Modelling the fate and transport of antibiotics and antibiotic resistant genes in agriculture

MSc thesis – Environmental Sciences

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Summary

The effects of antibiotics and antibiotic resistance have increased concerns over the last decade. One of its main drivers is the usage of veterinary antibiotics in agriculture. Antibiotics are excreted in manure applied to agricultural land and this spreads antibiotics in the environment. The increase in antibiotics' concentrations can make bacteria resistant due to the transfer of antibiotic resistant genes (ARGs). Antibiotics and ARGs have been studied by many model and observational studies, but these studies are not yet synthesised. The usage of antibiotics and the presence of antibiotic resistance has decreased significantly over the last decade in the Netherlands, but antibiotics are still detected in the environment and experts are still concerned about the presence of resistant bacteria. More insight into the pathway of antibiotics and ARGs is therefore relevant to predict where antibiotics and ARGs will end up and to prioritize environmental policies.

The objective of this study is *to develop an exploratory model to assess the pathways and environmental risks of antibiotics and ARGs from agricultural inputs in the Netherlands.* The following research questions were addressed:

Methods: To achieve this objective, the scientific literature on models related to antibiotics and ARGs was systematically reviewed. This review also strived to develop a parameter overview of the necessary input parameters to antibiotic modelling. Multiple linear regression models were constructed for sorption coefficients (Kd, Kf) and the half-life times in soils, which links these parameters to different independent variables found in literature. Based on the review, an exploratory model was developed for the fate and transport of antibiotics. This model was tested and applied to four different scenarios: 1) current situation; 2) increased risk (only manure from veal calves); 3) further increased risk (only veal calves, shorter storage); and 4) increased fraction of manure in treatment.

Antibiotics findings: The literature review found different models varying from simple exposure models to more advanced pesticide models, all with different data requirements. The models' equations used in these models are summarized to help develop my exploratory model. I created an overview of available model parameters, which depend on environmental and soil characteristics. Ways to quantify parameters based on these characteristics were limited. The parameter overview also indicated a lack of transparency on antibiotic usage and a need for more parameters on non-equilibrium sorption, transformation products and ionization of antibiotics.

Regression models for the sorption coefficients (i.e. Kd and Kf) and the half-life in soils were explored as a suitable option for relating parameters to environmental and soil characteristics. Sorption is significantly related to manure application, the percentage clay, the percentage organic carbon, the pH and the cation exchange capacity, which depends on the model (R^2adj 0.39-0.82). The half-life in soils was associated with the initial concentration, the percentage organic carbon, the percentage clay, aerobic/non-aerobic conditions and sterilized/non-sterilized soils (R^2adj 0.93). These models can be improved with a larger sample size for more antibiotics, a broader range of values in the independent variables and the exploration of non-linear models.

ARG findings: The systematic literature review found few models for ARGs and antibiotic resistant bacteria (ARB). The available models addressed specific relationships (e.g. manure storage), were highly data intensive and have so far only be applied for few limited conditions. My overview indicated complex relationships between antibiotic usage and resistance in manure, dependent on former exposure to antibiotics and other risk factors, such as the number of animals and dietary factors. This relationship was further complicated by co-selection and co-resistance. The effect of manure storage depended on the temperature, manure type and ARG type. The soil concentration was linked to the growth rate in bacteria, the rate of selection, the rate of gene transfer, the rate of cell death and losses to infiltration and runoff. Few studies were found on leaching and runoff, but both processes have been detected and depend on rainfall patters, slope and soil type. More research into the rates which lead to growth and death of bacteria and selection of resistance, is necessary. Existing models must be combined and tested for more cases and creative modelling approaches are crucial to include the relevance of former exposure to antibiotics.

Model findings: An exploratory model for the pathways of antibiotics was developed for different combinations of land use and soil types for wet and dry conditions. The scenario of the current situation showed low concentrations of antibiotics in soils, below the threshold values for risks to organisms or risk of resistance selection. Even when increasing the risk by only using veal calf manure or veal calf manure with a shorter storage time resulting concentrations were still below the risk threshold values. My model is more advanced than simple exposure models, but does not consider non-equilibrium sorption, ionization of antibiotics and transformation products. When their parameters are available, these processes can be included in the model. The model can also be linked to hydrology to obtain concentrations in surface and groundwater. An important further research step is model validation to determine the robustness of the model's results.

To conclude, the findings of the literature review allowed the development of an exploratory model for antibiotics but not for ARGs. Further research into the parameters required for antibiotic modelling and the relationships and parameters for ARG modelling is suggested. However, the foundation for modelling antibiotics and ARGs provided by this thesis is already relevant to guide policies that prevent adverse effects of antibiotics and antibiotic resistance.

Chapter 1. Introduction

1.1 Background

Antibiotics and antibiotic resistance

The extensive use of antibiotics in human healthcare and agriculture has increased the presence of antibiotics in the environment and the development of antibiotic resistance (Bueno et al., 2018; O'Neill, 2016). The effects of antibiotics and antibiotic resistance have been of increasing concern over the last decade (Berglund, 2015; Williams-Nguyen et al., 2016). Resistance micro-organisms can survive exposure to a drug and continue spreading (O'Neill, 2016). Drugs have therefore become less effective and the increase in resistance has led to a decrease in the development of new antibiotics (Berglund, 2015; O'Neill, 2016). More than 700,000 people die every year of drug-resistant bacterial infections and this number is predicted to increase further up to 10 million in 2050 unless actions are taken (O'Neill, 2016).

Antimicrobial resistance means that a microorganism (e.g. bacteria or virus) no longer responds to a drug it was originally sensitive for (WHO, 2014). This is a problem, since treatments against microorganisms such as antibiotic resistant bacteria (ARB), will no longer work (WHO, 2014). Bacteria can gain antibiotic resistance through the transfer of antibiotic resistance genes (ARGs). Apart from leading to the development of resistance, antibiotics can also have toxic effects. The toxicity in humans was found to be negligible (Cunningham et al., 2009; Schwab et al., 2005). Organisms can be affected, however, by concentrations in mg/L (Bengtsson-Palme & Larsson, 2016). The concentrations that enhance resistance are lower than the environmentally toxic concentrations, indicating that low, non-toxic concentrations can still result in a risk through the development of antibiotic resistance (Bengtsson-Palme & Larsson, 2016).

Contribution of agriculture

In agriculture, antibiotics have been used for the prevention, control and treatment of infections and for improvement of growth and feed efficiency worldwide (Berglund, 2015; Economou & Gousia, 2015). Although this is still under debate, agricultural inputs of antibiotics are often seen as the main driver for antibiotic resistance (ter Kuile et al., 2016). After application of antibiotics to livestock, a fraction is excreted in urine and manure (Berglund, 2015; Economou & Gousia, 2015; Kumar et al., 2005). Manure is stored and redistributed on land, consequently antibiotics may enter surface and ground waters (Kumar et al., 2005). Most antibiotics do not degrade rapidly and are strongly absorbed in soils (Kumar et al., 2005; Rougoor et al., 2016). The role of the environment is particularly important for the development of resistance, since it serves as a pool for the ARGs (Economou & Gousia, 2015). ARGs and ARB can reach humans through: the food chain, direct contact with animals and manure contaminated environments and aquaculture (Economou & Gousia, 2015).

The situation in the Netherlands

In the Netherlands, the usage of antibiotics has decreased significantly over the last decade which has also resulted in a decrease in antibiotic resistance (Veldman et al., 2017). Antibiotics are still detected, however, in sludge, ground and surface waters and soil samples (Lahr et al., 2014; Schilt & Van de Lagemaat, 2009; ter Laak et al., 2017) and there are still concerns about the presence of ARB (Veldman et al., 2017). More insight into the pathway of antibiotics and ARGs is therefore relevant to predict where antibiotics and ARGs will end up and to prioritize environmental policies.

Modelling antibiotics/ARGs

Modelling is a useful method to predict environmental concentrations under different scenarios. Insights into the presence and risks of antibiotics can be given without having to extensively monitor concentrations in the field. Modelling the pathways of antibiotics/ARGs would therefore be valuable, but some issues arise. The pathways of antibiotics have already been described and quantified by different scientists, but there is limited data availability on relevant model inputs and parameters (Lahr & Van den Berg, 2009; Rougoor et al., 2016). Furthermore, an overview of studies and existing models on the quantification of the pathway seems to be missing. The development of antibiotic resistance is still lacking a method of quantification (Berendonk et al., 2015; ter Kuile et al., 2016). There is, however, an increased attention for understanding the relevant ecological and environmental processes of the development of antibiotic resistance (Berglund, 2015); an overview of these studies is still missing. Opportunities for modelling have been identified, but this thesis will provide a better base for modelling by tackling some of the formerly mentioned issues. With the development of an exploratory model, environmental risks can be better assessed.

1.2 Objectives and Research Questions

The study's objective is *to develop an exploratory model to assess the pathways and environmental risks of antibiotics and ARGs from agricultural inputs in the Netherlands.* The research will focus on the Netherlands, because of the high availability of existing models, but the study will also touch upon models in other countries and on how to extrapolate the proposed model to other areas. This objective and scope are further specified in the following research questions (RQs):

<u>RQ1</u>: What are the pathways of antibiotics from agricultural inputs to soil and ground and surface waters and to what extent are these pathways quantifiable?

<u>RQ2</u>: What are the pathways of ARGs from agricultural inputs to soil and ground and surface waters and to what extent are these pathways quantifiable?

<u>RQ3</u>: How can the knowledge from questions 1 be applied in a model describing the pathways of antibiotics in agriculture to be applied to different land use and soil types?

<u>RQ4</u>: What are the predicted environmental concentrations of antibiotics and the related environmental risks in the Netherlands under different scenarios?

1.3 Guidance

To answer these RQs, a systematic literature review will be conducted to present an overview of the available literature on the models and studies on the pathways of antibiotics and ARG/ARB. Chapter 2 focusses on the fate and transport of antibiotics (RQ1) and Chapter 3 on antibiotic resistant genes and bacteria (RQ2). An overview of the necessary model parameters for antibiotics is created based on the literature review (RQ1, Chapter 2). Some parameters require regression models, which will be explored in Chapter 4. Chapter 5 describes the development of an exploratory model for the fate and transport of antibiotics (RQ3). Chapter 6 explores the model application by the exploration of different scenarios and an assessment of the environmental risks (RQ4). Every Chapter includes its own introduction, methodology, results, discussion and conclusion. Chapter 7 discusses the overall outcomes and the answers to the research questions are addressed in Chapter 8.

Chapter 2. Fate and Transport of Antibiotics

2.1 Introduction

This Chapter will describe the fate and transport of antibiotics based on a systematic literature research, addressing RQ1. The conceptual framework (Figure 1) will be used to describe the different processes and the way to quantify these, following the order of the numbers in front of the different processes. Degradation in surface water and sedimentation will not be discussed here, since these processes are already frequently addressed when discussing human pharmaceuticals and their fate in wastewater treatment.

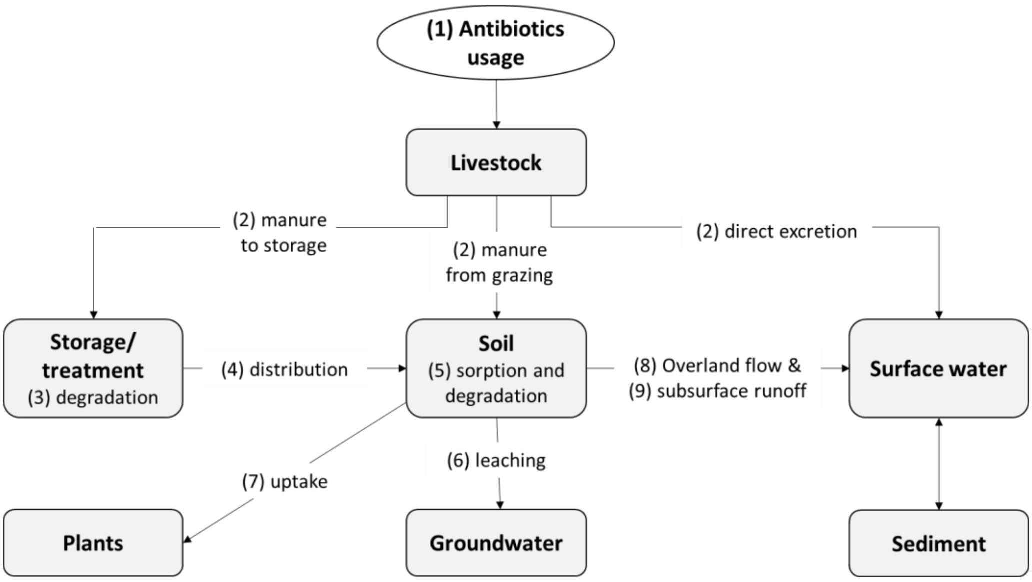


Figure 1 Conceptual model for the fate and transport of antibiotics in agriculture

This Chapter will discuss different types of antibiotics, which all belong to different classes and have their own abbreviations. Table 1 gives an overview of the different classes, their most common antibiotics and the abbreviations used in this thesis (Boxall et al., 2002; Kumar et al., 2005). The focus will be on sulfonamides and tetracyclines, because of their high usage and dissimilar environmental behaviour.

Table 1 Classes of antibiotics and their major active ingredients and abbreviations

Class	**Antibiotic**	**Abbreviation used**
Tetracyclines	Chlortetracycline	*CTC*
	Oxytetracycline	*OTC*
	Tetracycline	*TC*
Sulfonamides	Sulfadiazine	*SDZ*
	Sulfamethazine (Sulfadimidine)	*SMZ*
	Sulfamethoxazole	*SMX*
	Sulfadimethoxine	*SDM*
Macrolides	Tylosin	*TYL*
	Erythromycin	*ERY*
Fluoroquinolones	Enrofloxacin	*ENR*
	Tiamulin	-
b-lactams	Penicillin	-
	Ampicillin	-
Chloramphenicol	Florfenicol	-
Trimethoprim	Trimethoprim	-
Cephalosporin	Ceftiofur	-

2.2 Method

Search strategy

The search with search terms from Table 2 was conducted in Scopus based on the title, abstract and keywords, in March 2018.

Table 2 Search terms used for the literature review of Chapter 2

Related to antibiotics		Related to agriculture		Related to modelling		Related to fate and transport
Antibiotics OR tetracyclines OR sulfonamides	AND	Livestock OR Farming OR Manur*OR Veterinary OR Agricultur*	AND	model*	AND	Transport *OR* Pathway *OR* Flow *OR* Fate *OR* Dissemination *OR* Monitoring *OR* Behaviour

Results

The search gave 372 results, of which 54 were considered relevant based on their abstract for at least one of the sections. Interviewed experts also provided a number of key publications. Other articles, mostly concerning parameters, were selected with the snowball method. Table 3 gives the number of relevant articles per section also including articles outside of the systematic review.

Table 3 Relevant articles found per section

Topic	Relevant articles
Antibiotic inputs	7
Excretion in manure	12
Manure storage/treatment	18
Manure distribution	-
Sorption and degradation	29
Leaching	14
Uptake in plants	4
Subsurface runoff	-
Overland flow	9

2.3 Results and discussion

2.3.1. Antibiotic usage (process 1 in Figure 1)

Antibiotics are applied for prevention, control and treatment of infections or as a growth promoter (Kumar et al., 2005). In the latter utilisation, antibiotics are added to feed although this is prohibited in many countries (Berglund, 2015). Transparency on the usage of specific antibiotics is generally lacking. Different models on the fate of antibiotics were found in the literature review, which differ in their inputs. Some studies measured the concentrations in manure (Knäbel et al., 2016; Küçükdoğan et al., 2015), while others looked at concentrations in feed (Wang et al., 2014). To estimate usage in a country, antimicrobial sales data and animal populations can be used (Carmo et al., 2017; Zhang et al., 2015).

The usage of antibiotics can depend on the season. In general, there are more antibiotics applied in winter (Hu et al., 2010). Usage differs per livestock sector; in the Netherlands, pigs and veal calves receive the highest number of antibiotics (Veldman et al., 2017).

2.3.2. Excretion to manure (process 2 in Figure 1)

Antibiotics are not completely metabolized by livestock and a percentage is excreted via faeces and urine (Knäbel et al., 2016; Zhang et al., 2015). The largest fraction of antibiotics in manure is still in unchanged and active forms (Zhang et al., 2015). The metabolism depends on the animal and the type of antibiotic

(Boxall et al., 2002; Massé et al., 2014). The excretion data relevant to *SDZ* and *TC* are summarized in Table 4 (Arikan et al., 2007; Boxall et al., 2002; Kumar et al., 2005; Massé et al., 2014; Zhang et al., 2015). All excretion rates are available in Table S1 (Supplementary material). Pigs were found to have the highest excretion, followed by cattle and then poultry (Table S1). The type of antibiotic is also relevant; tetracyclines are excreted in higher percentages than sulfonamides (Table S1) (Boxall et al., 2002).

Table 4 Excretion factors of antibiotics to manure

Antibiotic	**Sector**	**Excretion (%)**	**Source**
SDZ	Pigs	44	Zhang et al. (2015)
TC	Pigs	75	Zhang et al. (2015)
SDZ	Chickens	29	Zhang et al. (2015)
TC	Chickens	53	Zhang et al. (2015)
OTC	Dairy manure	23	Masse et al. (2014)
CTC	Cattle faeces	75	Masse et al. (2014)
TC	General	25, 50-60	Masse et al. (2014)

Concentrations of antibiotics in manure can be calculated by multiplying the usage with the excretion factor and dividing that by the manure production (Montforts, 2003; Wang et al., 2014; Zhang et al., 2015). The usage is often estimated based on the dosage, body weight and the days of treatment, as performed in the risk assessment model for veterinary pharmaceuticals of the European Medicines Agency (EMA, 2016) and in the RIVM model which is used for risk assessment in the Netherlands (Montforts, 2003). Concentrations in manure are found in micro to milligrams per kilogram (Domínguez et al., 2014; Engelhardt et al., 2015; Kumar et al., 2005; Massé et al., 2014; Wohde et al., 2016).

2.3.3. Manure storage/treatment (process 3 in Figure 1)

Processes

Before being spread on the land, manure is often stored or treated. This can lead to degradation of antibiotics. The extent of degradation depends on a variety of factors, including pH, temperature, light and other storage conditions (Kuhne et al., 2000; Wohde et al., 2016). Degradation in manure is often determined with a half-life (DT50), which indicates the time it takes to degrade to half of the original concentration. A larger half-life describes slower degradation. In open storage conditions, leaching can occur under rainfall conditions, decreasing the concentration in storage (Szatmári et al., 2011), but this process is rarely discussed in other studies.

Antibiotics can transform to similar products, their transformation products (TPs), but limited literature was available on this. *SDZ* can be inactivated (acetylation) to *Acetyl-SDZ* and transformed to another less toxic form *4-OH-SDZ* (hydroxylation) (Engelhardt et al., 2015). The fractions of *Acetyl-SDZ* and *4-OH-SDZ* in manure were found to be respectively 26% and 21%, but these changed to 0 and 40% after storage (Engelhardt et al., 2015).

Models

Models take into account the half-life and the storage time to calculate the concentration in manure after a certain time (Bao et al., 2009; EMA, 2016; Montforts, 2003). Equation 2.1 is taken from EMA (2016), but other models use a similar relationship.

$$Mt = Mi \cdot e^{\frac{-\ln(2)\cdot Tst/2}{DT50}} \qquad \text{(Eq. 2.1)}$$

Where:

Mt	is the mass of active substance in manure/slurry after the mean storage time (mg);
Mi	is the mass of active substance in manure/slurry (mg);
Tst	is the length of time manure is stored (days); and
Dt50	is half-life of active substance in manure (days).

Parameter overview

A parameter overview was constructed (Table S2) combining literature reviews from Wohde et al. (2016), Massé et al. (2014), Schmitt et al. (2017), Boxall et al. (2002) and Ter Laak et al. (2017). Tetracyclines and quinolones have the longest half-lives in manure, followed by aminoglycosides, sulfonamides, macrolides and β-lactams (Chee-Sanford et al., 2009). Table S2 shows a lot of variation in half-lives, indicating the importance of manure type and storage conditions.

There are different ways of storing manure, which can lead to varying half-lives. The following order of degradation was found: composting > anaerobic digestion > manure storage > soil (Massé et al., 2014). This was also found in the literature review, since most studies for composting also show low half-lives (Arikan et al., 2007; Bao et al., 2009; Wu et al., 2011). Dolliver et al. (2008) compared storage with composting and found shorter half-lives for composting, but only by a couple of days (Table S2). Anaerobic digestion shows fast degradation in some cases, but there are also cases where storage in piles has a lower half-life. There is a distinction between aerobic and anaerobic conditions. Anaerobic experiments generally demonstrated longer half-lives than aerobic studies (Wohde et al., 2016). Temperature shows to be a more important factor, with significantly faster degradation under higher temperatures (Table S2). Shelver and Varel (2012), for example, examined degradation under different temperatures and found degradation of *CTC* increased from 7 to 98% with a temperature increase from 22 to 55 °C. Li et al. (2011) found a similar relationship for ceftiofur.

Biodegradation is often found to be the most important factor of degradation, which increases with increasing temperatures (Chee-Sanford et al., 2009). Furthermore, b-lactams, macrolides and sulfonamides are susceptible to hydrolysis and quinolones and tetracyclines are susceptible to photolysis (Chee-Sanford et al., 2009). Whether manure is stored in the light or in the dark can also play a role in degradation. The influence was difficult to determine, as there were no studies found which only compare varied light conditions, keeping all other factors constant, but this process of degradation is also relevant to take into account. Massé et al. (2014) state that except for some studies on *OTC* and *CTC*, information on transformation products (TPs) is missing. One other study also considers the TPs of *SDZ* (Lamshöft et al., 2010).

The animal type the manure comes from affects the degradation. Storteboom et al. (2007) found shorter half-lives for dairy manure compared to beef manure. Van Dijk and Keukens (2000) show faster degradation for broiler faeces than for layer hen. Bao et al. (2009) have shown faster degradation in layer hen and broiler manure compared to hog manure.

2.3.4. Manure distribution (process 4 in Figure 1)

After storage and treatment, manure is distributed on land. Since different livestock types are subject to different types of antibiotics, it is relevant to not only know the amount of manure, but also the livestock type and its former storage conditions. There are models available for manure inputs, such as INITIATOR, which gives the total amount of Nitrogen applied per hectare, distinguishing between different types of manure: stable (mostly cattle), pig, poultry and pasture (mostly cattle) (De Vries et al., 2003; 2015). The soil and land use types are relevant for the amount of manure. Sand gets a higher amount of manure than clay and the application is the lowest for peat (De Vries, personal communication). Grassland generally gets higher manure than arable land and maize receiver more manure than other arable land (De Vries, personal communication).

2.3.5. Processes in soil: sorption and degradation (process 5 in Figure 1)

<u>Sorption</u>

Processes

The amount of the antibiotic that gets absorbed in the soil depends on a variety of factors. Antibiotic specific characteristics are the water solubility and soil affinity (Chen et al., 2017; Wang et al., 2014). The sorption can be expressed with a sorption coefficient, such as the distribution coefficient Kd. Tetracyclines have a high distribution coefficient (Kd) and their strong affinity for soil and sediment means they are easily adsorbed (Chen et al., 2017; Wang et al., 2014). The Kd for sulfonamides, on the other hand, is lower so their adsorption capacity is also weaker (Chen et al., 2017; Wang et al., 2014). Sorption coefficients can change depending on different soil characteristics such as soil texture, organic carbon content, pH and cation exchange capacity (Fan et al., 2011).

The soil pH, which is affected by the pH of manure and rainwater, is an important characteristic, because the pH determines whether the antibiotic occurs in its neutral, cationic or anionic form (Pan & Chu, 2017; Wang et al., 2015). With a lower pH, the antibiotic occurs in its cationic form, which is more likely to adsorb to the soil. A higher pH gives the opposite effect by making the antibiotic more anionic (Lou et al., 2016; Maszkowska et al., 2015; Wang et al., 2014). The pH at which an antibiotic changes from neutral to anionic or cationic differs per antibiotic and is expressed with their pKa (Wang et al., 2015). Sulfadiazine, for example, has a pKa1 of around 2 and a pKa2 of around 6.5 (Wang et al., 2015). This indicates that at a pH lower than 2, sulfadiazine mostly occurs in its cationic form, while at a pH above 6.5, the anionic form dominates. The cation exchange capacity (CEC) indicates the extent a soil can hold cations by negative charges on the soil particle surfaces. The sorption of antibiotics increases with a higher CEC, since it will be able to hold more cations (Maszkowska et al., 2015; Park & Huwe, 2016).

Antibiotics can adsorb to organic matter and higher organic matter (OM) or organic carbon (OC) contents will thus increase the sorption coefficients (Białk-Bielińska et al., 2012; Lou et al., 2016; Maszkowska et al., 2015; Park & Huwe, 2016; Wang et al., 2014). Under a higher pH (of around 7, depending on the type of antibiotic), antibiotics become more anionic and bind less to organic carbon, showing less of an effect on an increase in organic carbon (Wang et al., 2014). Manure also contains organic matter, increasing the sorption capacity of a soil (Park & Huwe, 2016; Wang et al., 2015). The effect of manure on the migration through soil is not always clear (Engelhardt et al., 2015). Apart from increasing sorption, OM can also enhance migration by facilitated transport through the soil with the mobile dissolved organic matter (DOM) fraction (Engelhardt et al., 2015; Fan et al., 2011). In one study, the addition of manure reduced the transport for SDZ and SMPD, while increasing it for SMOX (Zhou et al., 2016).

Models

Different models to express sorption exist. Their main distinction is between equilibrium and non-equilibrium models. Equilibrium models state that there is an equilibrium between the concentration in soil and the concentration in soil solution, while non-equilibrium models account for, for example, irreversible sorption to soil. Examples of equilibrium models are Henry's linear model, the Freundlich model and the Langmuir model (Wang et al., 2015). These models have been tested for antibiotics and the Freundlich and Linear model fitted best, depending on the experiment (Białk-Bielińska et al., 2012; Maszkowska et al., 2015; Park & Huwe, 2016; Zhang et al., 2014). The two models differ in their linearity: Henry's model (Eq. 2.1) is linear, while the Freundlich model (Eq. 2.2) has a coefficient for nonlinearity.

$$Henry's\ model: Cs = Cw * Kd \qquad \text{(Eq. 2.2)}$$

Where:

Cs is the equilibrium concentration of antibiotic adsorbed in soil; and
Cw is the concentration of an antibiotic and Kd is the adsorption coefficient.

$$Freundlich\ model: Cs = Kf * Cw^{\wedge}n \qquad \text{(Eq. 2.3)}$$

Where:

Cs is the equilibrium concentration of antibiotic adsorbed in soil;
Cw is the concentration of an antibiotic;
Kf is the Freundlich adsorption coefficient; and
n is the linearity coefficient.

Non-equilibrium models account for equilibrium sorption with Freundlich or Henry's model, but also account for other processes. Sorption of *SDZ*, for example, was found to be time dependent and nonlinear (Wehrhan et al., 2010). Desorption, where the substance is released, was slower than the sorption and 41 days were found to be insufficient to reach desorption equilibrium (Wehrhan et al., 2010). Different models use a three-site model, with two reversible sites and one irreversible site, as illustrated in Figure 2a (Chu et al., 2013; Unold et al., 2010; Wehrhan et al., 2007). The reversible sites are both an instantaneous site, using equilibrium sorption with a sorption coefficient (i.e. Kf or Kd) and a rate-limited site, which uses a constant for the rate of sorption. The irreversible site accounts for irreversible sorption with a first-order kinetic rate. This model, using the Freundlich model for reversible instantaneous sorption, was found to adequately describe the sorption of *SMZ* (Chu et al., 2013), *SDZ* (Unold et al., 2010; Wehrhan et al., 2007), *4-OH-SDZ* and *An-SDZ* (Unold et al., 2010). Another study on such models for *SDZ*, however, found that the adsorption and desorption of *SDZ* could best be described with a new model with two sorption domains, as illustrated in Figure 2b (Wehrhan et al., 2010). There is thus some discussion on which model to use for non-equilibrium sorption, but both models account for irreversible sorption, instantaneous reversible sorption and rate-dependent reversible sorption.

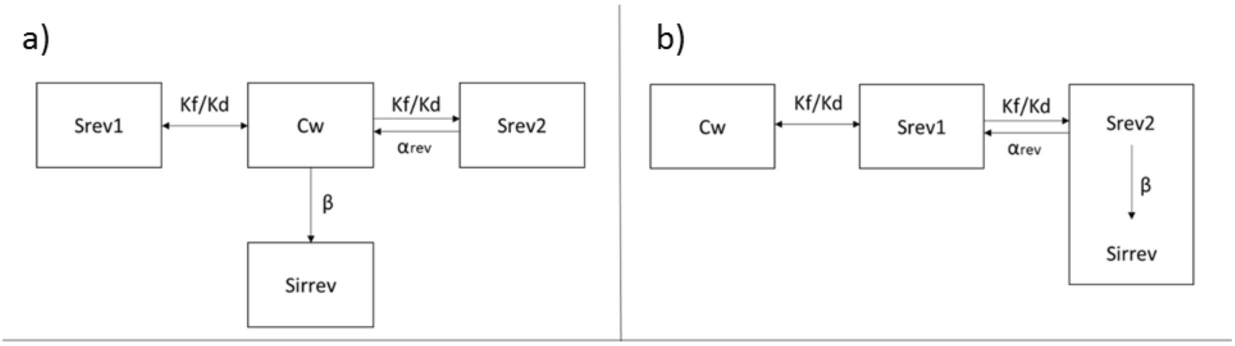


Figure 2 Non equilibrium sorption models with Srev1 (reversible, instantaneous), Srev2 (reversible, rate-limited) and Sirrev (irreversible)

Transformation products and ionization

Transformation of antibiotics into their transformation products (TPs) also occurs in soils (Wehrhan et al., 2007). These TPs can also persist in the environment and some are ecotoxic (Wohde et al., 2016). The processes of transformation are dependent on a variety of factors, such as the temperature, pH, aerobic or anaerobic conditions and microbiology (Wohde et al., 2016). The non-equilibrium models mentioned in the former section do not account for transformation products of the antibiotics. Some of Freundlich and Henry's parameters have been determined for transformation products: *CTC* epimer (Kd) (Halling-Sørensen et al., 2005), *4-OH-SDZ* (Kf) (Kasteel et al., 2010), *An-SDZ* (Kf) (Kasteel et al., 2010), but this is still very limited. Zarfl et al. (2009) created a non-equilibrium model with one reversible and one irreversible site, accounting for *SDZ*, *4-OH-SDZ* and *An-SDZ*. Rates of reversible sorption, desorption and irreversible sorption were determined for all transformation products and additionally, rate constants which account for the transformation were used (Zarfl et al., 2009). A transformation rate constant for *SMZ* and its metabolite was also used by (Fan et al., 2011).

Apart from their transformation products, antibiotics can exist in their neutral, cationic or anionic forms, as described previously (Engelhardt et al., 2015; Maszkowska et al., 2015; Wang et al., 2015). The mole fractions of these forms can be calculated using their pH and pKa (Wang et al., 2015). Cations have a higher sorption coefficient, but most sorption coefficients are not specific to the cationic/anionic/neutral forms of antibiotics, making it difficult to include this phenomenon into models.

Use in existing models

Different models are available for the risk assessment of veterinary pharmaceuticals. The European Medical Agency (EMA) suggests a simple exposure model (EMA 2016), which is adjusted by RIVM to the Netherlands (Montforts, 2003). These models calculate the initial concentration in soil similar to Eq. 2.4, accounting for the concentration in manure, the spreading of manure, the bulk density of soil and the penetration/mixing depth in soil. The mixing depth determines how deep the antibiotics will be spread in the soil and the dry bulk density of soil is necessary to calculate the concentration per kg of soil. The concentration in soil solution, depending on sorption, is not accounted for in these models.

$$PECsoil = \frac{Cmanure * Qmanure}{RHOsoil * DEPTHfield} \qquad \text{(Eq. 2.4)}$$

Where:

PECsoil	is the predicted environmental concentration in soil;
Cmanure	is the concentration in manure;
Qmanure	is the quantity of manure applied;
RHOsoil	is the dry bulk density of soil; and
DEPTHfield	is the mixing depth in soil.

In case of high exposure, EMA (2016) suggests to use the more advanced FOCUS models Pearl, PRZM, MACRO and PELMO (EMA, 2016; Leistra et al., 2001). These are pesticide models, which are of interest for veterinary medicines because of their similar use and mode of action. Pearl will be discussed here as an example. Pearl allows calculations for different layers in the soil at different depths. A distinction is made between equilibrium and non-equilibrium sorption (Leistra et al., 2001). Equilibrium sorption is described with the Freundlich model (Eq. 2.3). Non-equilibrium sorption takes into account the desorption rate. The quotient between non-equilibrium and equilibrium sorption is defined. Pearl also allows for the incorporation of the effect of pH on sorption, similar to Wang et al (2015), as discussed in the former section (Leistra et al., 2001).

Parameter overview

Data on transformation products and non-equilibrium parameters are very limited, while these are necessary for more advanced modelling such as in Pearl. The sorption coefficients Kd and Kf have been examined for a large group of antibiotics, accounting for different soil characteristics (pH, CEC, OC, soil type). These sorption coefficient are presented in Tables S3 and S4. Multiple linear regression models on sorption coefficients, accounting for soil characteristics as independent variables, have been constructed by different studies. There are models for *OTC* (Jones et al., 2005; ter Laak et al., 2006) and sulfachloropyridazine (ter Laak et al., 2006), which will be further discussed in Chapter 4.

<u>Degradation</u>

Processes

There are different processes of degradation in soil: hydrolysis, photolysis and biodegradation (Chee-Sanford et al., 2009). β-lactams, macrolides and sulfonamides are most susceptible to hydrolysis, while quinolones and tetracyclines are more susceptible to photolysis (Chee-Sanford et al., 2009). Biodegradation has shown a varying significance (Chee-Sanford et al., 2009), but a study examining differences between sterile and non-sterile soil showed the importance of biological processes (Y. Zhang et al., 2017). Aerobic biological transformation was also found to be the most important route of degradation (Sittig et al., 2014).

Other soil characteristics were found to be important as well. Organic matter facilitates contaminant removal and the content of organic matter/organic carbon in the soil thus enhances degradation (Strauss et al., 2011a; Zhang et al., 2017). This also indicates that the addition of manure, which is high in organic matter, will accelerate degradation (Zhang et al., 2017). A higher initial antibiotic concentration, on the other hand, was found to result in a longer degradation time and a higher persistence in soil (Pan & Chu, 2016). Sorption also affects the degradation: Sulfonamides have a high Kow (octonal water partitioning coefficient), indicating their lower sorption and a higher amount available for removal, increasing their rate of degradation. A comparison of laboratory and field experiments for *SDZ* found that biodegradation increases under field conditions, which is relevant for selecting model parameters (Engelhardt et al., 2015).

Models

The degradation data can be introduced into models to examine the concentration of the antibiotic over time. A commonly used model is the exponential decay model (Eq. 2.5) (Accinelli et al., 2007; Pan & Chu, 2016; Zhang et al., 2017). Others explain the relationship better with a bi-exponential curve (Eq. 2.6) (Blackwell et al., 2005, 2007; De Liguoro et al., 2003). The half-life (DT50) can be calculated from the degradation rate: DT50=ln2/k. The exposure models (EMA, 2016a; Montforts, 2003) and pesticide models use similar equations.

$$Ct = C0 * e^{-kt} \qquad \text{(eq. 2.5)}$$

Where:
Ct is the concentration of the antibiotic at time t; and
C0 is the initial concentration and k is the degradation rate.

$$Ct = A * e^{-k1t} + B * e^{-k2t} \qquad \text{(eq. 2.6)}$$

Where:
Ct is the concentration of the antibiotic at time t;
A and B are constants; and
k1 and 2 are degradation rates.

Parameter overview

Many studies determined the half-life of an antibiotic in soil, also reporting on different conditions: light/dark, aerobic/anaerobic, sterilized/not sterilized, initial concentrations, pH, organic carbon, soil texture and the application of manure (Table S5). Regression model looking at the combined effects of these models were not found. Chapter 4 will look into such a regression model.

2.3.6. Leaching to groundwater (process 6 in Figure 1)

Process

When antibiotics are present in soil solution, they can leach to the groundwater. Leaching potential is the highest for chemicals with negative charges, a Koc (soil organic carbon-water partitioning coefficient) value of <300-500 cm^3/g and a half-life in soil of more than 2 to 3 weeks (Pan & Chu, 2017). Leaching potential can be estimated with the GUS-value (Eq. 2.7) (Pan & Chu, 2017; Wang et al., 2015). A GUS value lower than 1.8 indicates that the antibiotic does not migrate, values between 1.8 and 2.8 suggest migration under proper conditions and values higher than 2.8 indicate high mobility (Wang et al., 2015). Other factors relevant for leaching are rainfall, rainfall pH and soil texture (Pan & Chu, 2017).

$$GUS = DT50 * (4 - \log Koc) \qquad \text{(eq. 2.7)}$$

Where:
GUS is the leaching potential;
DT50 is the half-life (in days); and
Koc is the sorption coefficient normalized to OC content in the soil.

Sulfonamides often have a GUS-value above 2.8, while tetracyclines have a value below 1.8 (Pan & Chu, 2017). Sulfonamides are also more frequently detected in groundwater measurements. In a study by Domínguez et al. (2014), *SDM* was detected in leachates, but *OTC* was not. Kay et al. (2005a) also found SCP in much higher concentrations in the leachate than *OTC*. Antibiotics have been detected in higher concentrations close to and underneath manure storage facilities (Szatmári et al., 2011). Pre-tillage can reduce leaching, because of the breakage of the macro pores in the soil, which results in more interaction between solutes and the soil (Kay et al., 2005a).

Models

Different studies have used Hydrus1-D to calculate the water flow and the transport to groundwater (Engelhardt et al., 2015; Strauss et al., 2011; Unold et al., 2009; Zhou et al., 2016). Hydrus-1 was found to describe manure colloid-affected transport well (Zhou et al., 2016). Domínguez et al. (2014) used a kinetic model to quantify the leaching process, which assumes the rate of leaching to be proportional to the remaining leachable content. This model needs specific parameters, as calculated in the experiment, which are not widely available.

The EMA and RIVM exposure models assume that the predicted environmental concentration (PEC) in groundwater equals the PEC in pore water, which is calculated with the concentration in soil and the partitioning coefficient between soil and water (EMA, 2016; Montforts, 2003). The model by Küçükdoğan et al. (2015) on the environmental risks of antibiotics in Turkey uses this relationship from the RIVM exposure model. Not all water in soil solution ends up in the groundwater, however. In INITIATOR, leaching is calculated with the concentration in soil solution and the precipitation access (personal communication, Wim de Vries). Leaching to groundwater is estimated based on a fraction of this total leaching, of which the other fraction will go to surface water. These models are based on estimates and do not account for, for example, the depth of the groundwater table.

As discussed before, the more advanced pesticide models are of interest for veterinary medicines, and also for antibiotics, because of their similar use and mode of action. Pearl, PELMO, PRZM and MACRO were mentioned as possibilities for modelling leaching to groundwater, but Pearl was found most suitable (EMA, 2016). The Pearl pesticide model has been tested for leaching. *SCP* was found not to leach in the model, while experimental data showed otherwise (Blackwell et al., 2009). Two explanations were given: 1) pH dependency of sorption and degradation could not have been represented well in the laboratory, 2) the interaction with the colloidal OM from the slurry could enhance transport, which the model did not take into account (Blackwell et al., 2009). The spatial edition of Pearl, GeoPearl, was also tested in its ability to model the fate and transport of antibiotics. Same as for Blackwell et al. (2009), the leaching concentrations were low and no leaching was found for *SDZ* and *SMX* (Lahr & Van den Berg, 2009). The importance of sufficient and good quality input parameters was emphasized (Lahr & Van den Berg, 2009). Suggestions for the model were 1) to include only areas where manure from certain livestock sectors was applied, 2) to include scenarios for manure injection and ploughing (Lahr & Van den Berg, 2009). GeoPearl requires data on the molecular mass, vapour pressure, water solubility, sorption and half-life of the antibiotics (Lahr & Van den Berg, 2009). PEARL, on the other hand, is more suitable for calculating the fate in a specific location, but needs more inputs, such as organic matter per soil layer, mineral composition and moisture characteristics (Lahr & Van den Berg, 2009).

2.3.7. Uptake by plants (process 7 in Figure 1)

Process

Plants take up water from the soil solution, which can result in the accumulation of antibiotics in vegetables (Chen et al., 2017; Domínguez et al., 2014; Hu et al., 2010; Wang et al., 2014). Antibiotics from different classes, including *OTC*, *CTC*, *ENR* and *SMR* were all found to be high in vegetables (Wang et al., 2014). These compounds have different physical and chemical properties, however, indicating that these properties, such as water solubility and half-life, had little effect on the uptake by vegetables (Wang et al., 2014). Other studies do not agree with that statement, relating the accumulation of antibiotics in vegetables with the octanol-water partitioning coefficient (Kow) (Hu et al., 2010; Wang et al., 2014). Kow is a measure for hydrophobicity an antibiotics with a higher Kow are less soluble and more likely to absorb.

Models

The CF (stem or leaf concentration factor) is calculated with constants A and B (depending on plant species and growing environment) and the Kow (Hu et al., 2010; Wang et al., 2014). Data on these A and B parameters are still limited to few studies. Another model uses uptake factors, calculated by dividing the concentration in plant by the concentration in soil, to estimate uptake in plants. A database on these uptake factors is still lacking (Boxall et al., 2006). Chen et al. (2017) attempted a pharmacokinetics approach to model the uptake of antibiotics by plants. This approach is data intensive and looks at absorption, distribution, metabolism and elimination processes. The bioaccumulation factors (BAF) were calculated for two different plant species. BAFs were significantly lower for *SMX* than for tetracyclines, agreeing with the link between solubility and uptake (Chen et al., 2017).

The RIVM and EMA exposure models do not account for uptake in crops. INITIATOR and STONE both look at uptake in crops, but parameters specific to antibiotics are necessary. Pearl uses Briggs relationship as described by Wang et al. (2014) and Hu et al. (2010) (Leistra et al., 2001).

2.3.8. Overland flow (process 8 in Figure 1)

Processes

In the case of rainfall after the application of manure, antibiotics can be transported to surface water directly through overland flow. These values are often higher for grassland than arable land, because of the incorporation or injection of manure in arable land (Kreuzig et al., 2005; Montforts, 2003). Broadcasting (surface application) of manure was found to lead to higher runoff than injection or incorporation (mixing) (Joy et al., 2013). The amount transported depends on the precipitation, the slope and the sorption characteristics of the antibiotic. Table 5 demonstrates different percentages found in surface water. Conclusions are difficult to make due to limited data availability, but tetracyclines clearly show less runoff than sulfonamides and a gentler slope seems to results in less runoff.

Table 5 Studies quantifying the overland flow of antibiotics

Author	Antibiotics and Percentage transported to surface water	Study notes
Kreuzig et al. (2005)	*SDZ*: 0.1-2.5% for arable land, 14.4-27.6% for grassland Sulfadimidine: 0.1-1.2% for arable, 15.8 for grassland *SMX*: 0.1-1.6% for arable, 13.3% for grassland	Clayey silt luvisol, slope between 7.7 and 8.2% on arable land and 9.0% on grassland.
Kay et al. (2005b)	*TYL* not detected, *OTC* ~0.05%, *SCP* 0.09-0.4%	Clay, slope around 4-7degrees, 3-4 mm/h over parts of 2 days.
Blackwell et al. (2007)	*OTC* was only detected in overland flow water 6 days after application whereas *SCP* was detected in all the samples collected up to 41 days after application. *TYL* not detected.	Sandy loam, 41 days, 313 mm total rainfall.
Davis et al. (2006)	*TC* 0.005%, *CTC* 0.009%, *STZ* (sulfathiazole) 0.056%, *SMZ* 0.059%, erythromycin 0.096%, *TYL* 0.057%, monensin 0.219%	Sandy clay loam, slope ~2%, sprayed 1 h before rainfall simulation, 60mm/h for 1h.

Models

Overland flow is not taken into account in EMA and RIVM exposure models. The FOCUS model, according to EMA risk assessment, was tested for predict runoff concentrations. The predicted concentrations were much higher than the measurements for most arable land, while they were underestimated for grassland (Knäbel et al., 2016). An explanation for the underestimation is that manure which is excreted during grazing was not incorporated for grassland. A reason for the overestimation is that the models are meant for pesticides, while antibiotics are applied with manure which is high in organic matter and enhances sorption of antibiotics (Knäbel et al., 2016). The modelling approach was similar to Pearl; formerly mentioned studies stated an underestimation of leaching in Pearl with similar explanations (Blackwell et al., 2009). MACRO was also tested and results show adequate simulation of concentrations comparing model results with microplot experiments (Larsbo et al., 2008). Some results were poor however, which was explained by the fact that re-infiltration is not accounted for in the model.

2.3.9 Runoff (subsurface) (process 9 in Figure 1)

The process of subsurface runoff was not found to be described in any studies. Some models do take it into account: EMEA and RIVM exposure models estimate subsurface runoff to surface water with a dilution factor. The concentration in surface water is estimated by dividing the concentration in pore water with the dilution factor. This factor is 3 for EMEA and 10 for RIVM. INITIATOR calculated this with a similar approach as leaching: it is dependent on the concentration in soil solution, the precipitation access and a fraction which determines how much flows to the groundwater and how much flows to the surface water.

Pearl allows for a more advances analysis, since it account for more hydrological processes (Leistra et al., 2001). The Stone model, another Dutch model, looks at heavy metals and nutrients from agricultural land to surface water for different combinations of land use, soil types and hydrological circumstances. Soil characteristics and a chemical equilibrium model are used to calculate concentrations in soil solution. Then the hydrological models SWAP (top system) and NAGROM (deep groundwater) calculate the transport to surface water, taking into account uptake by crops. Necessary inputs include precipitation, evaporation and temperature.

2.4 Discussion and conclusion

This Chapter provided an overview of the available studies on quantifying the fate and transport of antibiotics based on a systematic literature review. The search was only done in SCOPUS and search terms were selected with the goal to find antibiotic models, creating a risk to miss some information on specific relationships. This risk was limited by using the snowball method to find relevant publications in other articles. Most articles were on sorption and degradation. A limited number of articles was found on subsurface runoff, overland flow and uptake in plants. This indicates more uncertainty when modelling these processes, as will be addressed in Chapter 5.

The parameter data show some variation, as will be discussed in this paragraph. Limited data were available on the excretion rates, but the percentages varied. The value largely depends on whether urine is included or not: Massé et al. (2014) show and ER of 25% of *TC* in pig faeces, but Zhang et al. (2015) found an additional 50% for urine. There is also some general variation in values: Massé et al. (2014) found 50-60% for *TYL* in pig urine, but Zhang et al. (2015) show almost 0% for urine and 40% for faeces. Data to make a sufficient comparison are lacking, so values for excretion rates will have to be estimated. The ranges in the half-life in manure could largely be explained by the dependency on a variety of factors; storage type, temperature, aerobic/anaerobic conditions, light and animal type. The sample size is insufficient to conduct a regression analysis, making it difficult to determine to what extent the range in half-lives can be linked to the factors it depends on. The Kd and Kf significantly differ per antibiotic, with tetracyclines having values about a thousand times higher than sulfonamides. The sorption depends on factors such as the OC, clay, CEC and manure application. A range of values is visible: the Kd of OTC for example was measured between 40 and 12000 L/kg. The regression analysis in Chapter 4 finds a standard error of 1647 for the regression model, indicating that most variance can be explained by the independent variables. The half-life in soil is also subject to a range of values. *SDZ*, for example, has values between 3 and 19 days. This also depends on a variety of factors (clay, OC, initial concentration, manure, light, air, sterilization). The regression model constructed in Chapter 4 finds a standard error for all sulfonamides of 3.68 and a R^2-adj of 0.93, implying limited uncertainty in the measurements.

This overview indicates that quantifying the processes can be done in different ways, varying from simple exposure models to more advanced models (e.g. pesticide models). The advanced models take into account more factors, but are also more data intensive. The overview indicates that certain parameters are lacking. Regression models are a way to estimate parameters based on environmental and soil characteristics. This will be addressed in Chapter 4. Non equilibrium sorption describes the sorption process more adequately than equilibrium sorption, but data on the relevant parameters for these models are limited. A similar issue was found for parameters relating to transformation products and positive and negative ions. Furthermore, models that accurately describe the transformation process were also scarce. Despite some missing parameters, sufficient information is provided by this Chapter to develop an exploratory model on the fate and transport of antibiotics in agriculture, which will be further discussed in Chapter 5.

Chapter 3. Antibiotic Resistance

3.1 Introduction

The main interest in the fate and transport of antibiotics is related to concerns about the development of antibiotic resistance. Manure contains relatively high levels of antibiotics, resistant micro-organisms and metals, which are known to co-select for resistance (Fahrenfeld et al., 2014). A distinction will be made between antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs). Resistant bacteria acquire their resistance through ARGs, since the genes give the bacteria resistant characteristics (WHO, 2014). When this Chapter talks about ARGs, this also includes the ARB which contain ARGs. ARGs are often specific to a certain type of antibiotic. Examples of tetracycline resistant genes are *tet(W), tet(O), tet(X)* and *tet(M)*, while the most common sulfonamide resistant genes are *sul1* and *sul2*. *Erm*, the erythromycin resistant gene, will also be mentioned in this Chapter. Micro-organisms can also be resistant to multiple antibiotics: multidrug resistance.

ARGs are known to follow similar routes as antibiotics (Berglund, 2015), which means that the framework for Chapter 2 can also be used to describe the fate and transport of ARGs (RQ 2). There are, however, some important distinctions between the two. While antibiotic concentrations only decrease after their first input, ARGs are present in bacteria, which can multiply and thus increase the concentrations (Economou & Gousia, 2015; Fahrenfeld et al., 2014). Bacteria can also transfer their genes through horizontal gene transfer to other bacteria, enhancing the amount of bacteria with resistance and also increasing the risk of antibiotic resistance in pathogenic bacteria (Fahrenfeld et al., 2014). Another important factor which can increase the concentration of ARGs, is that concentrations of antibiotics, low or high, can select for resistant bacteria, since these are not sensitive to the antibiotic, while other bacteria are (Berglund, 2015). This results in a bacteria population with a higher dominance of the resistant bacteria. These factors complicate modelling approaches for ARGs and ARB, as will be discussed in this Chapter.

3.2 Method

Search strategy

The search with search terms from Table 6 was conducted in Scopus based on the title, abstract and keywords, in March 2018.

Table 6 Search terms for models related to antibiotic resistance

Related to resistance		Related to agriculture		Relating to modelling
"Antibiotic resistance" OR "Antimicrobial resistance" OR "bacterial resistance" OR ARG OR ARB OR "antibiotic resistance bacteria"	AND	livestock OR farming OR manur*OR veterinary OR agricultur*	AND	model*

Results

The search gave 577 results. Only articles from 2008-2018 were selected, which resulted in 450 results, of which 51 were considered relevant for at least one of the sections. Interviewed experts also provided more key publications and some articles were selected with the snowball method. Table 7 gives the number of relevant articles per section also including articles outside of the systematic review.

Table 7 Relevant articles per section found in the literature review

Topic	Relevant articles
Excretion in manure	20
Manure storage/treatment	8
Application to soil	6
Accumulation and degradation	6
Leaching	4
Uptake in plants	2
Subsurface runoff and overland flow	3

3.3 Results

3.3.1. Excretion in manure (process 2 in Figure 1)

Due to the presence of antibiotics, the resistant bacteria in the gut of livestock can become dominant, increasing the percentage of resistant bacteria in manure and thus also increasing the concentration of ARGs. The first step in modelling is to know the relationship between antibiotic usage and the extent of resistance in manure.

Concentrations in manure

Manure of pigs which were fed an extensive list of different antibiotics contained around 10^6 and 10^10 copies per gram, while this was between 10^5 and 10^9 for dairy manure, indicating differences between animal types (Sandberg & LaPara, 2016). These differences can also be explained by antibiotic usage, however, as swine often receive more antibiotics. Of the different ARGs examined, *tet(W)* was the highest, followed by *tet(X)*, *tet(A)* and *erm(B)* (Sandberg & LaPara, 2016). *Tet(W)* was found in the highest concentrations in other studies, followed by *sul1* and *sul2* and the other tetracycline resistance genes (Alexander et al., 2011; Chen et al., 2010; Storteboom et al., 2007). ARB are often expressed in a percentage of the total bacteria population. The MARAN reports look at resistance in the Netherlands and found bacteria populations in faeces with up to 50% resistance against tetracyclines (Veldman et al., 2017).

Relationship antibiotic usage and resistant bacteria in manure

The studies linking usage to resistance are summarized in Table 8. In general, increased usage leads to higher resistance, but there are also studies which do not show a significant association (Mourand et al., 2014; Snow et al., 2012). The extent of the relationship between increased usage and increased resistance is also unclear: trimethoprim/sulfonamides had low usage, but showed relatively high resistance (Gibbons et al., 2016). This indicates the relevance of other factors in determining resistance. Noyes et al. (2016) tried ten modelling strategies linking antibiotic usage with resistance to a variety of antibiotics, pointing out the inherent complexity of modelling these associations with real-world data. The correlation between ARGs and antibiotics was found to be affected or lost when either antibiotics or ARGs were initially present at low concentrations (Storteboom et al., 2007). Furthermore, there are other risk factors affecting the development of resistance, such as the number of animals, change of housing, cleaning/on-farm hygiene, the status of the previous group, the presence of wild bird populations on farms and dietary factors (Mo et al., 2016; Santman-Berends et al., 2017; Snow et al., 2012). Co-resistance can also occur, meaning that the usage of one antibiotic results in resistance to another antibiotic, further complicating the modelling process (Ozawa et al., 2012).

A mathematical bacterial kinetic model for the amount of resistant enterobacteria in the faeces of piglets exposed for five days to ciprofloxacin was developed by Nguyen et al. (2014). The model examines the growth of resistant bacteria and the loss of susceptible strains, accounting among other things for the fitness of the bacteria, growth rates, arrival of bacteria in the digestive tract and the maximum number of bacteria (Nguyen et al., 2014). This seems to be the first model examining this process. The model predicted the development of resistant bacteria well (Nguyen et al., 2014). Data intensity is high and the authors also state that there is still uncertainty related to the measurements.

Table 8 Associations between antibiotic usage and resistant in livestock faeces

Author	Antibiotic given	Bacteria	Resistance/ARG	Relationship	Study context
Alexander et al. (2011)	chlortetracycline or chlortetracycline/ sulfamethazine or tylosin	-	*tet(B), tet(C), tet(M), tet(W), sul1* and *sul2, erm(A), erm(B), erm(T), erm(X), erm(F)*	Antimicrobials generally selecting for higher levels of determinants -lack of consistent differences between treatment and control samples. Most obvious for tet (*tet(M) and tet(W)*), *then sul*. Not very clear for *erm.*	Steers in Canada
Boyer and Singer (2012)	Ceftiofur	-	bla_{CMY-2}	Higher mean quantities than untreated animals.	Dairy cattle in USA
Gibbons et al. (2016)	Separate classes of antibiotics	E.Coli	to the separate classes of antibiotics	Highest resistance for penicillin, followed by tetracycline and streptomycin. Relatively high resistance for Trim/Sulpha, despite low usage. Low for fluoroquinolones, gentamicin, cephalosporins (low usage).	Pig herds in Ireland
Guenther et al. (2017)	-	E.Coli	colistin (*mcr*-1)	Found positive strains from manure, boot swabs, dog faeces, stable flies	Swine farms in Germany
Herrero-Fresno et al. (2017)	-	E.Coli	to ampicilin and tetracycline	Pigs without treatment high proportion of resistant strains	Nursery pigs in Denmark
Lutz et al. (2011)	Ceftiofur (high, moderate or rare)	E.Coli	to ceftriaxone	Rare: lower odds	Swine barns in USA
		Salmonella spp.	to ceftriaxone	Highest (6%) in Common use group, followed by Rare (4.1%) and Moderate (0.15%)	
Makita et al. (2014)	13 different antibiotic classes	E.Coli	To 13 classes	Direct associations: - aminoglycosides in reproduction farms and resistance to kanamycin - tetracyclines in larger farms and resistance to oxy-tetracycline - beta-lactams and resistance to ampicillin - phenicols and resistance to chloramphenicol - fluoroquinolones and resistance to nalidixic acid and enrofloxaci	Pigs in Japan
Mourand et al. (2014)	Oxytetracycline	Campylobacter	*tet(O)*	Significantly more frequent	Chickens in France
	Enroflaxin	Campylobacter	to enroflaxin (Thr86Ile mutation in the gyrA)	Increased	
	Amoxicillin	Campylobacter	-	No impact	
	Amoxicillin	Enterococcus faecium	to ampicillin and sulfadimethozine/ trimethoprim	Selected for	

Table 8 Associations between antibiotic usage and resistant in livestock faeces (continued)

Author	Antibiotic given	Bacteria	Resistance/ARG	Relationship	Study context
Ozawa et al. (2012)	Macrolides	Campylobacter	to enrofloxacin, erythromycin and chloramphenicol	Higher enrofloxacin and erythomycin resistance	Pigs in Japan
	Tetracyclines			Higher chloramphenicol resistance	
	Phenicols			Higher chloramphenicol resistance	
Santman-Berends et al. (2017)	All (daily dose of AMU per farm per year)	E.Coli	ESBL/AmpC producing	Organic: 13%, Conventional: 41%. No association between the use of different types of antimicrobials and ESBL/AmpC herd status.	Organic dairy herds in the Netherlands
Sapkota et al. (2011)	Organic (erythromycin and tylosin) and Conventional (ciprofloxacin, gentamicin, nitrofurantoin, penicillin, and tetracycline)	E.faecalis	Multi-drug resistance	Forty-two percent of isolates from conventional poultry houses, 10% of isolates from newly organic poultry houses	Broiler farms in USA
Scherer et al. (2013)	Streptomycin	E.Coli and staphylococcus spp.	Streptomycin-resistance	E.Coli: Significantly higher than control group (39.9% compared to 22.3%) & Staphylococcus spp.: only detected after application & Increased multidrug resistance	Sheep faeces and nasal cavities in Switzerland
Snow et al. (2012)	3rd or 4th generation caphaolsporin (ceftiofur, cefoperazone and cefquinome)	E.Coli	ESBL	4 times more likely if treatment in the last 12 months	Dairy farms in England
	1st or 2nd generation cephalosporins	E.Coli	CTX-M	No significant association	
Storteboom et al. (2007)	Tetracylines (TC, OTC, CTC)		*Tet(O)* and *tet(W)*	Positive correlation (R^2~0.65) between levels of tet(W) in dairy manure and feedlot manure. Positive correlation (R^2=0.83) tet(O) in feedlot manure, not for dairy manure.	Horses in USA
Ström et al. (2017)	Greater diversity of antimicrobials at median-scale farms compared to small-scale farms + higher extent of antibiotics in feed	E.Coli	To 10 antibiotics	Significantly higher resistance levels in median-scale farms + higher level of multidrug-resistance	Sows in Thailand

3.3.2 Manure storage/treatment (process 3 in Figure 1)

During storage, the concentration of ARGs in manure can either increase or decrease, depending on the situation. Environmental conditions, such as temperature or oxygen content, can lead bacteria to thrive or be affected (Chee-Sanford et al., 2009). ARGs have a degradation rate, but can also increase in bacteria when they multiply and the presence of antibiotics give selection pressure (Ruuskanen et al., 2016).

The effect of open-air storage lagoons and silos in Finland was examined, showing that the abundance of ARGs (Sul1, tet(M) and blaOXA-58) increased in 7 of 11 cases (Ruuskanen et al., 2016). Sul1 and tet(M) increased in most farms, while this was less consisent for blaOXA-58. The manure was stored for 9 months under 5 degrees Celsius. This lower temperature can induce horizontal gene transfer (Ruuskanen et al., 2016). Aerobic biofiltration was found to be effective in reducing the amount of erm(X), while mesophilic aerobic digestion and lagoon storage did not reduce any ARGs (*erm(X), erm(B), erm(F), tet(G)*) (Chen et al., 2010). Composting for 35 days was found to decrease the abundances of erm(A) and erm(B) with 0.4-1.2 logs and 1.2-1.6 logs respectively (Yin et al., 2017). Composting was also found to reduce ARGs in chicken manure, but less so in pig and bovine manure (Qian et al., 2018). Qian et al. (2018) classified six types of ARGs based on their fate during composting: Type I ARGs were reduced in all different types of manure, while Type II, for example, were only reduced in chicken and pig manure samples, but not in bovine manure samples.

Bacteria can be inactivated at higher temperatures: composting up to 60˚C showed inactivation of E.Coli and degrading DNA fragments, while the fragments persisted at temperature below 48˚C and ambient temperatures of 0-8˚C resulted in the persistence of the E.Coli (Guan et al., 2010). Furthermore, co-factors can lead to an increased development of multidrug resistance. Copper is an important factor (Hölzel et al., 2012; Hu et al., 2016; Yin et al., 2017); higher concentrations of copper (200-2000 mg/kg) were found to result in higher persistence of resistance in the bacterial community (Yin et al., 2017). Zinc and copper were found to elevate resistance to b-lactams, while mercury was associated with low resistance (Hölzel et al., 2012). A possible explanation for this effect of mercury is co-toxicity (Hölzel et al., 2012).

A mathematical model on the situation in a slurry tank in the UK was developed (Baker et al., 2016). The spread of resistance is related to the gene transfer rate and the inflow of antibiotics. A higher gene transfer rate controls resistance more than antibiotic inflow, but if the gene transfer rate is low, a reduction in antibiotics does control resistance (Baker et al., 2016). The model uses data on antibiotics use and related selective pressure, population growth, slurry inflow, horizontal gene transfer rates and fitness costs of antibiotics (Baker et al., 2016). Like the other models, there is potential in using this for other situations, but the model is high in data requirement and so far only applied to a specific case.

3.3.3. Application to soil (process 4 in Figure 1)

When manure which contains ARGs is applied to soil, this logically also increases the concentrations of ARGs in soil (Fahrenfeld et al., 2014; Ruuskanen et al., 2016; Wang et al., 2017). The abundance of ARGs in soil was shown to increase about fourfold after manure application in Finland (Ruuskanen et al., 2016). Results of a study examining manure application in a corn field show that initial abundances of *erm(F)*, *sul1*, and *sul2* were higher than expected, which was explained by either a rapid growth/selection of bacteria, or by an increase in the rate of horizontal gene transfer (Fahrenfeld et al., 2014). Low tet(O), on the other hand, was explained either by: cell death, selective sorption or decay (Fahrenfeld et al., 2014). These results indicate that the abundance in soil depends on background levels of ARGs and environmental conditions which affect bacterial growth and horizontal gene transfer.

There are also co-factors playing a role in the soil. Persistent insecticides in soil can increase the development of multidrug resistance (Rangasamy et al., 2017). Heavy metals are other co-selectors in soil (Hu et al., 2016; Knapp et al., 2011). Copper was found to have the strongest impact on ARGs and correlate with most ARGs, while chromium, nickel, lead, and iron were found to significantly correlate with some specific ARGs (Hu et al., 2016; Knapp et al., 2011). The highest abundances of ARGs were found for copper concentration between 100 and 400 mg/kg. The ARG concentrations under these conditions were about a quarter higher than under the control (Hu et al., 2016). For nickel, the highest concentrations were also recorded at treatments with around 400 mg/kg, with ARG abundances almost double the control (Hu et al., 2017). pH was also significant in some of the regression models for selecting resistance Knapp et al. (2011). Organic carbon, total phosphorus, dry ash, and silt, sand and clay content were also taken into account, but did not show significance in the models (Knapp et al., 2011).

3.3.4. Processes in soil: accumulation and decay (process 5 in Figure 1)

Accumulation

ARGs can already be found in soil before manure application, indicating background levels of ARGs and ARB in agricultural soils (Fahrenfeld et al., 2014; Ruuskanen et al., 2016). A microcosm study found that most of the ARGs did not reduce back to background levels after 96 days (Wang et al., 2017). Macrolide, lincosamide and streptogramin B resistant genes were found to persist for <20 says, while sul1 could stay up to 2 months (Fahrenfeld et al., 2014). Sulfachloropyridazine resistance was reported to be back to baseline levels 21 days after pig manure slurry treatments, while this was 6 months for tetracycline resistant isolates (Fahrenfeld et al., 2014).

Fahrenfeld et al. (2014) suggest a conceptual model for accumulation of ARGs in soil:

$$ARGacc = ARGload + ARGgrowth - ARGrunoff - ARGdecay - ARGinf$$

Where: 'ARGacc is the rate of ARG accumulation; ARGload is the ARG loading rate; ARGgrowth includes increase in ARGs due to growth or expansion of hosts, which may be a result of selection pressure or horizontal gene transfer; ARGdecay is the ARG decay rate; ARGrunoff is the rate of surface transport of ARGs into and out of the control volume; and ARGinf is the infiltration rate of ARGs.'

The data or relationships for this model are not provided, however, and there is limited knowledge on some of the rates. The ARG load can be determined based on the concentration in manure, as explained in the former sections. The runoff, infiltration and decay will be discussed later in this Chapter. Although there is limited data availability, estimates can be made for these rates. The largest difficulty seems to be in the factor on ARG growth, since this depends on the growth of bacteria, the horizontal gene transfer and the selection pressure (Fahrenfeld et al., 2014). Mathematical models including bacterial growth, death and horizontal gene transfer are available (Lanzas et al., 2011), but the literature review has not provided sufficient information to estimate these factors and selective pressure is missing in those models. Some studies state that a bacteria with antibiotic resistance has higher metabolic cost due to the maintenance of resistance, eliminating the ARGs from the population after removal of selective pressure, but this does not always occur and the carrying of some ARGs has also resulted in improved fitness of the bacteria (Chee-Sanford et al., 2009).

Decay

Different models describe the decay of ARGs in soil: a logarithmic decay (Fahrenfeld et al., 2014; Wang et al., 2017) and the Collins-Selleck model (Sandberg & LaPara, 2016). Soil microcosm experiments with dairy and swine manure showed the good fit of the Collins-Selleck model (Sandberg & LaPara, 2016). $log10\left(\frac{N}{N0}\right) = \Delta cs[log10t - log10b]$, where N is the number of ARG copies at time t, N0 is the initial quantity of ARGs, Δcs is the specific lethality coefficient and b is a lag coefficient. The ARGs in the dairy manure returned to baseline levels, but this was not the case for swine manure, indicating that accumulation in the soil can occur (Sandberg & LaPara, 2016). Logarithmic decay also showed a good fit in two studies looking at soil microcosm with poultry, swine, dairy and dry stack applied manure (Fahrenfeld et al., 2014; Wang et al., 2017).

$$N = mlnt + b$$

Where:

> 't is time (day);
> N is the number of gene copies/16S rRNA copies at any given time t;
> m is the slope [log10 (gene copies/16S rRNA copies)/ln time (days)];
> ln is the natural logarithm; and
> b is the y-intercept [log10 (gene copies/16S rRNA copies)].' (Wang et al., 2017)

Erm(F) had a dissipation rate 2.8-3.0 times higher than sul1 and sul2 in dry-stack-amended soil and 20-25 times higher for slurry-amended soils (Fahrenfeld et al., 2014).

3.3.5. Leaching to Groundwater (Process 6 in Figure 1)

After introduction into the soil, ARGs can be transported to bacteria in groundwater (Chee-Sanford et al., 2009). This is correlated with several factors, such as the water content of manure and soil, preferential water movement and the presence of macro pores (Chee-Sanford et al., 2009). Leaching of resistant bacteria to groundwater has not been studied extensively. Deep soil samples of field experiments with ARG application in manure were examined, but no significant increase in ARG abundance was shown, in spite of precipitation events (Fahrenfeld et al., 2014). ARG abundance has been found to increase in groundwater underneath lagoons in studies with tetracycline resistant genes (Chee-Sanford et al., 2001; Koike et al., 2007). These increased concentrations underneath lagoons would not show in a model looking at the application in soil, but do indicate the possibility of leaching at higher concentrations.

3.3.6. Uptake in plants (process 7 in Figure 1)

No literature was found on the uptake of ARGs by plants. Plants can have an important role in the abatement and pathway of ARGs in soil, however, for which more research is required, due to 'the complex nature of soil biological and abiotic conditions' (Liang et al., 2017). The rhizosphere, which is the soil affected by root secretions around plants, has been associated with increased gene transfer, enhancing the effects of antibiotics (Jechalke et al., 2014).

3.3.7. Overland flow (process 8 in Figure 1) and subsurface runoff (process 9 in Figure 1)

Similar to antibiotics, ARGs can be transported to surface water through overland flow and subsurface runoff (Bueno et al., 2018; Joy et al., 2013). A field study with controlled rainfall events immediately after the application of pig manure slurry found *tet(Q)* and *erm(B/F)* in overland flow in concentrations between 10 (injection of *erm*) and 33000 (broadcast of *tet(Q)*) copies/mL (Joy et al., 2013). Dissipation patterns in another field study, however, found almost no overland flow of ARGs after the application of manure, despite precipitation events (Fahrenfeld et al., 2014). This indicates the likely importance of other factors, such as rainfall patterns and soil type. The manure land application method (broadcast, incorporation or injection) also affects the overland flow, with broadcast resulting in a significantly higher total mass loading (Joy et al., 2013).

Bueno et al. (2018) conducted a systematic literature review on the impact of point sources on ARB in the environment, including studies on terrestrial agriculture. Two studies found higher concentrations downstream of livestock farms compared to upstream, but one study showed no significant difference, again demonstrating the importance of factors such as rainfall patterns and soil type.

3.4 Discussion and conclusion

The literature review conducted in this Chapter gives a good overview of the pathway of antibiotic resistant genes and bacteria and methods to quantify this pathway. The systematic literature review searched for models, so it could have missed out on general articles on the processes. To deal with this, relevant publications were also searched for in the selected articles. Most articles look at the excretion of ARGs/ARB in manure and the association with antibiotic usage. Limited articles were found on the other processes; mostly information on the uptake in plants and runoff/leaching was very limited.

Although some models and parameters on the different relationships between compartments are available, key information is missing to create a model for ARGs in agriculture. An important first step is to link resistance with usage, accounting for other factors such as on farm conditions and background conditions on concentrations of antibiotics and ARGs, since these were found to have a significant effect on the development of resistance. This process would be very data intensive, however, and it will be difficult to take all factors into account. Other processes still lack sufficient knowledge on the processes and a suitable method of quantification, such as the uptake in plants, leaching, overland flow and subsurface runoff. More research on these processes will be necessary for the development of an adequate model.

The development of a model to accurately predict environmental concentrations of ARGs will no doubt be a procedure with an extensive data requirement, further complicated by co-resistance, co-selection by biocides and heavy metals and the importance of environmental and on-farm conditions and soil characteristics. A suggestion could be to focus on the development of a model with more extreme scenarios to identify hotspots for resistance and allow prioritization of measures. The conceptual model by Fahrenfeld et al. (2014) that links accumulation in soil to the load, growth, runoff, decay and infiltration is suitable for this, when the necessary data are available. Concentrations of antibiotics can also be linked to the selection of ARB, as pursued with predicted no effect concentrations (PNEC) by Bengtsson-Palme and Larsson (2016), to examine these hotspots. These PNECs will be used to assess risk in Chapter 6.

Chapter 4. Regression models

4.1 Introduction

Chapter 2 pointed out that model parameters are strongly dependent on a variety of factors. The sorption coefficient Kf and Kd depend on the application of manure, the clay content, the organic carbon (OC) content, the pH and the cation exchange capacity (CEC) (Section 2.3.5). The half-life also depends on the application of manure, pH and clay and OC contents, but further varies with dark/light, sterilized/not sterilized, aerobic/anaerobic and the initial concentration (Section 2.3.5). Models to predict parameters based on those characteristics are still mostly lacking, as mentioned in Chapter 2. This Chapter attempts to create linear regression models for the Kd, Kf and half-life in soil (DT50soil) based on the available data collected from literature. These regression models will assist in quantifying the pathway of antibiotics (RQ 1).

4.2 Method

Data selection

Values for the parameters Kf, Kd and DT50soil were selected from literature. The factors affecting these parameters, their independent variables, are also reported in most studies, allowing a regression analysis. The literature was found through the literature review in Chapter 2. The data can be found in Tables S3, S4 and S5.

Multiple linear regression

Multiple linear regression analysis was done in SPSS. Some studies have missing values for one of the independent variables. In that case, these studies were still taken into account (pairwise inclusion). The model was selected based on highest adjusted R^2-value with the lowest number of variables. The models were tested for independence (Durbin Watson: $1.5<d<2.5$), homoscedasticity (plotting Zred to Zpred), linearity (plotting Zred to Zpred) and normality of the residuals (Q-Q-plot). Collinearity is explored with matrix scatterplots and will also be discussed. There were some slight violations of these assumptions, as will be discussed in the results and discussion.

Table 9 Method for multiple linear regression analysis

Analysis	Dependent variable	Independent variables
Analysis 1: Henry's linear sorption coefficient (Kd) dependent on soil characteristics	Kd (in kg/L)	- Manure: 0 for no, 1 for yes - % Clay - % OC - pH - CEC in cmol/kg
Analysis 2: Freundlich sorption coefficient (Kf) dependent on soil characteristics	Kf (in kg/L)	- Manure: 0 for no, 1 for yes - % clay - % OC - pH - CEC in cmol/kg
Analysis 3: half-life in soil based on soil characteristics and environmental conditions	DT50 (in days)	- Manure: 0 for no, 1 for yes - Air: Aerobic (1) or anaerobic (2) - Bio: Sterilized (0) or not sterilized (1) - Dark (0) or light (1) - pH - % clay - % OC - Initial concentration (ug/kg)

4.3 Results

4.3.1 Analysis 1 and 2: Kd and Kf

The regression models were made for the antibiotics with a relatively high number of measurements. For Kd, oxytetracycline (*OTC*) and tetracycline (*TC*) were selected with 66 and 28 measurements respectively. For Kf, sulfadimethoxine (*SDM*), sulfamethazine (*SMZ*) and sulfamethoxazole (*SMX*) were selected with 30, 31 and 26 measurements respectively. Sulfonamides were first approached as a class, but this gave a lower R^2-value of 0.35, supporting the decision to approach the antibiotics separately. The results of the linear regression models are given in Table 10; more information on their assumptions and data sources is given in Table 11. As stated in the methodology, different models were tested and the one with the highest adjusted R^2-value and the lowest number of variables was selected. The outcomes of the other models with lower R^2-values can be found in Appendix I. The regression models showed adjusted R^2-values between 0.39 and 0.82 (Table 10).

Table 10 Multiple linear regression models for analyses 1 and 2. Nd=not determined (no data available), (-)=no effect in the regression model. The grey colour indicates significance (p<0.05).

	Kd		**Kf**		
	OTC	***TC***	***SDM***	***SMZ***	***SMX***
Manure	nd	nd	2.159	4.386	2.212
%Clay	21.67	1368	0.126	0.073	-
%OC	488.5	-	-	-	0.225
pH	-480.9	-22189	-	-	-0.441
CEC	-	-	-	-	0.018
Constant	3501	138569	0.368	0.948	3.095
R^2-adj	0.39	0.40	0.68	0.59	0.82
SE	1674	60202	0.92	1.67	0.48

Table 11 Data sources and assumption for regression models Table 10

Model	**n**	**Sources**	**Violation of assumptions**
OTC	66	4 (Figueroa-Diva et al., 2010; Jones et al., 2005; Rabølle & Spliid, 2000; ter Laak et al., 2006)	Some violation of independence (d=1.371) and slight violation of homoscedasticity.
TC	28	3 (Figueroa-Diva et al., 2010; Pan & Chu, 2016; Sassman & Lee, 2005)	Some non-linearity and the residuals are not normally distributed, indicating some problems with the model.
SDM	30	4 (Białk-Bielińska et al., 2012; Park & Huwe, 2016; Wang et al., 2015; Zhang et al., 2014)	One clear outlier – when excluded, no violations.
SMZ	31	5 (Accinelli et al., 2007; Pan & Chu, 2016; Park & Huwe, 2016; Srinivasan & Sarmah, 2014; Wang et al., 2015)	Some violation of normality of residuals and homoscedasticity.
SMX	26	3 (Park & Huwe, 2016; Srinivasan & Sarmah, 2014; Wang et al., 2015)	Some violation of independence (d=1.278)

4.3.2 Analysis 3: DT50

The multiple linear regression analysis for the half-life was conducted for the antibiotic class sulfonamides, since this was the only group with a relatively high number of measurements (n=36). The regression model is based on data from seven different studies (Accinelli et al., 2007; Blackwell et al., 2005; Kreuzig & Höltge, 2005; Pan & Chu, 2016; Strauss et al., 2011b; Wang et al., 2006; Zhang et al., 2017). The results can be found in Tables 12 and 13. Two models were tried; light and pH were excluded based on the results from the first model. The assumptions were not violated.

Table 12 Multiple linear regression models for analysis 3

	R^2adj	SE	Significant ($p<0.05$)
C0, OC, Clay, pH, Light, Bio, Air, Manure	0.93	3.67	OC, Clay, Bio, Air
C0, OC, Clay, Bio, Air, Manure	0.93	3.68	C0, OC, Clay, Bio, Air, Manure

Table 13 Outcome of multiple regression model of analysis 3, the grey colour indicates significance ($p<0.05$)

Variable	Regression model
C0	0.000225
OC	-12.7
Clay	0.384
pH	-
Light	-
Bio (non-sterilized)	-26.2
Air (aerobic)	-14.684
Manure	-7.473
Constant	58.3
R2	0.93
SE	3.68

4.4 Discussion and conclusion

Kd and Kf

The models show significant associations between the independent variables (manure, clay, OC and pH) and the parameter Kd or Kf, as expected from the literature review in Chapter 2. The literature review also showed effects of CEC, but this does not clearly come back in the models. A possible explanation is the presence of some collinearity between CEC and clay and CEC and organic carbon (Parfitt et al., 1995) (Figure 3). The variance will in that case already be explained by either OC or clay. There is also some collinearity between clay and OC, as illustrated in Figure 3, which seems mostly driven by an outlier. This collinearity makes it rather difficult to explain the role of individual variables, which is shown in the regression models, since model 1 and 5 (*OTC* and *SMZ*) show a significant effect of OC, while models 2,3 and 4 (*TC, SDM* and *SMZ*) do not and models 2 and 3 did find a significant impact of clay.

The relevance of pH depends on the range that is considered, because species change from neutral to cationic/anionic at a specific pH (Wang et al., 2015). This relationship might therefore violate linearity, since a small change in pH will only lead to increased sorption at specific pH values. A solution could be to isolate specific pH classes, but this would require more data.

The sample size of all models is small, meaning that the models can be strongly affected by outliers and that the range in values of the independent variables is quite limited. Some assumptions were violated, indicating problems with the reliability of the models. A larger sample size is likely to improve this. The model for *TC* showed some nonlinearity, implying the need to explore a non-linear model, which was out of the scope of this report. Another issue could be that the input data is taken from other experiments examining the Kd and Kf. These experiments are often keeping some factors constant, which is important to take into account while doing a multilinear regression analysis, since this is more likely to lead to a significant model. Although data from a variety of experiments is used, a higher variety of studies and a higher sample size is likely to improve the reliability of the regression analysis.

Literature study identified two other regression models for the Kd: Ter Laak et al., (2006) conducted partial-least-squares regression modelling of the Kd of *OTC* and showed a model with significance of OC, clay, CEC, iron and aluminium oxyhydroxide and got a R^2-value of 0.69. The last two were not taken into account in models 1 and 2, due to limited data availability, which might explain the lower R^2. Another model on the Kd of *OTC* showed a significant impact of clay, CEC and iron, but not of aluminium and OC (Jones et al., 2005). 38.1% of variation was explained by the model, which is similar to the outcome of model 1 which used a more extensive dataset, indicating the need for further research.

Although some issues with the models were pointed out and further research and a larger sample size seems necessary, the relatively low standard error (except for TC) indicates that the model can be used to predict the sorption coefficients based on soil characteristics. No better methods are currently available. The model for the Kd of OTC will be used in Chapter 5.

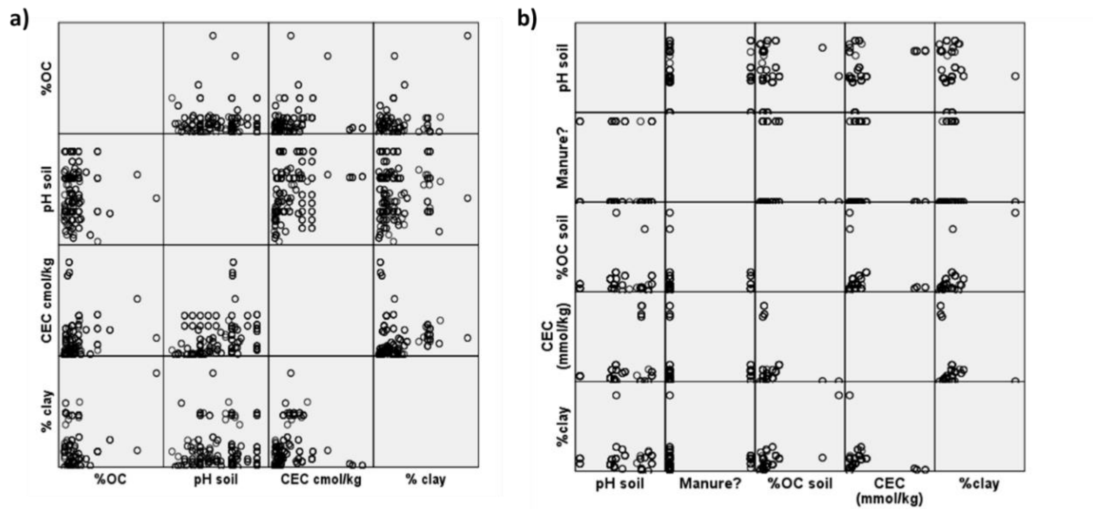


Figure 3 Collinearity scatterplots for independent variables of a) analysis 1 (Kd) and b) analysis 2 (Kf)

Half-life

The regression model for the half-life shows the dependency on many different factors. Biodegradation is an important process of degradation, since non-sterilized soil significantly decreases the DT50, as confirmed by the literature review (Sittig et al., 2014; Zhang et al., 2017). Furthermore, aerobic conditions lead to a lower half-time than anaerobic conditions, which is similar to the findings for the half-life of manure (Wohde et al., 2016). Light did not seem to be an important factor, which is confirmed by the fact that sulfonamides are hardly affected by photolysis (Chee-Sanford et al., 2009). Hydrolysis was not analysed as a separate factor, although this an important factor for sulfonamides (Chee-Sanford et al., 2009).

A higher initial concentration was found to lead to a longer half-time, as also demonstrated in the model (Pan & Chu, 2016). The beta coefficient is low, however, indicating that this effect mostly shows at high concentrations (in mg/kg). The addition of manure (OM) and the percentage of organic carbon both decrease the half-life, which is explained by the fact that organic matter facilitates contaminant removal, enhancing degradation (Strauss et al., 2011a; Zhang et al., 2017). A higher clay content, on the other hand, leads to increased sorption and a lower amount available for degradation. The model supports this: the half-life increases with a higher percentage of clay.

The high adjusted R^2-value of 0.93 indicates that the selected independent variables well describe the variance in the half-life; there is a good opportunity of using a regression model to determine the degradation. The applicability for prediction of this model can be questioned, however, since it was calculated with a small range of values for OC and clay. A high OC value will now lead the model to give a negative half-life, while there are many soils with a higher OC content. A larger dataset with a wider range of values is needed to develop a model with a higher predictive value.

Chapter 5: Modelling antibiotics

5.1 Introduction

This Chapter describes the exploratory model on the fate and transport of antibiotics, which has been developed based on the knowledge from the literature review in Chapter 2. RQ3, on the development of a model for different combinations of soil and land use types for the Netherlands, will be addressed. The model is developed in a way that the necessary model inputs and parameters for oxytetracycline (*OTC*) and sulfadiazine (*SDZ*) are available and those will be reported on as well. The processes within the blue box in Figure 4 will be discussed. The leaching to groundwater and the overland flow/runoff to surface water will be taken into account, but the actual concentrations in water will not be calculated, since this would require more elaborate data on the hydrology.

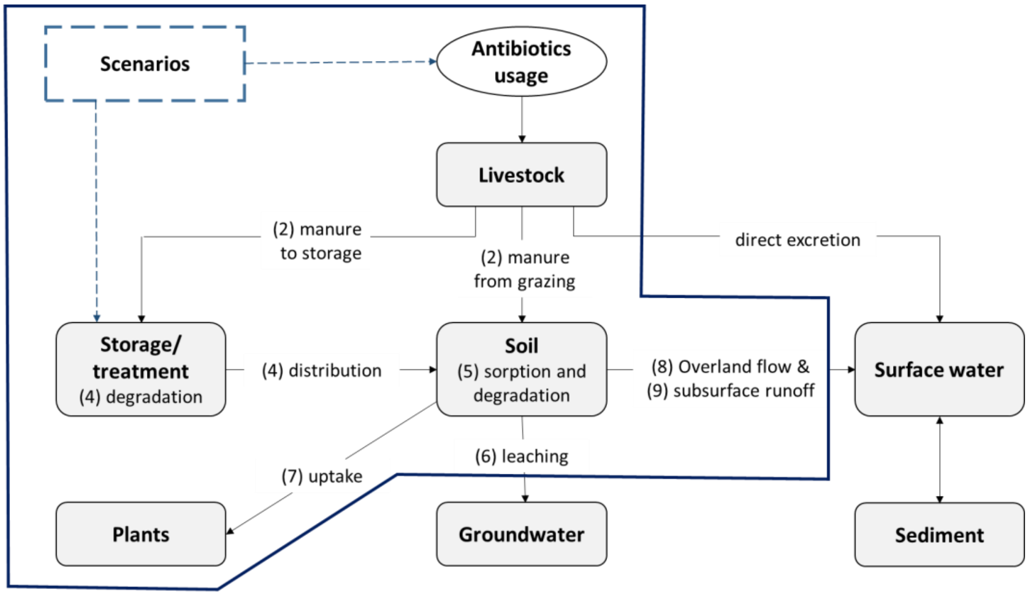


Figure 4 Conceptual model on the fate and transport of antibiotics in agriculture. The blue box represents the compartments and flows addressed in the exploratory model in this Chapter.

The model will be introduced in different sections, starting with the inputs and linking those to the concentrations in manure before and after storage. The application per m^2 is determined from which the concentration in soil and the corresponding concentration in soil solution can be calculated. The concentration in soil changes over time with degradation and losses linked to subsurface runoff, overland flow, leaching to groundwater and uptake by plants. These losses will all be discussed in their separate sections.

5.2 Methods

The results will describe the different formulas and necessary inputs and parameters. Figure 5 shows the different steps in the model with corresponding inputs, which will be discussed in this Chapter. The inputs are further elaborated on in Table 14 and the outputs are described in Table 15. The outputs can be calculated per day after manure application, allowing the possibility to examine how much antibiotics are left between application intervals of manure.

The parameters in the model are subject to some uncertainty, as discussed in Chapters 2 and 5. Therefore, a sensitivity analysis has been done to examine how a change in the input parameters will change the output of the model. The parameters which were be explored are: the sorption coefficient Kd, the half-life in soil (DT50soil), the half-life in manure (DT50manure), the storage time (Tst), the Runoff %, the uptake factor in plants (UF), the fraction lost to groundwater (fgw) and the mixing depth (d), since the literature review pointed out insecurity related to these factors. Half and double of the value were tried, assuming that these are reasonable ranges of the parameter. This is confirmed by the parameter overview in the literature review. The effects on the concentration in soil were examined, since this concentration is the main outcome of the model. The results are shown for OTC on wet sandy grassland.

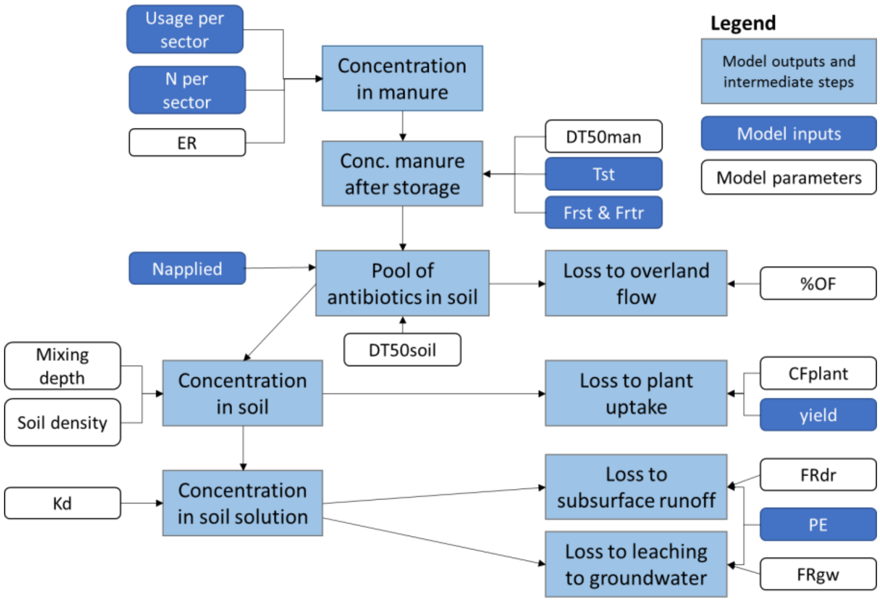


Figure 5 Model of the fate and transport of antibiotics including Model inputs and model outputs/intermediate steps. Abbreviations explained in Table 14.

Table 14 Model inputs and parameters

Input	Explanation	Source	Section
Usage per sector	Total antibiotic usage per sector per year (kg a.s.)	(Veldman et al., 2017)	5.2.1
N per sector	Annual nitrogen production in manure per sector (kg)	CBS (2012)	5.2.2
ER	Excretion rate of antibiotic in manure	Table 16	5.2.2
DT50man	Half-life of antibiotic in manure in storage/treatment (days)	Table 17	5.2.3
Tst	Storage time of manure (days)	Montforts et al. (1999)	5.2.3
Frst, Frtr	Fractions of manure in storage and treatment	Schmitt et al. (2017)	5.2.3
Napplied	Amount of manure applied (in kg nitrogen) on land	INITIATOR (2010)	5.2.4
DT50soil	Half-life of antibiotics in soil (days)	Table 20	5.2.5
Mixing depth	The mixing depth of manure in soil when applied (in m)	Montforts et al. (1999)	5.2.5
Soil density	The dry bulk density of soil (in kg/m^3)	Table 20	5.2.5
Kd	Linear sorption coefficient (kg/L)	Table 22	5.2.6
%OF	The percentage of antibiotics applied lost to overland flow	Table 25	5.2.8
CFplant	The concentration factor of antibiotics in plants, depending on the concentration in soil	Briggs formula	5.2.10
Yield	Yield per land use type (kg/m^2)	CBS (2018)	5.2.10
FRdr, FRgw	Fractions of leaching to surface water and groundwater	INITIATOR (Table 24)	5.2.7/9
PE	Precipitation access (m^3/m^2).	Table 23 (klimaatatlas)	5.2.7/9

Table 15 Model outputs

Output	Explanation	Section
CmanN	Concentration in manure per animal type (mg/kg Nitrogen)	5.2.2
CNstor	Concentration in manure after storage (mg/kg Nitrogen)	5.2.3
Cs	The concentration in soil per soil/land use type (ug/kg)	5.2.5
Css	The concentration in soil solution per soil/land use type (ug/kg)	5.2.6
OF	Overland flow per soil/land use type (ug/m^2)	5.2.8
UP	Plant uptake per soil/land use type (ug/m^2)	5.2.10
RU	Subsurface runoff per soil/land use type (ug/m^2)	5.2.7
LE	Leaching to groundwater per soil/land use type (ug/m^2)	5.2.9

5.3 Result: Model

5.2.1. Determining antibiotic inputs

The first step in determining the concentrations in manure is to establish the antibiotic usage. There is a lack of transparency on the amount of antibiotics applied per agricultural sector (animal type). Data on the Dutch usage of specific antibiotics were last provided in 2012, while information on the usage per antibiotic class is still made available on an annual basis (Veldman et al., 2017).

The fractions of oxytetracycline and sulfadiazine relative to their classes (tetracyclines and sulfonamides) were determined by calculating the average fraction over 2004-2012 for sows/piglets, fattening pigs, broilers and dairy cattle and for 2007-2012 for veal calves, based on the available data (MARAN, 2012). There was no data available for turkeys and other poultry and the assumption was made that the fractions were the same as for broilers. For other cattle, the average value for veal calves and dairy cattle was taken and for pigs, the average over sows/piglets and fattening pigs was determined. With these fractions, the usage per sector in 2016 can be determined by:

$$USE2016s,a = USE2016,s,c \cdot FRa,s \qquad \text{(Eq 5.1)}$$

Where:

USE2016s,a	is the usage in 2016 for sector s and antibiotic a;
USE2016s,c	is the usage in 2016 for sector s and antibiotic class c; and
FRa,s	is the fraction for total class of antibiotic a for sector s.

5.2.2. Concentrations in manure

The concentration in manure is determined per kg Nitrogen, as manure inputs later in the model (5.2.4) are provided in terms of kg N per hectare.

$$CmanN,s,a = \frac{ERs,a \cdot USE2016s,a}{(Nanimals,s \cdot ManureN,s)} \qquad \text{(Eq 5.2)}$$

Where:

CmanN,s,a	is the concentration in manure per kg Nitrogen for sector s and antibiotic a;
Nanimals,s	is the number of animals in sector s in 2016;
ManureN,s	is the excretion of Nitrogen per animal in sector s; and
ERs,a	is the excretion rate after metabolism in an animal for sector a and antibiotic a.

The number of animals is taken from Veldman et al. (2017) and the excretion of Nitrogen in manure from CBS (2012). The excretion rates were determined based on the literature review (Section 2.3.2) (Arikan et al., 2007; Boxall et al., 2002; Kumar et al., 2005; Massé et al., 2014; Zhang et al., 2015). Tetracyclines were found to have a higher excretion than sulfonamides. Pigs were found have the highest excretion, followed by cattle and then poultry. Excretion values from literature (Table 4 and Table S1) were taken and adjusted for antibiotic and animal type based on the relationships found in Table S1 (Table 16).

Table 16 Excretion rates of antibiotics (OTC, SDZ) for different animal types

Sector	**ER *OTC***	**ER *SDZ***
Broilers	0.5	0.3
Turkeys	0.5	0.3
Pigs	0.75	0.5
Dairy cattle	0.65	0.4
Veal calves	0.65	0.4
Other cattle	0.65	0.4
Other poultry	0.5	0.3

5.2.3. Concentrations in manure after storage/treatment

The concentration in manure decreases during storage and treatment, dependent on the half-life (DT50). As described in the literature review, this half-life is dependent on different factors, such as temperature, aerobic/anaerobic conditions and light. The storage time is different for grassland and arable land, because of the timing of application. Equation 5.3 is only applied to manure that will go through storage/treatment, so not for manure directly excreted on grassland through grazing.

$$CNstor,s,a = Cman,s,a \cdot \; e^{-\frac{ln2}{DT50stor,s,a} \cdot Tstor} \cdot Fstor + Cman,s,a \cdot \; e^{-\frac{ln2}{DT50tr,s,a} \cdot Ttr} \cdot Ftr \qquad \text{(Eq 5.3)}$$

Where:

CNstor,s,a	is the concentration in manure per kg Nitrogen after storage for sector s and antibiotic a;
DT50stor,s,a	is the half-life in manure in storage for sector s and antibiotic a;
Tstor	is the storage time of manure;
Fstor	is the fraction of manure in storage;
DT50tr,s,a	is the half-life in manure in treatment for sector a and antibiotic a;
Ttr	is the treatment time of manure; and
Ftr	is the fraction of manure in treatment.

The half-lives for storage and treatment were determined based on the literature review (Section 2.3.3 and Table S2). The values are shown in Table 17, which also describes the way they were selected. Some differences have been observed between different animal types, but data on this is limited and this is not taken into account in the model. In general, tetracyclines have a longer half-life than sulfonamides (Chee-Sanford et al., 2009). The fractions in storage and treatment (Table 18) were determined from RIVM data on Megatons of manure used on own farm, used on other ground and treated (Schmitt, Blaak, et al., 2017).

Table 17 half-lives of antibiotics in manure

Treatment	**Antibiotic**	**$t_{1/2}$**	**Notes**
Storage	*OTC*	30d	Based on aerobic light condition in manure heap of calf manure under ambient temperatures for OTC (De Liguoro et al., 2003)
	SDZ	17d	Based on aerobic dark conditions in cattle manure under 20 degrees for SDZ (Kreuzig & Höltge, 2005)
Composting under higher temperatures	*OTC*	4d	Estimated based on Dolliver et al. (2008)
	SDZ	2d	Estimated based on percentage reduction from Dolliver et al. (2008) and relative to OTC

Table 18 Fractions of manure in storage and treatment

Sector	**Used on own farm (MT)**	**Used on other ground (MT)**	**Total storage (MT)**	**Treatment (MT)**	**Fstorage**	**Ftreatment**
Broilers	0.1	0.05	0.15	0.6	0.2	0.8
Other poultry	0.1	0.05	0.15	0.6	0.2	0.8
Pigs	3.2	6.5	9.7	1.7	0.85	0.15
Dairy cattle	37.3	6.9	44.2	1.3	0.97	0.03
Beef cattle/ veal calves	3.1	0.8	3.9	0.9	0.81	0.19
Other cattle	0.3	0.6	0.9	0.6	0.6	0.4

5.2.4. Antibiotics applied

The model will look at three different soil types (sand, clay and peat) and three different land use types (grassland, maize and other arable land). Data from INITIATOR (2010) are available on the kilograms of Nitrogen applied per hectare for all nine combinations of these soil and land use types. The data distinguishes between manure from stables, manure from grazing (pastures), pig manure and poultry manure. Stable manure is assumed to be cattle manure from veal calves, dairy cattle and other cattle and pasture manure is assumed to be cattle manure, but not from veal calves since these barely graze (Montforts, 1999). The fractions of manure are calculated as a fraction of the total animal numbers (Table 19). The fractions for cattle are different for stable and pasture manure, because veal calves do not graze.

Table 19 fraction of animals per group

Animal (group)	**Fraction of total group**	**Fraction for grazing**	**Total animal numbers x1000** (Veldman et al., 2017)
Broilers (poultry)	0.46		48378
Turkeys (poultry)	0.01		762
Pigs	1.00		11881
Dairy cattle (cattle)	0.29	0.35	1794
Veal calves (cattle)	0.16		956
Other cattle (cattle)	0.55	0.65	3353
Other poultry (poultry)	0.54		57172

Equations 5.4-5.7 will calculate the application of antibiotics per hectare separately for the different types of manure. The amount of manure (Nman,so,lu) is the data from INITIATOR (2010).

$$ApplStable,so,lu,a = Nman,stable,so,lu \cdot (FRveal \cdot\ CNstor,veal,a + FRdairy \cdot CNstor,dairy,a + FRothc \cdot CNstor,othc,a) \quad \text{(Eq 5.4)}$$

Where:

ApplStable,so,lu,a	is the application of antibiotics from stable manure (in kg active substance/hectare) for soil type so, land use type lu and antibiotic a;
Nman,stable,so,lu	is the amount of stable manure applied (in kgN/hectare) for soil type so and land use type lu;
FRveal	is the fraction of manure from veal calves;
CNstor,veal,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for veal calves;
FRdairy	is the fraction of manure from dairy cattle;
CNstor,dairy,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for dairy cattle;
FRothc	is the fraction of manure from other cattle; and
CN,stor,othc,a	is the concentration of antibiotic a in manure after storage/treatment in kg a.s/kgN) for other cattle.

$$ApplPig,so,lu,a = Nman,so,lu \cdot\ CNstor,pig,a \quad \text{(Eq 5.5)}$$

Where:

ApplStable,so,lu,a	is the application of antibiotics from pig manure (in kg active substance/hectare) for soil type so, land use type lu and antibiotic a;
Nman,stable,so,lu	is the amount of pig manure applied (in kgN/hectare) for soil type so and land use type lu; and
CNstor, pig,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for pigs;

$$ApplPoultry, so, lu, = Nman, poultry, so, lu \cdot (FRbro * \ CNstor, bro, a + FRtur \cdot CNstor, tur, a + FRoth \cdot CNstor, othp, a) \quad \text{(Eq 5.6)}$$

Where:

ApplPoultry,so,lu,a	is the application of antibiotics from poultry manure (in kg active substance/hectare) for soil type so, land use type lu and antibiotic a;
Nman,poultru,so,lu	is the amount of poultry manure applied (in kgN/hectare) for soil type so and land use type lu;
FRbro	is the fraction of manure from broilers;
CNstor,bro,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for broilers;
FRtur	is the fraction of manure from turkey;
CNstor,tur,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for turkey;
FRothp	is the fraction of manure from other poultry; and
CN,stor,othp,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for other poultry.

$$ApplPast, so, lu, a = Nman, past, so, lu \cdot (FRdairy \cdot \ CNstor, dairy, a + FRothc \cdot CNstor, othc, a) \quad \text{(Eq 5.7)}$$

Where:

ApplPast,so,lu.a	is the application of antibiotics from pasture manure (in kg active substance/hectare) for soil type so, land use type lu and antibiotic a;
Nman,stable,so,lu	is the amount of pasture manure applied (in kgN/hectare) for soil type so and land use type lu;
FRdairy	is the fraction of manure from dairy cattle;
CNstor,dairy,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for dairy cattle;
FRothc	is the fraction of manure from other cattle; and
CNstor,othc,a	is the concentration of antibiotic a in manure after storage/treatment (in kg a.s/kgN) for other cattle;

Equations 5.4-5.7 express the separate contributions of the different types of manure. These are combined in Equation 5.8 to get to the total amount of antibiotics applied. The number of applications differs for grassland and arable land. For grassland, there are four applications with an interval between applications of about 71 days, while there is only one application per year for arable land (Montforts, 1999).

$$ApplAnt, a, so, lu = \frac{(ApplStable, so, lu, a + ApplPig, so, lu, a + \ ApplPoultry, so, lu, a + \ ApplPast, so, lu, a)}{Napplications \cdot 10000} \quad \text{(Eq 5.8)}$$

Where:

ApplAnt,a,so,lu	is the application of antibiotic a (in kg active substance/m^2) for soil type so, land use type lu;
N applications	is the number of applications per year.

5.2.5 Concentration in soil

The initial concentration is calculated based on the application of antibiotics (Eq. 5.9). The mixing depth is based on information and data on agricultural practices. The mixing depth is 0.05 m for grassland and 0.2 m for arable land (maize and other arable) (Montforts, 1999). The bulk densities also differ slightly per soil type. The high organic matter content of peat decreases the bulk density (Hossain et al., 2015).

$$CIsoil, so, lu \ = \frac{ApplAnt, a, so, lu}{\rho soil \cdot d, lu} \quad \text{(Eq 5.9)}$$

Where:

CIsoil,a,so,lu	is the initial concentration in soil for antibiotic a, soil type so and land use type lu (in ug/kg);
ρsoil,so	is the dry bulk density of soil for soil type so (in kg/m^3); and
d,lu	is the mixing depth for land use type lu (in m).

Table 20 bulk densities per soil type

	Bulk density (kg/m^3)	**Source**
Sand	1.5	Yu et al. (2015)
Peat	0.6	Hossain et al. (2015)
Clay	1.4	Yu et al. (2015)

This concentration decreases because of runoff, overland flow, leaching to groundwater and uptake in plants (Eq. 5.10). Furthermore, degradation of antibiotics in soil occurs and this also affects the total amount. The model assumes first-order degradation kinetics. Equation 5.10 describes the amount of antibiotics in soil over time. This can be converted to the concentration in soil with Equation 5.11. The losses through subsurface runoff, overland flow, leaching and uptake by plants will be discussed in their respective sections following this section.

$$Apool,a,so,lu(t+1) = Apool,a,so,lu(t) \cdot e^{-\frac{ln2}{DT50soil,a}} - RUa,so,lu(t) - OFa,so,lu(t) - LEa,so,lu(t) - UPsa,so,lu(t) \quad \text{(Eq 5.10)}$$

Where:

Apool,a,so,lu(t)	is the total amount of antibiotics present in the soil at time t for antibiotic a, soil type so and land use type lu (in ug/m^2);
DT50soil,a,so	is the half-time in soil for antibiotic a and soil type so(in days);
RUso,lu	is the loss due to runoff for antibiotic a, soil type so and land use type lu (in ug/m^2);
OFso,lu	is the loss due to overland flow for antibiotic a, soil type so and land use type lu (in ug/m^2);
LEso,lu	is the loss due to leaching for antibiotic a, soil type so and land use type lu (in ug/m^2); and
UPso,lu	is the loss due to uptake by plants for antibiotic a, soil type so and land use type lu (in ug/m^2).

$$Csoil,a,so,lu(t) = \frac{Apool(t)}{\rho soil \cdot d,lu} \quad \text{(Eq 5.11)}$$

Where:

Csoil,a,so,lu(t)	is the concentration in soil at time t for antibiotic a, soil type so and land use type lu (in ug/kg).

The half-time is dependent on soil characteristics, as discussed in Chapter 2 (section 5). The multiple linear regression model (Chapter 4) for sulfonamides showed the dependency on the initial concentration, the organic carbon content, the percentage clay, the application of manure, sterilized/non-sterilized and aerobic/anaerobic. The conditions are assumed to be aerobic and non-sterilized. The DT50 values (Table 21) are estimated based on Table S5 and adjusted based the relationships as shown with the regression model. Peat is likely to have a lower DT50, due to the high OM content and clay will have a slightly higher DT50, because of the higher sorption.

Table 21 Half-time in soil (in days)

	DT50 soil (d)	
	OTC	***SDZ***
Sand	20	10
Peat	15	7.5
Clay	25	12.5

5.2.6 Concentration in soil solution

The concentration in soil solution is dependent on the sorption to soil, which can be expressed by different models. The literature review showed that Freundlich and Henry's linear model both seemed suitable to model the sorption and to determine the concentration in the soil solution (Białk-Bielińska et al., 2012; Maszkowska et al., 2015; Park & Huwe, 2016; Zhang et al., 2014) . In this model, the linear model is used (Eq. 5.12).

$$Css,a,so,lu(t) = \frac{Csoil,a,so,lu(t)}{Kd} \quad \text{(Eq 5.12)}$$

Where:

Css,a,so,lu(t)	is the concentration in soil solution for antibiotic a, soil type so, land use type lu at time t (in ug/L); and
Kd	is the linear adsorption constant (in L/kg).

The Kd can depend on a variety of factors, such as soil type, soil organic matter or carbon content, soil pH and the cation exchange capacity (Section 2.3.5). A multiple linear regression was done in Chapter 4 to determine the influence on these different factors on the adsorption, but due to limited data availability this cannot be determined for all antibiotics and an estimation has to be made for the input values for Kd, based on the characteristics of the soil. The Kd values for *OTC* were estimated based on a calculation with the regression model. (Table 22) The Kd values for *SDZ* are taken from S3 and adjusted based on soil characteristics (Table 22).

Table 22 Linear sorption coefficients (in L/kg)

	Kd *OTC*	**Kd *SDZ***
Sand	2500	0.5
Peat	9000	2.5
Clay	3000	1.2

5.2.7 Subsurface runoff

Subsurface runoff is dependent on the concentration in soil solution and the amount of water transported, depending on for example soil type and precipitation excess. Precipitation was determined based on climate data for the Netherlands (Table 23) (KNMI, n.d.). The fraction of subsurface runoff to surface water is taken from INITIATOR (2010) and differs for the different soil and land use types (Table 24). The subsurface runoff is significantly lower for sand, because of the higher infiltration in sand.

$$RUa,so,lu(t) = PE \cdot Css,a,so,lu(t) \cdot frdr \quad \text{(Eq 5.14)}$$

Where:

RUa,so,lu(t)	is the loss due to runoff at time t for antibiotic a, soil type so and land use type lu;
frdr	is the fraction of the soil solution that will leach away to surface water; and
PE	Precipitation access (in m^3/m^2).

Table 23 Precipitation access under wet (max) and dry (min) conditions for different areas in the Netherlands.

		PE (mm/month)	**PE ($m^3/m^2/day$)**
NB (sand)	Dry	47.2	0.0015
	Wet	78.5	0.0026
ZH (peat)	Dry	45.4	0.0015
	Wet	96.2	0.0032
FL (clay)	Dry	46.1	0.0015
	Wet	85.5	0.0029

Table 24 Fractions leaching to ground and surface water (INITIATOR, 2010)

Soil type	**Land use type**	**Fraction leaching to groundwater**	**Fraction subsurface runoff (frdr)**
Sand	Grass	0.818	0.182
	Maize	0.791	0.209
	Other arable	0.605	0.395
Peat	Grass	0.095	0.905
	Maize	0.195	0.805
	Other arable	0.118	0.882
Clay	Grass	0.101	0.899
	Maize	0.312	0.688
	Other arable	0.078	0.922

5.2.8 Overland flow

Overland flow is calculated based on a percentage of the total amount of antibiotics applied with manure. This percentage (Table 25) is estimated based on the literature review (Table 5, Section 2.3.8). It is higher for *SDZ* than for *OTC* due to the lower sorption to manure. Furthermore, the overland flow is higher for grassland than for arable land since manure is more incorporated for arable land (Montforts, 1999). The model assumes that overland flow occurs for the first four days after application, after which it is set to 0. Two different weather conditions are explored: a drier and a wetter condition, affecting the fraction lost to overland flow.

$$\mathrm{OFa, so, lu(t)} = ApplAnt, a, so, lu \cdot (FRof, a, so/4) \quad \text{(Eq 5.15)}$$

Where:

OFa,so,lu(t) is the loss due to overland flow at time t (for t=1,2,3 and 4) for antibiotic a, soil type so and land use type lu (in ug/m^2); and

FRof,a,so is the fraction of antibiotics applied in manure lost to overland flow.

Table 25 Fraction of manure applied lost by overland flow for antibiotic OTC and SDZ under dry and wet conditions.

	***OTC* wet**	***OTC* dry**	***SDZ* wet**	***SDZ* dry**
Grassland	0.01	0.005	0.1	0.05
Arable land	0.005	0.0025	0.05	0.025

5.2.9 Leaching to groundwater

Similar to runoff through drainage, leaching to groundwater depends on the concentration in soil solution and the precipitation access. The fraction of this leaching to groundwater is taken from Table 24 (INITIATOR, 2010).

$$\mathrm{LEa, so, lu(t)} = Css, a, so, lu(t) \cdot PE \cdot frgw \quad \text{(Eq 5.16)}$$

Where:

LEa,so,lu(t)	is the loss due to leaching at time t for antibiotic a, soil type so and land use type lu (in ug/m^2); and
frgw	is the fraction of the soil solution that will leach away to groundwater.

5.2.10 Uptake in plants

The uptake in plant is calculated with the concentration in plants and the yield (Eq. 5.19). The concentration in plants is determined based on the concentration in soil and the concentration factor (Eq. 5.17) calculated with a formula as given by Briggs (Hu et al., 2010; Wang et al., 2014). (Eq. 5.18). The yield is based on Dutch statistics data (CBS, 2018). The Kow is determined from literature (Chee-Sanford et al., 2009; Domínguez et al., 2014; Zhang et al., 2017). Due to limited data availability, a similar concentration factor is assumed for all soil types and Briggs constants are not specified for specific plants. The concentration factor is constructed for a longer time period. To get to uptake values per day, the concentration per plant is divided by the time between applications (Eq. 5.18).

$$CFplant, a = A \cdot logKow, a - B \qquad \text{(Eq 5.17)}$$

Where:

CFplant,a	is the concentration factor in plants for antibiotic a (in ug(plant)/ ug(soil))
A and B	are constants in Briggs formula (A=0.6, B=1.55); and
Kow	is the octanol/water partition coefficient.

$$Cplant, a, so, lu(t) = (CFplant, a \cdot \ CIsoil, so, lu(t))/tmax \qquad \text{(Eq 5.18)}$$

Where:

Cplant,a,so,lu(t)	is the concentration in plants at time t for antibiotic a, soil type so and land use type lu (in ug/kg).

$$Equation\ 4.20{:}\, UPa, so, lu = yield, lu \cdot Cplant, a, so, lu(t) \qquad \text{(Eq 5.19)}$$

Where:

UPa,so,lu(t)	is the loss due to uptake by plants for antibiotic a, soil type so and land use type lu (in ug/m^2); and
Yield,lu	is the crop yield for land use type lu (in kg/m^2);

5.2.11 Sensitivity Analysis

The results of the sensitivity analysis are shown in Figure 6. Doubling the half-life in soil resulted in an increase in the concentration in soil with a factor of 2.36. The half-life in manure, the storage time and the mixing depth were also found to have a significant effect on the concentration in soil. These effects were very limited for the Kd, the uptake factor in plants, the overland flow percentage and the fraction leaching to groundwater.

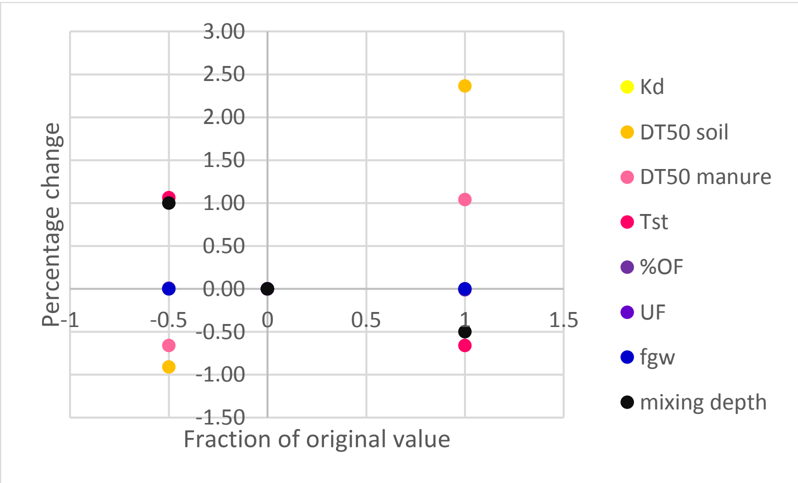


Figure 6 Sensitivity analysis of model parameters on wet sandy grassland

5.3 Discussion and conclusion

Sensitivity analysis

The sensitivity analysis indicated that a change in the half-life in soil had the largest impact on the concentration in soil, followed by the half-life in manure, the storage time and the mixing depth. The half-life in soil is most directly related to the concentration in soil with exponential decay, explaining the high impact. These are all factors that are subject to uncertainty, indicating that selecting appropriate values for these parameters is important. A concentration in soil 250% higher would still not be a problem in this case, as the value would still stay far below the risk value of 100 ug/kg. The Kd, the runoff percentage, the uptake factor in plants and the fraction leaching to groundwater were found to have almost no effect on the concentration in soil. A change in these parameters is likely to have a more significant effect on concentrations in water and plants, however, which should be taken into account when those concentrations are of interest.

Model assumptions and uncertainties

Some assumptions that could affect the outcome of the model, were made. Equilibrium sorption was assumed and this leads to a slightly higher concentration in soil solution than non-equilibrium. The effect on the concentration in soil is likely to be limited, but this could have an effect on the amount of antibiotics leaching to ground and surface waters. Furthermore, transformation products were not accounted for, while some are known to also be toxic. Future research should focus on these toxic transformation products. Ionization of antibiotics was also not included and this could further affect sorption. The effect of the pH was considered in the estimate of the Kd, however, which already partly accounts for this. An important assumption for the sorption is the homogeneity of the soil; the soil is likely to differ in for example organic matter and clay content and this affects the sorption. This assumption is more relevant when the model is translated into a spatial model. The scale will have to be chosen with the model application in mind.

The effect of the way manure is applied (broadcasting/injection/incorporation) is only accounted for with the mixing depth and a change in the percentage overland flow; this is a rough estimate of the impact and the model could be improved to further take these management options into account. Overland flow is only assumed for 4 days. Some studies show that the process occurs for more days, but the %OF is estimated based on those longer-term studies, so this won't have a large effect on the total amount of antibiotic leaching, only to the concentration at a certain day. Another uncertainty is related to the assumption that daily precipitation is the monthly precipitation divided by the number of days in that month. Peak precipitation is likely to results in higher overland flow and leaching. This was partly resolved by the inclusion of wetter conditions, but these are still averages over a wetter month. The model does not assume for leaching during storage; this could affect the concentration in manure after storage and the concentration in the soil and water close to storage facilities. More studies could focus on the effects of this on groundwater and manure concentrations to see whether these processes will have a significant effect on model outcomes.

The amount of antibiotics was estimated based on a percentage of the total class determined before 2012. The model assumes that this percentage is also representative for the current situation. The uptake in plants in based on Briggs model, which is dependent on the Kow, but this Kow was not specified per soil type. I assumed that this simple model is sufficient, but other soil-plant models should be explored to further establish this relationship. The other parameters also have their own uncertainties: the literature review (Chapter 2) found ranges in for example the Kd and the half-lives, which was examined in the sensitivity analysis. The half-life in manure for treatment was assumed to be only for composting, but different types of treatment have different half-lives.

Chapter 6: Model application

6.1 Introduction

In this chapter, the model from Chapter 5 will be applied for the Netherlands. The antibiotics taken into account are sulfadiazine (*SDZ*) and oxytetracycline (*OTC*). Apart from reporting on the current situation, several scenarios will be explored and a sensitivity analysis will be conducted to examine the effect of uncertainties in the parameters.

6.2 Method

6.2.1 Model application: scenario analysis

To test the model and to get a better understanding of the situation in the Netherlands, different scenarios are explored, which are discussed in Table 26. The first scenario looks at the current situation in the Netherlands, with the antibiotics usage in 2016. The concentrations in manure in this scenario are calculated as an average over the year based on the yearly use per sector. However, the concentration in manure could be higher if it comes from a group of animals that are all treated with antibiotics. For that reason the second and third scenario examine an increased risk, by assuming that all manure applied comes from veal calves, which have the highest concentration of antibiotics in manure. The third scenario further increases this risk by shortening the storage time to 10 days, which will result in higher concentrations of manure being applied to land. Scenario 4 explores the effect of increased treatment of manure by assuming that half of the manure from veal calves and pigs will be treated.

Table 26 Scenarios explored in this Chapter

Scenarios	Description	Change in parameters
1) Current situation in the Netherlands	Current situation, utilizing the parameters from Chapter 5	-
2) Increased risk	Only manure from veal calves	CNstor only for veal calves (highest value)
3) Further increased risk	Only manure from veal calves, shorter storage	CNstor = only for veal calves (highest value) Tst = 10 days, was 71 (grassland) and 152 (arable land)
4) Increased treatment	Increase in fraction of treatment of veal calves and pigs	Ftr = 0.5 for veal calves and pigs, was 0.15 (pig) and 0.19 (veal calves)

6.2.2 Risk Analysis

The concentrations in soil and soil solution will be analysed on their risk. The EMA (2016) base their risk assessment on a threshold value of 100 µg/kg soil. If the concentration in soil is higher than this value, further risk assessment is deemed necessary. The model results will be compared against this value, but some values from literature are also taken into account (Table 27). The lowest value found for concentration in water was for the development of resistance (0.5 ug/L) for *OTC* (Bengtsson-Palme & Larsson, 2016). Values for risk to organisms are significantly higher, with most in concentrations in mg/L or mg/kg. No value was found which discussed the development of resistance for *SDZ*, but the value for sulfamethoxazole, another sulfonamide, is 16 ug/L (Bengtsson-Palme & Larsson, 2016).

Table 27 Risk analysis values for oxytetracycline (OTC) and sulfadiazine (SDZ)

Compound	Affected	Effect	Concentration	Source
OTC	Biomass	85% inhibition	10mg/L	Thiele-Bruhn (2003)
OTC	Biomass in sandy loam	56% inhibition	160ug/g	Thiele-Bruhn (2003)
OTC	Bacteria in sand soil	71%	10ug/g	Thiele-Bruhn (2003)
OTC	Silty sand/loamy sand	ED10 SIT	0.81-31.2ug/g	Thiele-Bruhn (2003)
OTC	Springtails, earthworms, enchytreids	LC10/EC10	>5000ug/g	Thiele-Bruhn (2003)
SDZ	- (Soil)	NOEC	60mg/L	Thiele-Bruhn (2003)

OTC	- (Soil)	EC50	1.2 mg/L	Thiele-Bruhn (2003)
SDZ	- (Soil)	EC50 0/10h	0.12/0.27mg/L	Thiele-Bruhn (2003)
OTC	- (Water)	PNEC resistance	0.5 ug/L	Bengtsson-Palme & Larsson (2016)
OTC	- (Water)	Size adjusted lowest NOEC	4 ug/L	Bengtsson-Palme & Larsson (2016)
SDZ	Daphnia Magna	PNEC	10ug/L	Nguyen et al. (2015)

6.3 Results

6.3.1 Usage

Table 28 shows the usage of antibiotics in the Netherlands in 2016. For both *SDZ* and *OTC*, pigs and veal calves are the sectors with the highest usage and there is an overall higher usage of *OTC* than *SDZ*.

Table 28 Usage of OTC and SDZ per section in the Netherland in 2016 (in kg active substance)

	broilers	**turkeys**	**pigs**	**dairy cattle**	**veal calves**	**cattle**	**Other poultry**
OTC	198.4	162.3	16755	1707	17798	3270	145.8
SDZ	0.2	0.0	8867	2458	8673	1235	0.0

6.3.2 Concentrations in manure

Before storage

The concentrations per kg nitrogen are shown in Table 29. Veal calves had the highest usage and a relatively small number of animals has resulted in the highest concentration in manure. Pigs, which had a similar usage per sector, also show high concentrations, but lower than for veal calves because of the higher number of animals per sector.

Table 29 Concentration of OTC and SDZ in manure before storage (in mg/kgN)

	broilers	**turkeys**	**pigs**	**dairy cattle**	**veal calves**	**cattle**	**Other poultry**
OTC	4.10	62.7	70.5	6.18	605	10.6	1.59
SDZ	0.00	0.00	24.9	5.48	181	2.46	0.00

After storage

Storage of an average of 71 days for manure to be spread on grassland and 152 days for manure on arable land has significantly decreased the concentrations (Table 30). The higher storage time for arable land has led to clearly lower concentrations in the manure.

Table 30 Concentrations of OTC and SDZ in manure after storage (in mg/kgN)

	broilers	**turkeys**	**pigs**	**dairy cattle**	**veal calves**	**cattle**	**Other poultry**
OTC arable land	0.024	0.374	1.79	0.179	14.6	0.189	0.010
OTC grassland	0.159	2.43	11.6	1.16	95.0	1.23	0.062
SDZ arable land	0.000	0.000	0.043	0.011	0.299	0.003	0.000
SDZ grassland	0.000	0.000	1.17	0.294	8.13	0.082	0.000

6.3.3 Concentrations in soil

Wet/dry

The initial concentrations in soil were calculated based on the application of manure and the concentrations in manure. Wet and dry conditions were both compared, since wet conditions were expected to result in higher losses related to overland flow, subsurface runoff and leaching to groundwater. Figure 7 looks at the initial concentrations and shows limited differences between wet and dry conditions, indicating that losses to water have a small effect on the concentration in soil. The higher concentration of *OTC* in manure has also resulted in higher concentrations in soil. Furthermore, the elevated concentrations in grassland can be explained by the shorter storage and the contribution of grazing, while the higher concentrations in peat are linked to the lower density of soil.

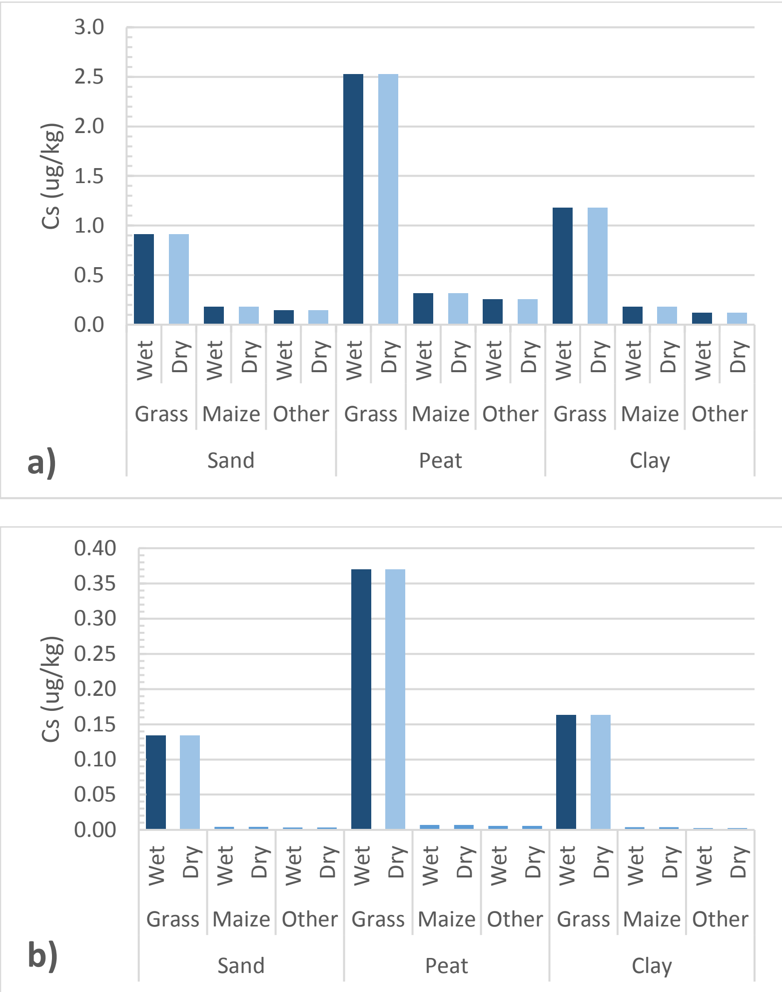


Figure 7 Initial concentrations of a) OTC and b) SDZ in soil

Initial vs end

The rest of the figures will only look at the situation under wet conditions, since there is little impact on the concentration in soil and this gives more insight on leaching and runoff. Figure 8 compares the initial concentration in soil with the concentration at the end of an application interval, just before a new application of manure. The residue in soil seems negligible for *SDZ*, while *OTC* shows some risk of accumulation in soil, mostly on grassland (Figure 8).

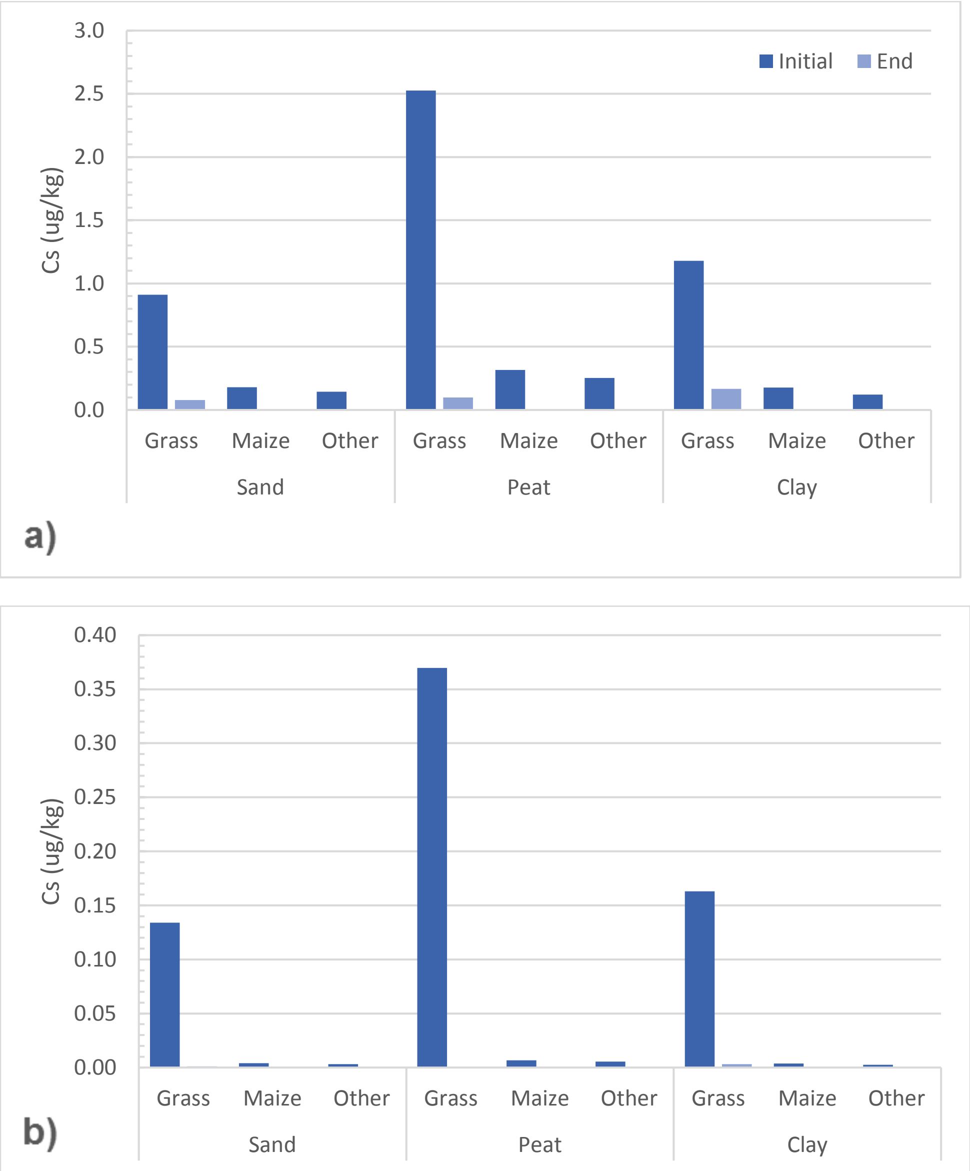


Figure 8 Initial concentrations and concentrations after application interval (71 d for grassland and 365 d for arable land) for a) OTC and b) SDZ in soil

6.3.4 Concentrations in soil solution

For *OTC* all concentrations in soil solution were lower than 1 ng/L, even at time of application of manure. *SDZ* has a lower sorption capacity, however, resulting in higher concentrations in soil solution, as shown in Figure 9. By the time that a new manure application starts, the concentrations are negligible.

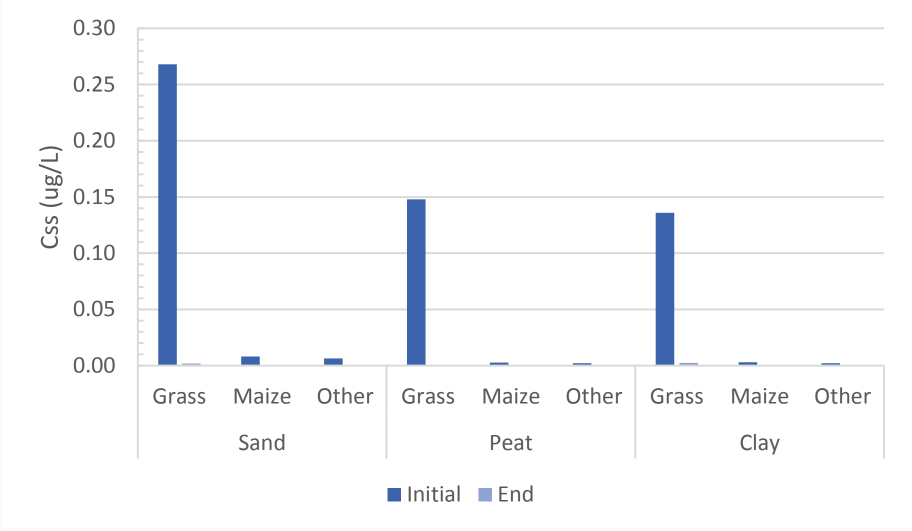
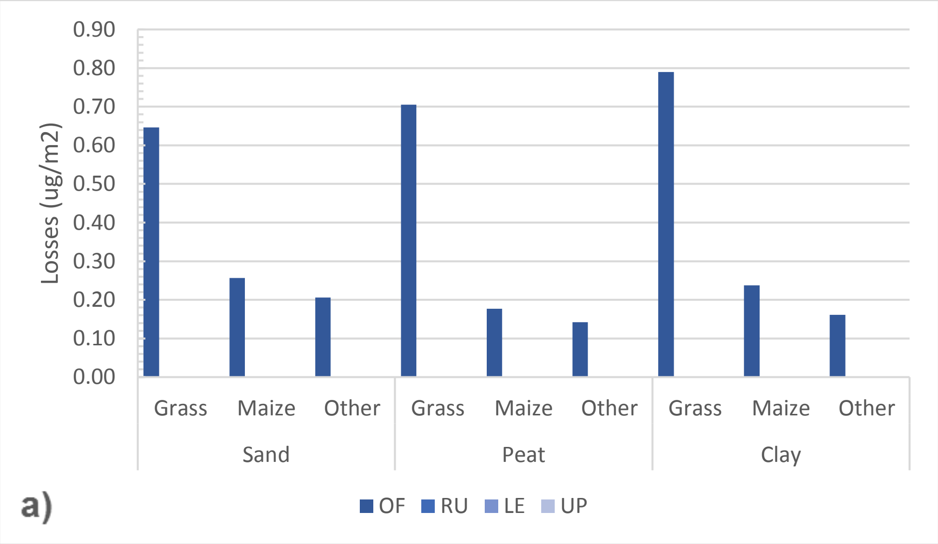


Figure 9 Concentrations of SDZ in soil solution at time of application (initial) and time after the application interval (end) (71 d for grassland and 365 d for arable land)

6.3.5 Other losses

Total losses

Figure 10 shows the losses of antibiotics to overland flow (OF), subsurface runoff (RU), leaching to groundwater (LE) and uptake by plants (UP). Overland flow is the most important source of loss from soil, likely to contribute most to the concentrations in surface water. Leaching and uptake by plants cannot be ignored, however, since these could still lead to environmentally relevant concentrations in groundwater and plants. Leaching to groundwater was only found for *SDZ* in low concentrations in sandy grassland (5-8 ng/m^2) or clay grassland under wet conditions (1ng/m^2). Uptake in plants resulted in concentration in ng/kg, with higher concentrations for *SDZ*.



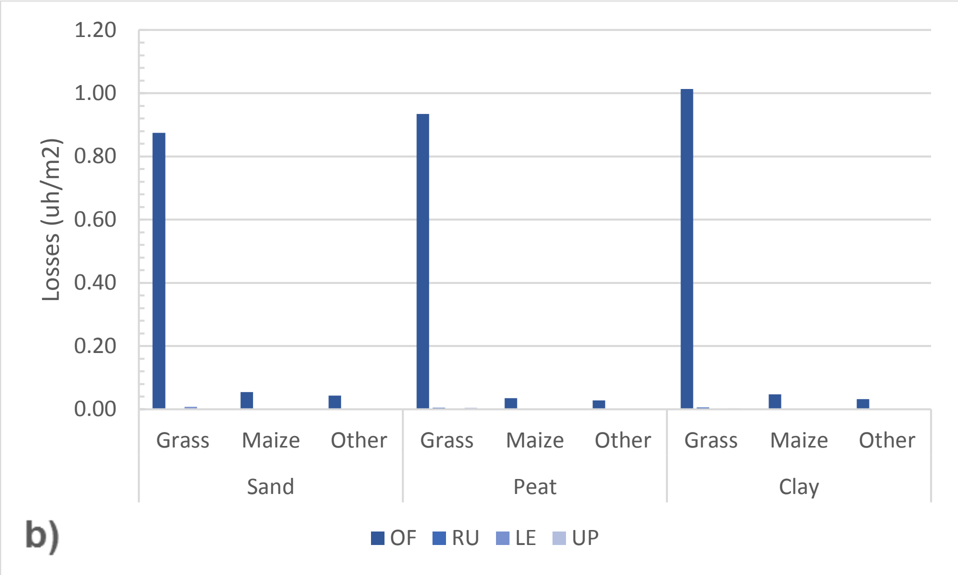


Figure 10 Total losses of a) OTC, b) SDZ from soil to overland flow (OF), subsurface runoff (RU), leaching to groundwater (LE) and uptake by plants (UP)

6.3.6 Scenario 2: Increased risk: only manure from veal calves

The environmental concentrations in soil and water under the model considering the current situation were below the risk value of 100 ug/kg for soil concentrations and even below the PNECs for resistance selection in water of 0.5 and 16 ug/L for *OTC* and *SDZ* respectively. Therefore, scenario 2 explored the impact of only applying the high concentration manure from veal calves. The results are shown in Tables 31 and 32 and Figure 11 compares the *OTC* values for grassland with the current situation. The figures of the scenarios only show the soil concentrations of OTC on grassland, since these were found to be the highest concentrations, closest to the risk threshold values. The Tables do not show values for other arable land, because there were limited differences with maize. The concentrations are clearly higher under scenario 2, but they are still below the values as formerly identified as risk (Table 31/32).

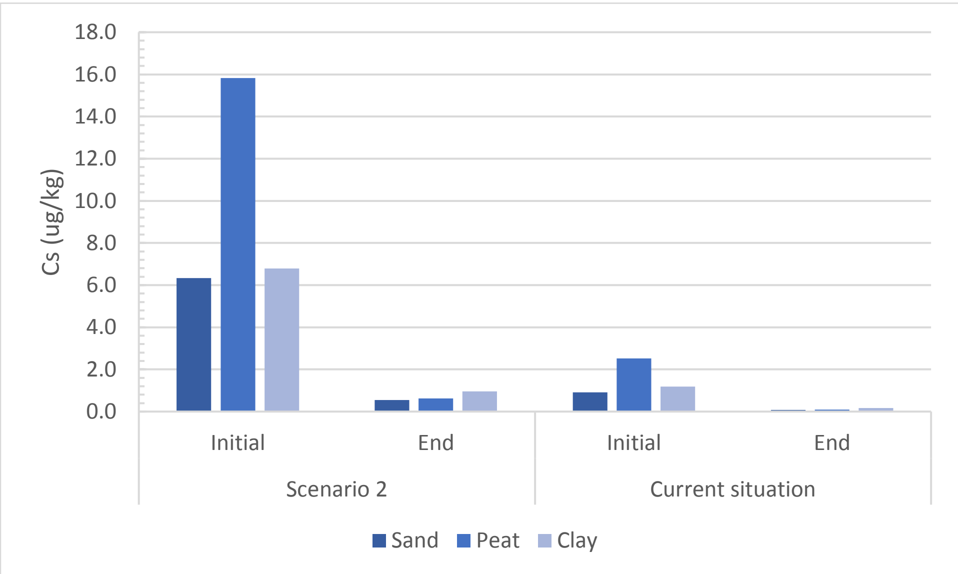


Figure 11 Initial and end concentrations in soil of OTC on grassland, comparing the current situation with scenario 2

Table 31 Initial and end concentrations of OTC under scenario 1. Cs=concentration in soil, Css=concentration in soil solution, OF=overland flow, RU=subsurface runoff, LE=leaching to groundwater, UP=uptake by plants.

		Cs (ug/kg)		Css (ug/L)		OF (ug)	RU (ug)	LE (ug)	UP (ug)
		Initial	End	Initial	End	Sum	Sum	Sum	Sum
	Sand	6.33	0.55	0.00	0.00	4.50	0.00	0.00	0.00
	Peat	15.8	0.62	0.00	0.00	4.42	0.00	0.00	0.01
Grass	Clay	6.79	0.96	0.00	0.00	4.54	0.00	0.00	0.01
	Sand	0.97	0.00	0.00	0.00	1.38	0.00	0.00	0.00
	Peat	2.43	0.00	0.00	0.00	1.36	0.00	0.00	0.00
Maize	Clay	1.04	0.00	0.00	0.00	1.40	0.00	0.00	0.00

Table 32 Initial and end concentrations of SDZ under scenario 1. See explanation Table 31.

		Cs (ug/kg)		Css (ug/L)		OF (ug)	RU (ug)	LE (ug)	UP (ug)
		Initial	End	Initial	End	Sum	Sum	Sum	Sum
	Sand	0.54	0.00	1.08	0.01	3.52	0.01	0.03	0.01
	Peat	1.35	0.00	0.54	0.00	3.41	0.02	0.00	0.02
Grass	Clay	0.58	0.01	0.48	0.01	3.59	0.02	0.00	0.01
	Sand	1.00	0.00	2.00	0.00	13.30	0.02	0.06	0.01
	Peat	2.50	0.00	1.00	0.00	12.88	0.03	0.01	0.01
Maize	Clay	1.07	0.00	0.89	0.00	13.57	0.03	0.01	0.01

6.3.7 Scenario 3: Further increased risk: only manure from veal calves under shorter storage

Scenario 3 further increases the risk by shortening the storage time to 10 days. The concentrations are significantly higher, but concentration in soil are still not reaching the risk values of 100 ug/kg, although this value is close for the initial concentration of *OTC* in peaty grassland (Figure 12). The concentrations in soil solution are also approaching the 0.5 and 16 ug/L, but are still below these PNECs (Table 33/34).

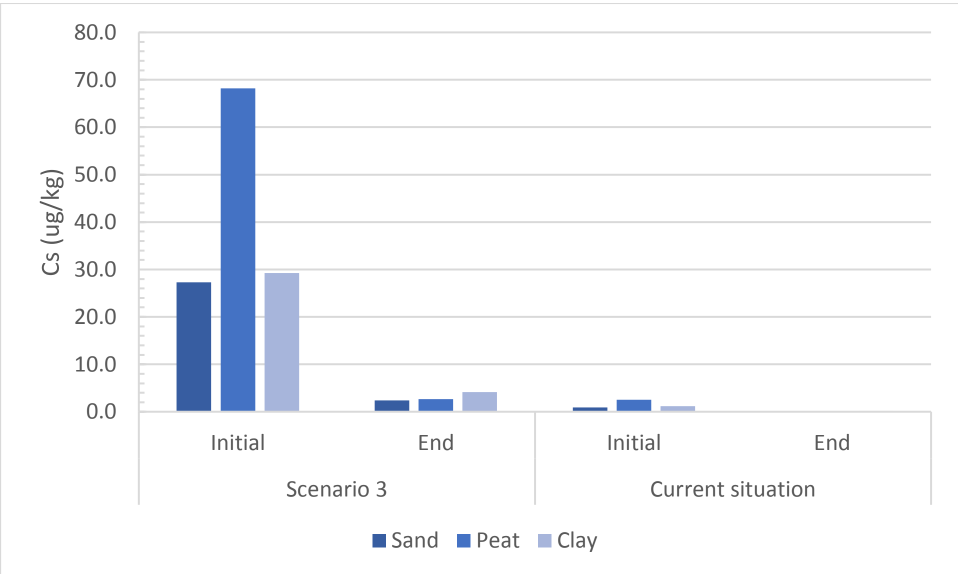


Figure 12 Initial and end concentrations in soil of OTC on grassland, comparing the current situation with scenario 3

Table 33 Initial and end concentrations of OTC under scenario 2. See explanation Table 31.

		Cs (ug/kg)		Css (ug/L)		OF (ug)	RU (ug)	LE (ug)	UP (ug)
		Initial	End	Initial	End	Sum	Sum	Sum	Sum
	Sand	27.3	2.39	0.011	0.001	19.4	0.000	0.001	0.018
	Peat	68.2	2.66	0.008	0.000	19.1	0.000	0.000	0.036
Grass	Clay	29.2	4.15	0.010	0.001	19.6	0.001	0.000	0.023
	Sand	27.3	0.000	0.011	0.000	38.8	0.000	0.001	0.008
	Peat	68.2	0.000	0.008	0.000	38.2	0.000	0.000	0.015
Maize	Clay	29.2	0.001	0.010	0.000	39.2	0.001	0.000	0.010

Table 34 Initial and end concentrations of SDZ under scenario 2. See explanation Table 31.

		Cs (ug/kg)		Css (ug/L)		OF (ug)	RU (ug)	LE (ug)	UP (ug)
		Initial	End	Initial	End	Sum	Sum	Sum	Sum
	Sand	6.52	0.045	13.0	0.091	42.6	0.083	0.375	0.106
	Peat	16.3	0.022	6.52	0.009	41.2	0.195	0.021	0.202
Grass	Clay	6.99	0.129	5.82	0.107	43.4	0.251	0.028	0.139
	Sand	6.52	0.000	13.0	0.000	86.7	0.101	0.383	0.043
	Peat	16.3	0.000	6.52	0.000	84.0	0.182	0.044	0.082
Maize	Clay	6.99	0.000	5.82	0.000	88.5	0.206	0.093	0.058

6.3.8 Scenario 4: Increased treatment

Scenario 4 explores the impact of an increased fraction of pig and veal calf manure in treatment. Figure 13 illustrates that the concentration in soil slightly decreases under increased treatment.

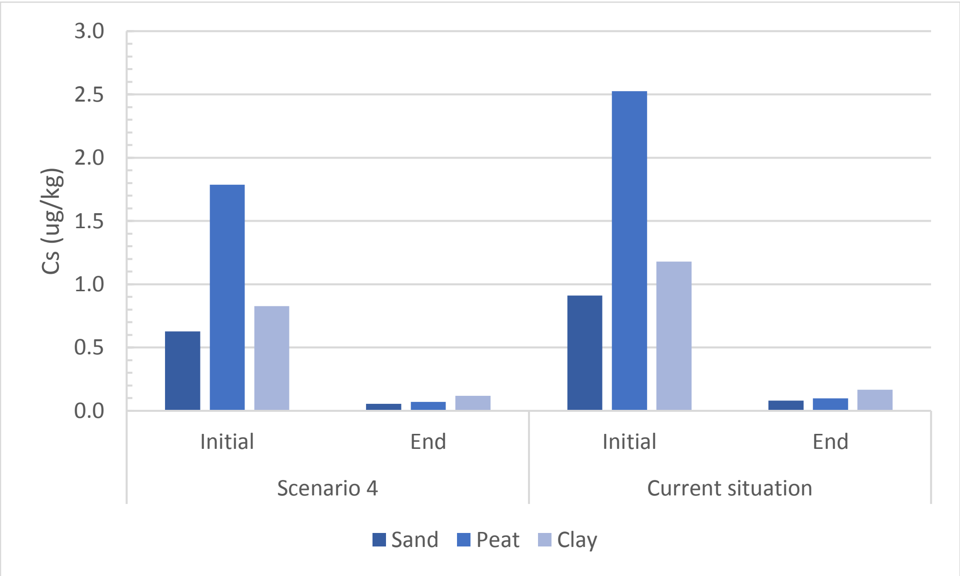


Figure 13 Initial and end concentrations in soil of OTC on grassland, comparing the current situation with scenario 4

Table 35 Initial and end concentrations of OTC under scenario 3. See explanation Table 31.

		Cs (ug/kg)		Css (ug/L)		OF (ug)	RU (ug)	LE (ug)	UP (ug)
		Initial	End	Initial	End	Sum	Sum	Sum	Sum
	Sand	0.628	0.055	0.000	0.000	0.446	0.000	0.000	0.000
	Peat	1.79	0.070	0.000	0.000	0.499	0.000	0.000	0.001
Grass	Clay	0.827	0.118	0.000	0.000	0.554	0.000	0.000	0.001
	Sand	0.111	0.000	0.000	0.000	0.158	0.000	0.000	0.000
	Peat	0.201	0.000	0.000	0.000	0.113	0.000	0.000	0.000
Maize	Clay	0.114	0.000	0.000	0.000	0.152	0.000	0.000	0.000

Table 36 Initial and end concentrations of SDZ under scenario 3. See explanation Table 31.

		Cs (ug/kg)		Css (ug/L)		OF (ug)	RU (ug)	LE (ug)	UP (ug)
		Initial	End	Initial	End	Sum	Sum	Sum	Sum
	Sand	0.108	0.001	0.216	0.002	0.704	0.001	0.006	0.002
	Peat	0.306	0.000	0.122	0.000	0.773	0.004	0.000	0.004
Grass	Clay	0.133	0.002	0.111	0.002	0.826	0.005	0.001	0.003
	Sand	0.003	0.000	0.005	0.000	0.034	0.000	0.000	0.000
	Peat	0.004	0.000	0.002	0.000	0.023	0.000	0.000	0.000
Maize	Clay	0.002	0.000	0.002	0.000	0.031	0.000	0.000	0.000

6.4 Discussion

The results show a relatively low risk, with concentrations in soil of maximum 2.5 ug/kg, far below the threshold risk value of 100 ug/kg. Some risk of accumulation for OTC on grassland was pointed out. Although there is limited data available to compare the model outcomes to, concentrations of *OTC* in Dutch soils were found between 0.2 and 0.7 ug/kg and *SDZ* was not detected in soil samples (Schilt & Van de Lagemaat, 2009). These values also show low concentrations, similar to the results, supporting the model outcomes. Initial concentrations are likely to be higher, however, but no values were found for the initial concentrations in soils in the Netherlands. There is limited data available for validation, which should be performed to examine the reliability of the model. The most significant losses are associated with overland flow. The model does not allow examination of the contribution of the losses to ground and surface water to the aqueous concentrations. Monitoring data found higher concentrations of sulfonamides in ground and surface water than tetracyclines (Schilt & Van de Lagemaat, 2009; Verhagen & Ottow, 2016), as is also shown in the model with higher leaching of SDZ. An important next step could be linking the model to the hydrology to include concentrations in water.

Scenarios 2 and 3 increase the concentration in manure to examine the risk, but the concentrations in soil and soil solution were still below the risk factors. The concentrations used in scenarios 2 and 3 were calculated based on averages over a total sector. If manure from only a treated group or individual will be used, concentrations in manure will still be higher, although it should be examined how realistic that situation would be. Scenario 4 looks at the impact of increasing the fraction of storage. The concentration in soil indeed decreased, but only around two thirds of the former concentration, which could be considered as a small effect for such an intensive measure.

The values were below the PNECs for resistance selection in water. The PNEC for *SDZ* was based on the PNEC of another sulfonamide and should be better specified. The fact that the model outcomes are below the PNEC also does not mean that resistance is not a problem; ARGs can still enter the environment during excretion in manure, increase in manure storage, grow in bacteria and spread through horizontal gene transfer.

Chapter 7. Discussion

Research contribution

This thesis presents an overview of the available literature describing the fate and transport of antibiotics and ARGs and methods to quantify these pathways. Concerns on the effects of antibiotics and particularly resistance have increased over the last decade. The problem definition explained that although many studies are available, a comprehensive overview was still lacking. An overview of these studies is now provided and based on this, uncertainties and recommendations for further research could be determined, as will be discussed in the next section. The exploratory model suggested in this thesis can be used to predict environmental concentrations and risks of antibiotics under different scenarios, which can is relevant for assisting policy making.

Several existing models with suitability to model antibiotics were identified from literature with a varying degree of complexity and data intensity. This will assist in developing and selecting models in the future. An exploratory model was developed based on the knowledge gathered during the literature review. This model is a simple model with its own uncertainties, as elaborated on in the next section. The model is more complicated than the simple exposure models (EMA, 2016; Montforts, 1999), as it accounts for overland flow, leaching and uptake by plants. Although advanced models are likely to describe the situation more accurately, these also have more excessive data requirements. The concentrations as calculated with the model were found to be similar to some concentrations in the field, indicating an advanced model might not be necessary, depending on the desired application. A dataset to validate the model is lacking, but the results indicate a good opportunity for using a similar model. The application for the exploration of different scenarios can aid decision making, for example by prioritizing policy choices. Different soil and land use types were accounted for in the model, allowing the possibility to identify risk locations. The model outputs on overland flow, subsurface runoff and leaching can be coupled to the hydrology to obtain concentrations in surface and groundwater.

The literature review presented an overview of the current state of knowledge concerning models on antibiotics resistant genes and bacteria in agriculture. No model has currently been developed for resistance, because of the lack of clear relationships and parameters. The overview provides a better understanding of what exactly is lacking and what will be necessary to develop a model in the future, as will be discussed in the next section.

Uncertainties and recommendations

Antibiotics

The literature overview on antibiotics identified the importance of many different factors contributing to the fate and transport, such as the type of manure, the way of spreading manure on land, the characteristics of the soil (e.g. organic matter content and soil texture) and the environmental conditions (e.g. temperature and light). Modelling antibiotics is dependent on the accuracy of the parameters related to those factors. Chapter 2 found some parameters lacking. Some ranges in parameter values were detected for all parameters, but most of the variance could be explained by the factors they depend on, such as soil characteristics and manure storage conditions.

The sorption capacity (Kf and Kd) and half-life in soil were found to strongly depend on soil characteristics, but methods to calculate these parameters based on these characteristics are limited. Chapter 5 explored the option of developing a multilinear regression model using available data from literature. These models were found to be a feasible approach to estimating parameters, but some issues related to model assumptions and applicability were identified. A larger sample size with a broader range in the independent variables would likely improve these models. For sorption, aluminium and iron oxide were not taken into account due to limited data availability, despite being found to be relevant factors (Jones et al., 2005; ter Laak et al., 2006). The model for the half-life in soil did not consider hydrolysis. Inclusion of these factors should further improve the regression models.

In general, parameters were missing for some antibiotics, especially for those least commonly used. The literature review indicated the presence of non-equilibrium sorption; some of the sorption to soil is irreversible. Parameters to account for this non-equilibrium sorption in models are limited, making them a complex addition to models. A similar problem occurs for the incorporation of transformation products. Only a limited amount of TPs are known to be toxic; further research should focus on identifying parameters for these specific TPs (Wohde et al., 2016). Antibiotics can occur in cationic, neutral and anionic forms depending on the environmental pH (Wang et al., 2015). This form also affects the sorption, since cations absorb more, but this is only taken into account in a limited number of studies. Future studies could also develop parameters more specific to certain pH ranges.

Model

The exploratory model gave interesting insights into the pathways of antibiotics. The results showed concentrations in soil and soil solution below the risk threshold values, even when increasing the risk with different scenarios. The limited risk of antibiotics for organisms was confirmed by experts, but there is still a concern for the selection of ARB. The risk for antibiotic resistance was examined by comparing with PNECs for resistance selection (Bengtsson-Palme & Larsson, 2016) and found to be lower than these PNECs, indicating low risk. These PNECs are only available for concentrations in water and can be compared with the concentration in soil solution, but it would be good to further examine PNECs for resistance selection in soil. This is of particular importance for tetracyclines, which sorb strongly to soil.

The sensitivity analysis showed that doubling the half-lives in soil and manure had the most significant effect on the concentration in soil, followed by the storage time of manure and mixing depth. The half-lives found in literature reviews showed quite some variation leading to uncertainty in the model outputs. Doubling the half-lives led to a concentration more than twice as high, but this concentration still stays below the risk threshold value. To examine risk more conservatively, the decision can be made to include high values for the half-lives.

An important step in the model is to validate the model outcomes. Data for validation have to be collected. The model can be further improved by including hydrology which enables the calculation of concentrations in surface and groundwater. Some models involving hydrology, such as Pearl, include more characteristics such as information on macro pores to improve the estimation of leaching. If parameters are available, transformation products and non-equilibrium sorption can also be accounted for. The inclusion of seasonal differences related to the application of manure, concentrations in manure and environmental temperatures could advance the model. Right now, the model allows for daily calculations, but the parameters such as the half-lives and percentage overland flow, which are dependent on factors such as temperature and rainfall, do not differ per season. The model has the assumption that the soil is heterogeneous; the same soil type was assumed over a hectare. There are likely to be variations in characteristics such as organic matter, pH and soil texture. A change in the scale of input values can make this more accurate, but whether this is necessary depends on the desired model application.

Antibiotic resistance

The literature overview for antibiotic resistance showed high variation in the outcomes of studies. The link between usage and resistance varied with factors such as on-farm hygiene, farm size and former exposure to antibiotics. Co-resistance and co-selection further complicated the association. Concentrations in manure storage also increased or decreased depending on the manure type, the antibiotic type and storage conditions such as temperature. Some models are available, but these are highly data intensive and have only been applied to a limited number of cases. Increased knowledge is necessary on specific parameters such as the rate of horizontal gene transfer, the growth in bacteria and the concentrations at which selective pressure occurs. Whether concentrations of antibiotics increase the dominance of resistant bacteria also depends on the former exposure of those bacteria to antibiotics. Including former exposure is prone to many uncertainties. The number of studies on leaching and runoff is minimal. Further research should focus on new or more advanced modelling approaches to 1) link antibiotic resistance in manure to antibiotic usage, 2) to examine the effect of manure storage conditions on the growth or degradation of ARGs, 3) to determine the concentrations at which selection of resistance occurs and 4) to quantify leaching and runoff. Including former conditions would be highly data intensive, however, raising the question on what type of model is required to address the risk assessment of ARGs in the environment.

Chapter 8. Conclusion

This thesis presented an exploratory model for the fate and transport of antibiotics that was based on an overview of the current state of literature on modelling antibiotics and ARGs. The research questions addressed 1) the pathways of antibiotic/ARGs, 2) the extent to which the pathways are quantifiable, 3) the application of these pathways in a model and 4) the model application for different scenarios. Here, the conclusions on these research questions are summarized.

Pathway of antibiotics

The systematic literature review indicated the possibility of using relationships as provided in both simple exposure models and more advanced models (e.g. pesticide models). After application of antibiotics, a fraction (25-90%) of the active substance is excreted to manure. In storage, the concentration in manure can decrease with first-order exponential decay. Manure is applied to land where a fraction is adsorbed to soil and the other fraction exists in soil solution, where it is available for leaching. Sorption was found to be well described with Henry's linear model or the Freundlich model, which are both equilibrium models. Non-equilibrium models, accounting for irreversible sorption were found to fit the data better. Degradation in soil, similar to degradation in manure, occurs with first-order or second-order exponential decay. Leaching and subsurface runoff are both dependent on the precipitation access, the concentration in soil solution and soil characteristics. Overland flow relates to the sorption to manure, the slope, precipitation access and the application method of manure (broadcasting, incorporation, injection). Uptake in plants can be fit to different models; most models describe low sorption to be relevant for uptake. The literature review indicated limited transparency on antibiotic usage and the lack of data availability on some parameters, mostly relating to transformation products, non-equilibrium sorption and the sorption of ions.

Pathway of ARGs

The overview of the available literature showed a low availability of existing models for ARGs. The available models on specific relationships (e.g. manure storage) were highly data intensive and have so far only be applied to few cases. Antibiotic usage was in most studies related to higher concentrations of ARGs in manure, but this association is also dependent on former exposure to antibiotics and other risk factors such as the number of animals and dietary factors. Co-selection and co-resistance further complicate the modelling process. Some ARGs were found to decrease in manure storage, while others increase, depending on the temperature, manure type and type of ARG. Application of manure was shown to lead to unexpectedly high or low concentrations in soil, linked to the rate of growth, the rate of selection, the rate of gene transfer and the rate of cell death. Accumulation related to these rates, but is also affected by runoff and infiltration. Decay was described by either logarithmic decay or the Collins-Selleck model. Few studies were found on leaching and runoff, but both processes have been detected, depending on rainfall patterns, slope and soil type. The decision was made that further research was necessary for the development of an exploratory model for ARGs.

Multiple linear regression models

Multiple linear regression models were constructed for the sorption coefficients (Kd and Kf) and the half-life in soil. Sorption has been significantly related to the application of manure, the percentage clay, the percentage organic carbon, the pH and the cation exchange capacity, depending on the model. Adjusted R-square values between 0.39 and 0.82 were found. The half-life in soil was associated with the initial concentration, the percentage organic carbon, the percentage clay, aerobic/non-aerobic conditions and sterilized/non-sterilized soil, with an adjusted R-square of 0.93.

Exploratory model and model application

An exploratory model on the fate and transport of antibiotics has been developed for different combinations of soil and land use types under wet and dry conditions in the Netherlands. This model is more advanced than the simple exposure models, but does not account for non-equilibrium sorption, ionization of antibiotics and transformation products. The model was applied to different scenarios. The scenario of the current situation shows low concentrations of antibiotics in soil, below the risk threshold values. Concentrations in manure were increased by only using manure from veal calves (scenario 2) or only manure from veal calves with short storage time (scenario 3). These scenarios resulted in higher concentration in soil, but these values were still below the risk threshold values. The concentrations in soil solution were also found to be below the PNEC for resistance selection. These results indicate limited risk of antibiotics in Dutch soil, but these findings should be addressed with caution; the model requires validation and the development of a larger number of risk scenarios. Risk of resistance is not ruled out; ARGs can still enter the environment during excretion in manure, increase in manure storage, grow in bacteria and spread through horizontal gene transfer.

Synthesis

The study objective was *to develop an exploratory model to assess the pathways and environmental risks of antibiotics and ARGs from agricultural inputs in the Netherlands.* The findings of the literature review allowed the development of such a model for antibiotics, but not for ARGs. Further research into the parameters required for antibiotic modelling and the relationships and parameters for ARG modelling is suggested. However, the foundation for modelling antibiotics and ARGs provided by this thesis is already relevant to guide policies that prevent adverse effects of antibiotics and antibiotic resistance.

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Appendix I: Multiple linear regression models

Table 37 Adjusted R-square and standard error of the estimate values for the a multiple linear regression model for Kd (OTC) for different combinations of independent variables

	R^2adj	SE	Significant ($p<0.05$)
pH, OC, clay, CEC	0.385	1687	pH and OC
pH, OC, clay	**0.394**	**1674**	**pH, OC, (clay 0.07)**
pH, OC	0.371	1707	pH and OC
OC, clay	0.3	1800	OC
pH, clay	0.065	2081	pH

Table 38 Adjusted R-square and standard error of the estimate values for the a multiple linear regression model for Kd (TC) for different combinations of independent variables

	R^2adj	SE	Significant ($p<0.05$)
pH, OC, clay, CEC	0.393	61750	pH
pH, OC, clay	0.385	61118	pH and clay
pH, OC	0.284	65978	pH
OC, clay	0.19	70146	Clay
pH, clay	**0.404**	**60202**	**pH and clay**

Table 39 Adjusted R-square and standard error of the estimate values for the a multiple linear regression model for Kf (SDM) for different combinations of independent variables

	R2adj	SE	Significant ($p<0.05$)
Manure, clay, pH, CEC, OC	0.934	1.15	Clay, (manure 0.06)
Manure, clay, pH, CEC	0.935	1.14	Clay and manure
Manure clay, pH, OC	0.936	1.13	Clay, (manure 0.06)
Manure, clay, pH	0.937	1.12	Clay and manure
Manure, clay, OC, CEC	0.936	1.13	Clay, (manure 0.06)
Manure, clay, OC	0.939	1.11	Clay and manure
Manure, clay, CEC	0.937	1.12	Clay and manure
Manure, clay	**0.94**	**1.10**	**Clay and manure**
Manure, OC	0.58	2.91	Manure and OC

Table 40 Adjusted R-square and standard error of the estimate values for the a multiple linear regression model for Kf (SDM) for different combinations of independent variables, excluding an extreme outlier

	R2adj	SE	Significant ($p<0.05$)
Manure, clay, pH, CEC, OC	0.65	0.96	Manure and Clay
Manure, clay, pH, CEC	0.665	0.94	Manure and clay
Manure clay, pH, OC	0.59	0.95	Manure and clay
Manure, clay, pH	0.672	0.93	Manure and clay
Manure, clay, OC, CEC	0.664	0.94	Manure and clay
Manure, clay, OC	0.667	0.93	Manure and clay
Manure, clay, CEC	0.677	0.92	Manure and clay
Manure, clay	**0.680**	**0.92**	Manure and clay
Manure, OC	0.547	1.09	Manure

Table 41 Adjusted R-square and standard error of the estimate values for the a multiple linear regression model for Kf (SMZ) for different combinations of independent variables

	R2adj	SE	Significant ($p<0.05$)
Manure, clay, pH, CEC, OC	0.543	1.75	Manure
Manure, clay, OC	0.586	1.67	Manure, (clay 0.06)
Manure, clay	**0.585**	**1.67**	**Manure, (clay 0.06)**
Manure, clay, CEC	0.561	1.72	Manure
Manure, clay, pH	0.571	1.70	Manure, (clay 0.06)

Table 42 Adjusted R-square and standard error of the estimate values for the a multiple linear regression model for Kf (SMX) for different combinations of independent variables

	R2adj	SE	Significant ($p<0.05$)
Manure, clay, pH, CEC, OC	0.815	0.49	Manure, pH and OC
Manure, OC, pH, CEC	**0.823**	**0.48**	**Manure, pH and OC**
Manure OC, pH, clay	0.811	0.49	Manure, pH and OC
Manure, OC, pH	0.817	0.48	Manure, pH and OC
Manure, OC, clay, CEC	0.719	0.60	Manure, (OC 0.06)
Manure, pH	0.699	0.62	Manure, (pH 0.053)

Tables S1-S5 in supplementary material