section 3.2).

Estimation of the excreted amount after 4 days is difficult: The administered doses are not known, there is no information about the excreted volumes, and only SDZ and AC concentrations have been measured. We can however estimate the fraction of the total dose excreted on days 1-4, Φ_4, under the assumption of a constant excretion volume, complete excretion after 10 days and an excretion of other metabolites which is proportional to the amount of excreted SDZ + AC:

$$\Phi_4 = \frac{\sigma_4}{\sigma_{10}} \tag{5.2}$$

where σ_n is the amount of SDZ equivalents excreted after n days,

$$\sigma_n = V \cdot \sum_{i=1}^{n} (C(SDZ)_i + C(AC)_i \cdot \frac{250.28\ g/mol}{292.30\ g/mol}) \tag{5.3}$$

V is the constant volume of manure excreted per day [l], $C(SDZ)_i$ is the concentration of SDZ in manure on day i [mg/l] and $C(AC)_i$ is the concentration of AC in manure on day i [mg/l]. In the calculation, V cancels out, and we obtain $\Phi_4 = 0.85$, i.e. 4 days after the first administration, pig **a** had excreted 85 % of the given doses. This is less than in the slow excretion scenario where 95 % excretion are predicted, but this difference is largely due to the SDZ concentration peak on day 8.

Comparison of the data for pig **a** and pig **b** (Figure 5.6) reveals a strange feature: SDZ and AC concentrations on the first four days are both extremely much lower for pig **b** than for pig **a**. This suggests that pig **b** excreted much less SDZ and AC than pig **a** though they both

received a SDZ dose of 30 mg/kg and though the drug was completely excreted after 14 days. A further investigation of this phenomenon is impossible because neither the absolute dose nor the manure mass of the single pigs are known. Extensive formation of the OH metabolite, a much lower absolute dose, or extensive manure production for pig **b** might explain the concentration differences.
With regard to these large individual differences, the lack of individual samples for the ^{14}C-SDZ

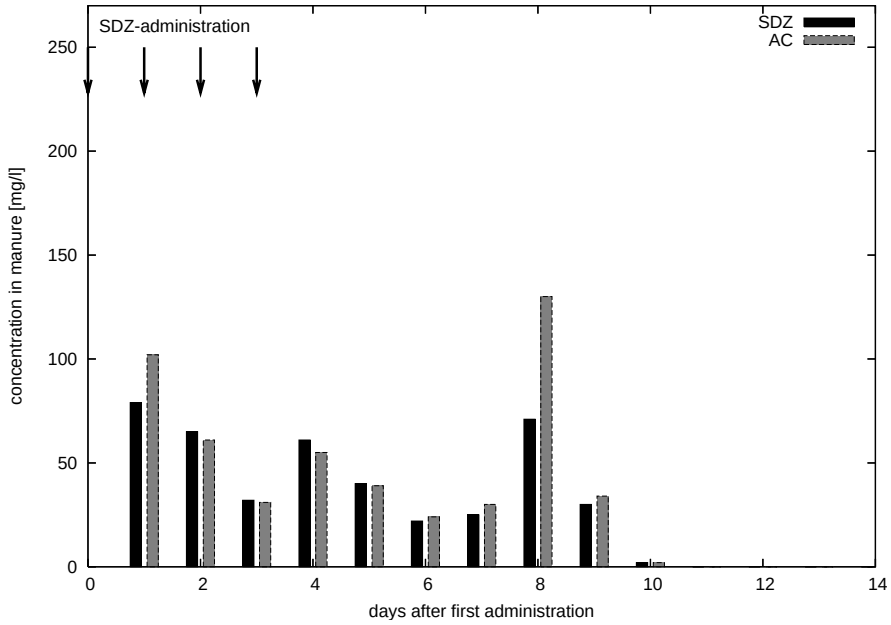

Figure 5.6: Concentrations of ^{12}C-SDZ and AC in manure of pig **b** determined by LC-MS-MS.

pigs is even more regrettable, since the ^{12}C-SDZ data suggest that the kinetics even for two pigs held in exactly the same conditions may be extremely variable. In case of the ^{14}C-SDZ pigs, the concentration course is so extreme that considerable differences in the individual concentration course appear unlikely, but they cannot be excluded.
Just like for the ^{14}C-SDZ data, there is evidence that the data for pig **b** correspond to the model on the first day and on the final days. The exponential decrease on days 8 - 11 has already been mentioned (see Table 5.3). Furthermore, calculation of Φ_1 analogously to Equation 5.2 reveals that excretion on the first day is 19 % of the total dose, or 76 % of the dose administered on the first day. This is very similar to the 81 % predicted in the slow excretion scenario.
The remaining concentration course does not correspond to the expectations: Concentrations on the administration days decrease instead of increasing; Φ_4 is 52 %, and therewith far from the expected 95 %. The highest concentrations are reached on day 8, but contrarily to the data for pig **a**, this peak mainly consists of AC and can therefore not be explained by incomplete absorption. These features correspond to the ^{14}C-SDZ data where the concentration course is even more pronounced. It is therefore probable that the same process than for the ^{14}C-SDZ data is present. Possible hypotheses for this process will be presented in the next subsections. In Subsection 5.2.2, the most obvious approaches are refuted before the most promising hypothesis is presented in Subsection 5.2.3.

5.2.2 Disprovable Hypotheses

Volume Variations A self-evident hypothesis is that the data correspond so badly to the model because the model assumes constant urine flow, whereas it has be shown in Section 5.2.1 that the daily urine volumes varied considerably. However, a comparison of the slow excretion scenario[5] with the ^{14}C-SDZ data (Figures 5.3 and 5.4) shows that the difference cannot solely be due to different excretion volumes. This impression is strikingly confirmed if we calculate the volumes that would be required so that the activities in the measured data correspond to those in the scenario: The (hypothetical) volumes excreted per day by the two pigs range from 19.5 l on day 3 to almost no excretion on days 6 - 10 (Table 5.4), which is obviously unrealistic, as well as the total urine volume of 51 l. Only if all rate constants in the scenario are scaled

Table 5.4: Urine volumes hypothetically required for the measured activity data in order to coincide with the slow and the very slow excretion scenario (in the "very slow" excretion scenario, the rate constants of the slow excretion scenario are scaled down by the factor of $\frac{1}{12}$)

	volume [l]	
day	slow	very slow
1	3.4	0.1
2	18.7	1.8
3	19.5	3.7
4	9.0	2.6
5	0.6	1.2
6	0.0	0.7
7	0.0	0.7
8	0.0	1.8
9	0.0	2.4
10	0.0	4.0
sum	51.2	19.0

down by a factor of $\frac{1}{12}$, we obtain a realistic total urine volume of 19 l, and imaginable values for the daily urine volumes between 0.1 l and 4 l. However, both prerequisites, a decrease of the minimum reported rate constants by a factor of $\frac{1}{12}$ and variations of the daily urine volumes by a factor of 40, are rather extreme. If this can occur at all, it is only imaginable in presence of an exceptional process that has yet to be identified.

Uptake of Urine and Feces It shall now be examined in how far the uptake of feces and urine which occured during the ^{14}C-SDZ feeding experiment could have affected the outcome of the experiment. Uptake of feces has few relevance in the context of this study: Concentrations of SDZ and metabolites in feces were negligibly small even in case of contact with urine. The only possible effect of such uptake might therefore consist in variations of the urinary pH due to the change in diet.

[5] The fast excretion scenario is even more extreme.

In contrast, the uptake of urine could indeed have affected the concentration curve. The high concentrations on days 5 - 7 could amongst others be caused by uptake of urine on the preceding days, under the condition that considerable masses were excreted into the drinking vessels. This is doubtful for days 2 - 4 because concentrations were low, but it cannot be excluded. In any case, uptake of urine cannot explain the extraordinarily low concentrations on days 2 - 4: Urine uptake may increase the urinary concentrations on the following days, but it can never cause a decrease of the concentration on the present day; only the mass of a substance in the collected urine is reduced. Consequently, the low concentrations on days 2 - 4 are definitively not caused by uptake of urine.

Delayed Absorption An obvious hypothesis is that the delayed concentration peaks are the result of delayed absorption in the small intestine. As mentioned above, delayed and therefore incomplete absorption may explain the concentration peak in the data for pig **a**. This is an exceptional case, as SDZ is normally rapidly absorbed in pigs, with absorption half-lives between 0.25 h and 2.2 h [32, 33, 34, 35], but it may have happened due to bad solubility of SDZ in the intestine.
In contrast, intestinal absorption cannot have caused a delay longer than 20 h in the ^{14}C-SDZ data: Since all orally administered drugs are absorbed principally in the small intestine [18] and since no relevant traces of SDZ have been detected in the feces, we can assume that absorption was almost complete after passage of the small intestine. Passage time of digesta through the small intestine is about 10 - 20 h in pigs [29], after which the absorption should thus be completed.
Therefore, it can be stated that if there was a relevant delay of absorption at all, this must have happened before SDZ entered the small intestine, namely in the stomach. This could be the case if the administered capsules were resistant to gastric juice. In this case, their passage into the small intestine could be considerably delayed if administered with food. In humans, Ewe et al. [59] report an emptying delayed by 10 h; in 2 of 10 persons, the capsules were still in the stomach after 16 h. However, the supervisors of the ^{14}C-SDZ and the ^{12}C-SDZ experiments, Köster [55] and Beddies [60], state that the administered gelatin capsules dissolve almost immediately in the stomach. Immediate dissolution of gelatin capsules was also reported in literature, e.g. [61, 62].

Reabsorption Another hypothesis is that reabsorption from the renal tubules slowed down the excretion of the ^{14}C-SDZ pigs during the first days. Reabsorption is pH-dependent, though this dependency is much less pronounced for SDZ than for other sulfonamides (see Section 3.5). It could be argued that during the first three days, when pH was low, SDZ was almost completely reabsorbed, while on the following days reabsorption did not take place because of the higher pH. This hypothesis can be refuted in several ways, some of which shall be presented here. Firstly, pH-dependency of sulfonamide reabsorption only refers to the parent compound; the excretion of the acetyl and hydroxy metabolites is not affected by urinary pH [49]. Therefore, this hypothesis can only explain the low concentrations of SDZ, but not those of AC and OH. Even if we assume that deacetylation is unusually fast, there should still be high concentrations

of OH. Secondly, the hypothesis does not apply for the data for pig **b**, where there was no pH step. And finally, the hypothesis does not explain the low concentrations on day 4: The pH on that day was 9.25 so that there was probably few reabsorption. As the plasma concentration was most probably highest on day 4, the urine concentrations should have attained their maximum then, and not two days later.

Saturation of Specific Processes As the experimental data do not conform to the model in the intermediate period, one of its fundamental assumptions, the linearity of the system, could be doubted. Nonlinearity in pharmacokinetics is mostly the consequence of saturation of specific processes such as metabolism, plasma protein binding, and carrier-mediated transport. It rarely occurs in the therapeutical dose range [24]. The given dose of 30 mg/kg corresponds to the therapeutical dose, and is even lower than doses administered in many literature experiments: Nielsen et al. [31] and Nouws et al. [6] applied about 40 mg/kg, while Friis et al. [37] and Nielsen et al. [38] administered 60 mg/kg, and Garwacki et al. [34] applied 30 mg/kg twice daily. It is therefore improbable that there was noteworthy nonlinearity due to saturation.
But even if one or several of these processes are saturated, the amount of SDZ equivalents excreted at high plasma concentrations should still be greater or equal to the amount excreted at lower concentrations: Saturation of plasma protein binding results in a higher fraction of free SDZ and therefore even accelerates the kinetics; saturation of metabolism or of carrier-mediated transport in the renal tubules results in a zero order kinetics, i.e. the same amount is metabolized or excreted per unit time, independently of the plasma concentration. Thus, if we assume that the compound is rapidly absorbed, the excreted amounts should be highest on the first 4 days when plasma concentrations are highest, and less or equal on the following days. As this is not the case, saturation of specific processes cannot explain the concentration course.

Delayed Excretion It could also be doubted that a substance is immediately urinarily excreted after being renally eliminated from the bloodstream. However, this hypothesis can also be refuted under normal circumstances: SDZ can be detected in human urine 30 minutes after oral administration [9]; after IV administration to 60-75 days old piglets, Friis et al. [37] recovered 48 % of the dose in urine after 4 h. Besides this literature evidence, the experimental data themselves indicate that there is no large delay under normal circumstances because concentrations on the first day are relatively high. A hypothesis for a process leading to an unusual delay of excretion will now be examinated in a new subsection.

5.2.3 Crystallization

We can presume that the process governing the experimental data is not one of the common processes, probably a side effect induced by the SDZ administration, and has not yet been considered in this study. Besides the unusual concentration course which can apparently not be explained with the considered processes, the unexpected pH step and the varying urine volumes corroborate this assumption.
The most frequent side effect of sulfonamides in the early days of sulfonamide application in humans was the renal impairment by crystalline deposits of the sulfonamide or its acetyl

metabolite[6]. In 29 % of the patients medicated with SDZ, crystalluria in the urine could be observed, in 2.5 % there were renal complications [63]. Krüger-Thieme and Bünger [16] report renal calculi in 4 % of the SDZ-medicated patients studied for this reaction, and classify SDZ as one of the most dangerous sulfonamides with respect to crystalluria.

Nowadays, the incidence of renal complications of sulfonamides is considerably reduced [64]. This may not only be due to "the development of sulfonamides with greater solubility and the availability of other antimicrobial agents" [64], but also to lower doses [16] and to refined admininistration techniques, which take care of an alkaline urine and a high urine flow [25]. Nevertheless, crystallization of SDZ is still a problem in AIDS patients, where very high daily doses of SDZ (4 - 8 g) are applied, provoking the intratubular crystallization of AC. Though the calculi may also contain SDZ, it is rarely the major component [64].

Vree and Hekster [25] review several cases with a sharp increase of the renal clearance after cessation of a sulfonamide therapy. They attribute this finding to crystallization: "It is possible that precipitated N_4-acetylsulfonamide crystals in the tubules have produced a 'mechanical' obstruction" [25] preventing active tubular excretion, so that excretion is limited to passive glomerular filtration. Precipitation of the the acetyl metabolite in the glomerulus may also impair the glomerular filtration. After termination of the therapy, the concentration decrease results in the dissolution of the precipitated crystals so that the full excretion capacity is reestablished [25].

As we have seen, low urinary concentrations during medication and high urinary concentrations some time after the last administration are also characteristics of the ^{14}C-SDZ data and of the data for pig **b**. Therefore, I will now examine the hypothesis that crystallization is responsible for the unusual concentration course of the experimental data. There are many signs endorsing this theory:

Firstly, the decrease in manure volumes can easily be explained by impairment of the glomerular filtration by SDZ and AC crystals. It also fits in that the ^{14}C-SDZ pigs, showing the most pronounced delay in excretion of SDZ, produced the least manure, whereas the ^{12}C-SDZ pigs, where a less pronounced delay was observed for pig **b** only, produced more manure, and the unmedicated pigs produced most.

Secondly, the concentration increase in the ^{14}C-SDZ data, beginning on day 4, coincides with the pH step. As SDZ is much better soluble at high pH, the pH increase could have provoked the dissolution of the crystals. This pH dependency could also explain the fact that the concentration course for pig **b** is less pronounced, because the urinary pH is much higher for this pig than for the ^{14}C-SDZ pigs.

Thirdly, the decrease of the ratio SDZ/AC in the data of Vockel et al. [51] can be explained with this theory if the AC crystals dissolve slower than the SDZ crystals. Finally, the ACOH metabolite is a strong indication for kidney impairment. ACOH has not been reported in any publication about SDZ in pigs which is not amazing because it is formed by hydroxylation of AC or acetylation of OH. This implies the presence of considerable amounts of AC or OH in plasma, but both substances are rapidly excreted under normal circumstances. AC and OH can only accumulate in plasma, and then be further metabolized to ACOH, if their main excretion

[6]No literature is available about crystalluria of SDZ in pigs.

pathway, the tubular secretion, is impaired.
On the other hand, there are many considerations challenging the theory of crystallization: Firstly, formation of sulfonamide crystals is favored by long term or high dose therapy [64], which is not the case in this experiment; the given dose of 30 mg/kg was even lower than in many other studies [6, 31, 34, 37].
Secondly, the mean SDZ and AC concentrations on day 2 and 3, when crystallization is supposed to occur, are lower than 35 mg/l, and therefore probably lower than the maximal solubility of SDZ and AC at pH 7. However, literature findings about SDZ and AC solubility are contradictory (see Chapter 2), so that the exact solubility of SDZ cannot be determined. Besides, pH of excreted urine does generally not correspond to the pH in the tubules: pH of voided urine has been shown to be about 0.7 higher than that of urine in the urinary bladder, at least in humans [50]. Moreover, Oster et al. showed that the pH of urine samples stored during 24 h is about 0.5 higher than the pH measured immediately after completion [65]. Within the nephron, where various secretion and reabsorption processes occur, the pH is also very likely to change. Given that small variations of the pH result in large variations of the solubility, it is almost impossible to determine the real solubility of SDZ in the tubules. The fast excretion scenario has also shown that maximum SDZ concentrations in urine may well exceed 2000 mg/l on the first day, which could be more than the maximum solubility. It also exemplifies the high temporal variability of concentrations in the tubules, so that the maximum concentration in the tubules may be much higher than the mean excreted concentration. On the other hand, Ylitato and Vapaatalo report that SDZ can be present in human urine to the extent of oversaturation [66]. In any case, the specific mechanism of crystallization is not known, and there may be considerable temporal and local concentration and pH changes. Therefore, it can be stated that too many parameters are unknown, and that the processes involved in crystallization are much too complex to exclude the possibility of crystallization merely based on 24 h-concentration and pH samples.
Another argument against the crystallization hypothesis is that crystallization of OH is not mentioned in any study. Though there is quite generally much less information about OH than about AC, and though the solubility of OH is not known, it is probable that the solubility is high and that OH does not form crystals. If this is the case, we expect the excreted percentage of OH to be relatively high on the days when the crystals are formed, because excretion of OH is reduced by renal impairment only, while excretion of SDZ and AC is reduced by renal impairment and storage in crystals. When the storage is released, we expect a decrease of the relative OH concentration. This is only partly confirmed by the data (Table 5.5), and to a much lower extent than one would have expected. If we assume that crystallization occured on days 2 - 4 while dissolution occured on days 5 - 7, we can see that the excreted percentage of OH is in fact markedly higher on days 3 and 4 than it is on days 5 - 7. On the contrary, the percentage on day 2 is much lower than it is on days 5 - 7. However, total concentrations on day 2 are very low, so that this might also be a measurement error.
Summarizing, it can be stated that there are many arguments in favor of the hypothesis of crystallization, especially the manure volume variations, the pH step and the presence of the ACOH metabolite. There are also considerations challenging this theory, but they can neither be confirmed nor refuted due to the data restrictions. Therefore, intratubular crystallization is the most probable explanation of the phenomenon observed in the ^{14}C-SDZ data and in the data

Table 5.5: Percentage of ^{14}C-SDZ and metabolites on total daily activity. 100 % is defined as the sum of these activities.

day	SDZ %	AC %	OH %	ACOH %
1	40.1	36.9	23.1	0.0
2	46.8	39.6	13.6	0.0
3	35.5	34.5	30.1	0.0
4	30.3	37.6	32.1	0.0
5	31.1	43.0	22.8	3.2
6	35.4	38.8	20.7	5.1
7	39.6	34.0	23.0	3.3
8	38.3	35.8	23.2	2.7
9	39.5	33.4	23.7	3.3
10	47.5	38.3	14.2	0.0

for pig **b**.
In the above argumentation, the question which excretion process is impaired to what extent by crystallization has been factored out. The variations in urine volume are an indication for an impaired glomerular filtration, whereas the delayed excretion of the OH metabolite (which does not form crystals) suggests that tubular secretion has also been largely impaired, inhibiting the efficient excretion of OH. The exact extent of each process cannot be determined with the given data, but would be mere speculation. Suggestions about the design of a new experiment delivering suitable pharmacokinetic data will be given in the next chapter.

Chapter 6

Summary and Outlook

Coming back to the aims defined in the introduction, it has to be stated that the concentration course of SDZ in pigs cannot be precisely predicted with a simple model, and it is even doubtful whether it can be predicted at all. The variability of the literature data presented in this work exemplifies the variability of pharmacokinetic processes and confirms the statement of Brown [20] "that it is virtually impossible to quantitatively predict the relative concentrations of metabolites that will be produced after administration of a drug in any given species." However, the parameter range is still narrow enough to allow qualitative statements, all the more since literature agrees as for the kinetics of the involved processes. Therefore, besides the usefulness of the developed model for a deeper understanding of the system, it can provide reference scenarios for the qualitative evaluation of the experimental data.

The comparison between model results and experimental data reveals that the data of the ^{12}C-SDZ pig **a** are in good accordance to the model except for a peak on day 8 which can be explained by excretion in feces.

Data for the ^{14}C-SDZ pigs and for the ^{12}C-SDZ pig **b** conform to the model at the end and partly at the beginning of the measurement period, but do not coincide with the model in the intermediate phase. As the model was developed similarly to literature models, and parameterized with values covering the whole range of parameters reported in literature, it can be assumed that it suitably models the common pharmacokinetic processes. The good accordance of the model results with the data for pig **a** corroborates this assumption. If the data are not erroneous, the process observed in the experimental data must therefore be an unusual process. It is probable that this process is intratubular crystallization of SDZ and AC, but this point cannot be fully elucidated due to the data restrictions. As long as detailed data are lacking and the processes governing the concentration course are consequently unknown, it is impossible to adapt the model structure so that it models the experimental data suitably. However, given the enormous individual variability of the experimental data, the construction of a model for merely qualitative prediction of the concentration course would probably be a very challenging task even if much more detailed data were available.

In the following, I will outline some requirements for an experiment designed in order to deliver data suitable for the examination of the pharmacokinetics of SDZ in pigs, and for the investigation of the question whether the unusual process is indeed crystallization. The most important points for this purpose are the determination of individual urine volumes and plasma concentra-

tions in a higher temporal resolution. Urine volumes, in combination with urine concentrations, are indispensable to calculate the mass of excreted SDZ and metabolites, and therewith for a mass balance. On the other hand, concentrations of SDZ and metabolites in blood plasma are necessary for a deeper understanding of the processes in the body. Absorption and excretion rates cannot be reliably estimated if plasma concentrations are not known.
Furthermore, an indication for crystallization or more generally for renal impairment could be gained from plasma concentrations: An increase of the concentration ratio OH/SDZ could suggest that the most efficient excretion mechanism for OH, namely tubular secretion, is impaired. The decrease of urine volumes, on the contrary, could be a sign of impaired glomerular filtration. More detailed information about the specific processes occuring in the kidneys during the experiment could be obtained by parallel measurement of creatinine (or inulin) and p-aminohippuric acid clearance.
Determination of urinary pH can also deliver valuable information, but measurement immediately after completion would be strongly preferable. Separation of urine and feces is also important, as the data for pig **a** suggest that considerable amounts of SDZ may be excreted in feces.
The experimental setup should naturally assure the same food and housing for all animals, and enough time to familiarize with these conditions. If the risk of crystallization shall be minimized during the experiment, this can be achieved by some simple measures: Lower doses, alkalization of the urine by parallel administration of alkalinizing substances, and securing of sufficient water uptake [64, 25]. It should however be noted that alkalization of urine not only changes the manure pH, but also the relative concentrations of SDZ and metabolites by hampering the reabsorption of SDZ.
An experiment considering these suggestions would be appropriate to examine the hypothesis of crystallization and to generate new knowledge about the pharmacokinetics of SDZ in pigs. It has been shown that the available experimental data contain so many uncertainties that it is improper to draw any conclusions on this basis. They can only serve for the generation of questions and hypotheses about the pharmacokinetics of SDZ in pigs, which has been done in this work.

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Appendix A

Analytical Solution of Model 2

The analytical solution of model **2** is presented here. In order to enhance its readability, the following abbreviations have been introduced:

$$\begin{aligned} \alpha &= k_{ac} + k_{dac} + k_{exA} + k_{exS} + k_{oh} \\ \beta &= \sqrt{\alpha^2 - 4(k_{ac}k_{exA} + (k_{dac} + k_{exA})(k_{exS} + k_{oh}))} \end{aligned}$$

```
mSI[t] = e^(-kab t) mabs
mSB[t] =
 -(e^(-1/2 t (2 kab+α+β)) kab mabs
     (2 e^(1/2 t (α+β)) (kab - kdac - kexA) β +
       e^(t (kab+β)) (kac (kdac - kexA) + kab (kac - kdac - kexA + kexS + koh - β) +
          (kdac + kexA) (kdac + kexA - kexS - koh + β)) +
       e^(kab t) (kac (-kdac + kexA) - (kdac + kexA) (kdac + kexA - kexS - koh - β) -
          kab (kac - kdac - kexA + kexS + koh + β)))) / (2 (kab^2 + kac kexA + (kdac + kexA) (kexS + koh) - kab α) β)
mAB[t] =
 -(e^(-1/2 t (2 kab+α+β)) kab kac mabs
     (-2 e^(1/2 t (α+β)) β + e^(kab t) (2 kab - kac - kdac - kexA - kexS - koh + β) +
       e^(t (kab+β)) (-2 kab + kac + kdac + kexA + kexS + koh + β))) /
  (2 (kab^2 + kac kexA + (kdac + kexA) (kexS + koh) - kab α) β)
```

```
mOH[t] =
 (kab koh mabs
    (-e^(-1/2 t (α+β)) (-1 + e^(t β)) kac^2 kdac kexoh + e^(-1/2 t (2 (kab+kexoh)+α+β)) (kdac + kexā) (kdac + kexā - kexoh)
       (e^((kab+kexoh) t) kexoh (kdac + kexā - kexS - koh - β) + 2 e^(1/2 t (2 kexoh+α+β)) (kexoh - kexS - koh) β +
         2 e^(1/2 t (2 kab+α+β)) (kexS + koh) β - e^(t (kab+kexoh+β)) kexoh (kdac + kexā - kexS - koh + β)) -
      e^(-1/2 t (2 kexoh+α+β)) kab^2
       (e^(kexoh t) (-kac (kdac - kexā + kexoh) - (kdac + kexā - kexoh) (kdac + kexā - kexS - koh - β)) -
         2 e^(1/2 t (α+β)) (kdac + kexā - kexoh) β +
         e^(t (kexoh+β)) (kac (kdac - kexā + kexoh) + (kdac + kexā - kexoh) (kdac + kexā - kexS - koh + β)) -
      e^(1/2 t (2 (kab+kexoh)+α+β)) kac
       (e^((kab+kexoh) t) kexoh (-2 kdac^2 + kexā (kexā - kexoh) - kdac (kexā - kexoh + kexS + koh - β) -
         2 e^(1/2 t (2 kab+α+β)) kexā (kdac + kexā - kexoh) β - 2 e^(1/2 t (2 kexoh+α+β)) (kdac + kexā) (-kexā + kexoh) β -
         e^(t (kab+kexoh+β)) kexoh (-2 kdac^2 + kexā (kexā - kexoh) - kdac (kexā - kexoh + kexS + koh + β)) +
      e^(-1/2 t (2 (kab+kexoh)+α+β)) kab
       (-e^((kab+kexoh) t) (kac^2 kdac + kac (2 kdac^2 - kexā^2 + kexoh^2 + kdac (kexā + kexS + koh - β)) +
            (kdac^2 + 2 kdac kexā + kexā^2 - kexoh^2) (kdac + kexā - kexS - koh - β)) +
         2 e^(1/2 t (2 kexoh+α+β)) (kac (kexā - kexoh) - (kdac + kexā - kexoh) (kexoh - kexS - koh)) β -
         2 e^(1/2 t (2 kab+α+β)) (kdac + kexā - kexoh) (kac + kdac + kexā + kexS + koh) β +
         e^(t (kab+kexoh+β)) (kac^2 kdac + (kdac^2 + 2 kdac kexā + kexā^2 - kexoh^2) (kdac + kexā - kexS - koh + β) +
            kac (2 kdac^2 - kexā^2 + kexoh^2 + kdac (kexā + kexS + koh + β)))))) /
  (2 (kab - kexoh) (kac (kexā - kexoh) - (kdac + kexā - kexoh) (kexoh - kexS - koh))
    (kab^2 + kac kexā + (kdac + kexā) (kexS + koh) - kab α) β)
```

Appendix B

Experimental Data: Tables

Table B.1: Concentrations of SDZ and metabolites in total manure. OH was not determined in ^{12}C-SDZ manure.

	SDZ [mg/ml]	AC [mg/ml]	OH [mg/ml]	method
^{14}C	126.4	12.9	45.7	radiodetection
^{12}C	41.0	39.0	-	LC-MS/MS

Table B.2: Urinary pH of the ^{14}C-SDZ pigs and pH of manure of the ^{12}C-SDZ pigs and one control pig

day	^{14}C-SDZ	^{12}C-SDZ (pig **a**)	^{12}C-SDZ (pig **b**)	control
1	7.2	8.5	9.1	6.0
2	7.1	8.3	9.0	8.4
3	7.1	9.0	8.7	8.8
4	9.3	8.9	9.1	8.6
5	9.1	9.1	9.1	8.6
6	9.0	8.3	9.1	9.0
7	9.0	pasty	9.5	9.8
8	9.0	8.6	8.9	8.2
9	8.9	7.9	9.2	8.7
10	9.0	8.5	8.8	8.8
11	-	8.4	8.8	8.4
12	-	8.0	8.3	8.8
13	-	8.3	8.6	8.8
14	-	8.3	8.6	8.9
total	9.0	-	-	-

Table B.3: Radioactivity in pig urine and feces after administration of ^{14}C-SDZ

day	urine [KBq/ml]	feces [KBq/g]
1	76.3	6.5
2	16.5	1.7
3	16.1	0.6
4	35.2	0.7
5	98.7	1.5
6	164.5	2.9
7	165.2	2.3
8	60.9	0.8
9	39.6	2.2
10	20.4	1.5

Table B.4: Concentrations of ^{14}C-SDZ and metabolites in pig urine determined by radioactivity quantification

	concentration [mg/l]			
day	SDZ	AC	OH	ACOH
1	137.4	146.7	84.0	0.0
2	34.7	34.1	10.7	0.0
3	25.7	29.0	23.1	0.0
4	48.0	69.0	53.8	0.0
5	137.9	221.1	107.0	17.4
6	261.5	333.2	162.2	46.5
7	294.2	293.2	180.9	30.5
8	105.0	113.6	67.4	9.0
9	70.2	69.1	44.8	7.3
10	43.7	40.8	13.8	0.0

Table B.5: Daily urinary concentrations of SDZ and metabolites in the slow excretion scenario, calculated according to Equation 4.26. Mean daily activities were calculated as the sum of the mean daily activities of SDZ, AC and OH, assuming a specific activity of 55.1 MBq/mmol (220 KBq/mg SDZ).

	concentration [mg/l]			activity
day	SDZ	AC	OH	[KBq/ml]
1	207.6	324.3	173.2	142.6
2	243.1	395.0	209.9	171.3
3	247.5	403.8	214.5	174.9
4	248.1	404.9	215.0	175.3
5	40.5	80.7	41.9	32.8
6	5.0	10.0	5.2	4.1
7	0.6	1.2	0.6	0.5
8	0.1	0.2	0.1	0.1
9	0	0	0	0
10	0	0	0	0

Table B.6: Concentrations of ^{12}C-SDZ and AC in manure (supernatant) of pig **a** and **b** determined by LC-MS-MS

	SDZ [mg/l]		AC [mg/l]	
day	pig **a**	pig **b**	pig **a**	pig **b**
1	94.7	79.2	206.2	102.9
2	148.7	65.3	176.5	61.8
3	224.0	32.3	140.9	31.6
4	144.5	61.5	90.5	55.9
5	17.1	40.0	14.0	39.3
6	16.0	22.1	15.5	24.5
7	25.9	25.8	31.6	30.4
8	69.7	71.3	10.8	130.7
9	5.9	30.8	4.8	34.9
10	1.1	2.7	1.1	2.2
11	0.4	0.2	0.4	0.2
12	0.2	0	0.2	0
13	0	0	0	0
14	0	0	0	0

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