EMISSIONS OF VETERINARY PHARMACEUTICALS FROM LIVESTOCK BREEDING IN THE NETHERLANDS

NIKOLA RAKONJAC

Propositions

1. Resolution of the so-called "Dutch nitrogen crisis" will reduce the emissions of veterinary pharmaceuticals to environment.
   (this thesis)
2. Preventing the emissions of veterinary pharmaceuticals to the environment is more effective than mitigating environmental consequences.
   (this thesis)
3. Scientific progress is always beneficial for society.
4. The democratization of AI technology will have a significant impact on the scientific publishing industry.
5. Pursuing a PhD postpones starting your own family.
6. Admission to top-ranked European universities is more challenging for individuals from non-EU European countries than for those from non-European countries.

Propositions belonging to the thesis, entitled

Emissions of Veterinary Pharmaceuticals from Livestock Breeding in the Netherlands

Nikola Rakonjac
Wageningen, 19 June 2023

Emissions of Veterinary Pharmaceuticals from Livestock Breeding in the Netherlands

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Emissions of Veterinary Pharmaceuticals from Livestock Breeding in the Netherlands

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Thesis

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Chapter 1

General Introduction

1.1 Veterinary pharmaceuticals

Veterinary pharmaceuticals (VPs) are substances used to treat or prevent diseases in animals. They might also be utilized to restore or modify physiological functions in animals, or to make a medical diagnoses. VPs are used worldwide and subject to regulation, requiring market authorization before use (EMEA, 2008). The type of compound used depends on the type of animal, geographic location, and purpose. Products that are beneficial to, but not administered directly to animals, such as disinfectants, are not considered to be VPs. The potential benefits of VPs for animal health and welfare may sometimes overlap with those of food additives, but these two categories are regulated separately and governed by different legislation (Lahr et al., 2019). It should be noted that VPs are used for both companion and livestock animals, but the types of VPs used and the amounts administered may vary significantly between the two. This thesis specifically focuses on the livestock sectors, and therefore the results and conclusions obtained may not be applicable to companion animals.

The most frequently used VP groups in livestock animals are antibiotics, antiparasitics, coccidiostats, and hormones (Boxall et al., 2004). Antibiotics are typically used in the treatment and prevention of bacterial diseases, antiparasitics and coccidiostats are used against parasites, and hormones to deal with e.g. fertility cycles. In the past, some hormones were also used as a growth promotors but this has been prohibited in the EU since 1996 (Directive 96/22/EC). Several other therapeutic groups with relatively high usage are antifungals, anesthetics, tranquilizers, analgesics, and euthanasia products (Boxall et al., 2004). The intensification of livestock farming has progressively increased the use of these compounds, often without considering possible ecological consequences. Their frequent and long-term use leads to an inevitable increase in drug resistance in the environment and eventually it may lead to complete resistance of local bacterial populations, which is particularly the case with antibiotics (de Greeff et al., 2021). It is recognized that VPs have the potential to cause negative impacts on the environment and human health, although their toxicity and ecotoxicity are not yet fully comprehended (Boxall et al., 2012). Furthermore, they are classified as emerging contaminants, meaning that they are an area of growing concern for the scientific and regulatory communities.

1.2 Environmental pathways

A major pathway by which VPs enter the environment is the excretion of urine and faeces from treated animals and application of contaminated manure to agricultural land (Boxall et al., 2004; Crane et al., 2009). After administration to animals, a considerable proportion of VPs may be excreted unchanged through urine and faeces. The amount of VPs excreted depends on various factors, including the

livestock sector, properties of the VPs, and the method of administration (Kim et al., 2011). Typically, after excretion, VPs residues end up in manure storage where they may further dissipate. The extent of dissipation is affected by various factors such as storage conditions, duration of storage, temperature, physical-chemical properties of the VPs, and manure handling and management practices. When the storage period ends, the remaining fractions of VPs (if any) are being applied to soil via manure. This pathway is summarized with Figure 1.1. It is worth noting that VPs residues can also end up on the soil directly through manure or urine deposited by grazing animals. This pathway should not be overlooked, as it bypasses storage and the associated dissipation processes.

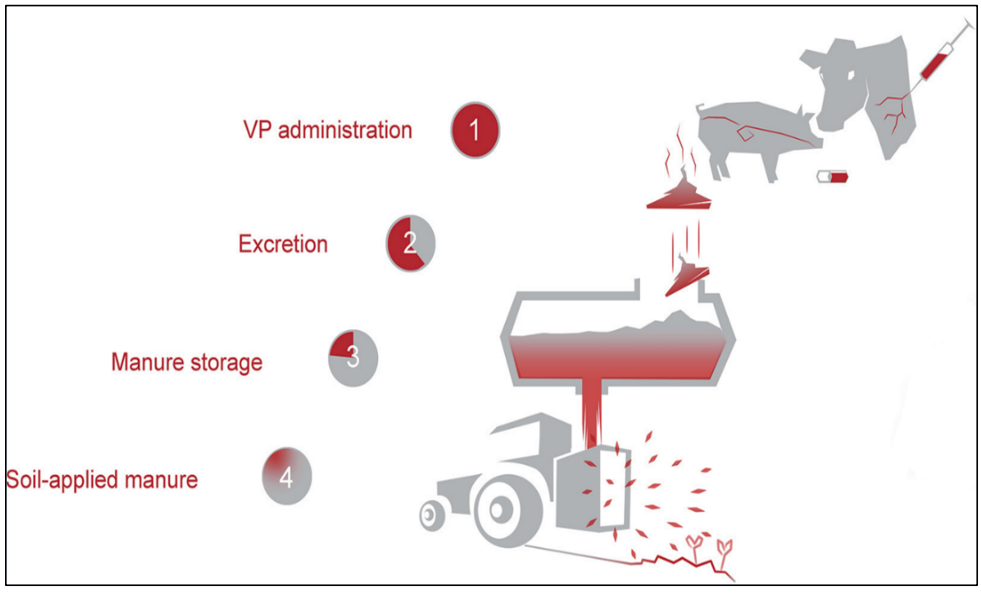


Figure 1.1: *Visualization of the entry route of VPs to the environment.*

After entering the terrestrial compartment, VPs residues can accumulate in the soil or be transported to surface water or groundwater. These residues may have negative effects on the chemical quality of water and can contaminate both surface and groundwater, which are sources of drinking water (e.g. Benotti et al. (2009)). Furthermore, VPs can also have an impact on the health of both terrestrial and aquatic ecosystems (e.g. Cycoń et al. (2019)). Leaching is a known environmental route for the transport of VPs to groundwater (Lapworth et al., 2012). However, the transport of VPs towards surface water is more challenging to estimate and may lead to pollution of surface water systems downstream of the application areas over long distances. Bailey (2015) conducted an extensive analysis of the transport mechanisms of VPs from agricultural fields to surface water. In principle, the contamination level of surface water is affected by several factors, including hydrology, physical

processes during transport, the method of manure application (such as spreading, injection, or incorporation), and the distance to surface water. The behavior of the compound and environmental conditions such as soil pH and organic matter also strongly influence both transport routes. Overall, the interactions between VPs, manure, soil, and water flow paths are not entirely understood and could be complex and diverse.

1.3 VPs in the Netherlands

In the Netherlands, the federation of the Dutch veterinary pharmaceutical industry (FIDIN) provides annual sales information for all antimicrobial veterinary medicinal products. These data are estimated to cover approximately 98% of all sales in the country. According to FIDIN, there has been a decrease in sales of these products by around 70% over the past decade. At the same time, usage data for antibiotics are publicly available, but they are typically reported in terms of antibiotic groups rather than individual compounds. For example, the Netherlands Veterinary Medicines Institute (SDa) provides an overview of the used quantities of veterinary antibiotic groups in different livestock sectors on an annual basis. SDa reports a general decline in antibiotic usage over the past decade, but livestock sector usage levels could differ substantially between individual farms (Diergeneesmiddelen, 2019). According to the SDa, the most commonly used antibiotic groups are tetracyclines, macrolides, trimethoprim/sulfonamides, penicillin, and quinolones. However, for antiparasitics and hormones, the usage data are not publicly available, and additional databases need to be reviewed to obtain an inventory of their usage.

In the Netherlands, manure from intensive livestock farming is applied onto arable land and grassland. The amount applied is restricted by emission limits for nitrogen and phosphorus, fertilizer recommendations, and soil type or crop tolerance for slurry (Montforts, 2006). In the European Union (EU), admissible nitrogen and phosphorus application rates are regulated by the EU Nitrates Directive 91/676/EEC (de Vries et al., 2021). With regard to that directive, the Netherlands as a whole is assigned as a vulnerable zone implying a strict maximum threshold for the use of animal manure of 170 kg N ha−1. However, the European Commission (EC) has granted a derogation for the Netherlands implying a maximum application rate for dairy farm (> 80 % grassland) ranging from 230 to 250 kg N ha−1, depending on soil type and region. This results in considerable amounts of applied animal manure, on average ca. 200 kg N ha−1 and 70 kg P2O5 ha−1. Consequently, manure application can potentially serve as a (major) source for VPs to enter the environment. In addition, the close proximity of agricultural land to waterways in the Netherlands increases the risk of VPs entering water bodies, making the Dutch water system vulnerable to such emissions.

The most comprehensive overview on the occurrence of VPs residues in manure, soil, and water bodies in the Netherlands (Lahr et al., 2019), shows that current knowledge and available data are useful but insufficient to determine and reflect on the complete picture. In particular, it highlights the scarce data on VP concentrations in the environmental compartments soil, surface water, sediment and groundwater, a lack of understanding on the compound sources and spreading routes, as well as uncertainties about the environmental risks, exposure quantities and effects on aquatic and terrestrial ecosystems that are exposed. Some other studies focused on the Netherlands affirm the occurrence of compounds associated with animal husbandry, namely in manure (e.g. Lahr et al. (2014) and Berendsen et al. (2015)), in soil (e.g. Lahr et al. (2018)), in groundwater (e.g. Kivits et al. (2018) and Van Loon et al. (2020)), and in surface water (e.g. Vethaak et al. (2005) and Lahr et al. (2018)).

1.4 Modelling approaches

Sources, pathways, fate, and behavior of VPs in the environment can be evaluated through dedicated experiments and monitoring studies or by using modelling approaches. The advantage of a modelling approach is that it is applicable to a broad range of environmental conditions and different types of VPs, whereas experimental efforts provide results for particular situations only. Furthermore, modelling can aid authorities and policymakers in identifying VPs that may be present in environmental compartments and assessing conditions that could increase their exposure. Conducting scenario studies is also a significant benefit of modelling. However, the accuracy and reliability of modelling results depend heavily on the availability and (un)certainty of input parameters. Furthermore, monitoring data is necessary to confirm the accuracy of model predictions or to provide input data for the model.

The European Medicines Agency provides a guideline on environmental impact assessment for veterinary medicinal products (EMEA, 2008). This guideline addresses different modelling perspectives per environmental compartment. In principle, it is recommended that when estimating the VPs residues in environment a stepwise (tiered) approach should be followed, using simple equations to provide an initial standard assessment and moving on to more complex modelling approach when a more refined estimate of exposure is required. Regarding the latter, a series of mechanistic environmental models and accompanying scenarios have been created by working groups in Europe known as FOCUS (Forum for the Co-ordination of Pesticide Fate Models and Their Use) to simulate the fate and transport of agrochemicals in the environment. Since FOCUS models are primarily designed for the exposure assessment of pesticides, before they can be used for modelling the exposure of VPs they have to be tailored likewise. FOCUS soil calculations are reasonably straightforward and are based

on the rate of degradation of the soil-applied chemical, implying that for VPs this model could be used if soil-applied amounts and degradation kinetics are established. FOCUS surface water calculations are performed using an overall calculation shell called SWASH which controls models which simulate runoff and erosion (PRZM), leaching to field drains (MACRO), and aquatic fate in ditches, ponds and streams (TOXSWA). These calculations provide detailed assessments of potential aquatic concentrations in a range of water body types in several geographic and climatic settings. FOCUS groundwater calculations involve the simulation of the leaching behavior of agrochemicals using a set of four models (PEARL, PELMO, PRZM and MACRO) in a series of multiple geographic settings with various combinations of crops, soils and climate. Detailed recommendations on how to implement VPs instead of pesticides in the aforementioned calculations, as well as how to interpret the modelling results, are provided in the EMEA guidelines.

Montforts (2005) provides an extensive overview on the exposure modelling approaches for VPs, reflecting primarily on Dutch agricultural situation, but also on the EMEA guidelines. Montforts discusses that agricultural practices and environmental conditions may vary from one country to the other, and that in order to assess the environmental fate of VPs an interdisciplinary approach is needed. He further states that some pesticide-targeted models encompass the same agricultural fields that are relevant for manure application, making them applicable also to VPs. However, Montforts identifies as a challenge that, at the time of publication (2005), none of the existing models was validated for VPs. He notes that successful validation would require an improved understanding of different parameters and modelling steps, namely animal husbandry phase (e.g. VPs dose administered), storage phase (e.g. slurry production), VPs behavior in slurry, emission to soil (e.g. VPs dosage applied), VPs behavior in soil and water, and environmental conditions (e.g. hydrology). Montforts further indicates that in the Netherlands farmers have to deal with restricted spreading times (spreading of slurry is allowed between February and September), an aspect that needs to be taken into account when defining modelling scenarios. At the end, the same author concludes that for the purpose of better environmental legislation in the Netherlands, and proper risk assessment of VPs, there is a need to quantify the impact of temporal and spatial differentiation in VPs emissions.

After 2005, several researchers attempted to model the environmental fate of VPs in the Netherlands. Lahr and Van den Berg (2009) and Hoeksma et al. (2020) investigated spatially distributed modelling to evaluate the VPs residues in soil and groundwater. In both cases, a pesticide-targeted model GeoPEARL (Tiktak et al., 2002) was used and the obtained results provided an aggregated national overview. Furthermore, Wöhler et al. (2021) estimated VPs loads to freshwater at regional scale via the VANTOM model (Bailey et al., 2015). Finally, a recent Dutch report (Kools et al., 2022) provided a

national scale overview on VPs emissions to surface water, by partially combining outputs of this thesis with the National Water Quality Model (Bolt et al., 2020). However, these studies primarily focused on individual environmental compartments or very limited number of VPs. Therefore, an automated and integrative approach is needed to follow the entire sequence of processes, from VPs usage to particular environmental compartment, while accounting for limited available information and processing efficiency. The main goal of this thesis is to accomplish that objective.

1.5 General outline

Previous sections emphasize the current knowledge and some of the challenges associated with assessing the emissions of VPs from livestock breeding. This thesis builds on the available knowledge and data, and goes a step further by developing tools for VPs prioritization and models to predict the occurrence of VPs in different environmental compartments. From the challenges and concepts mentioned above, four general objectives for this thesis were derived: (1) Understand and quantify the chain of processes that lead to VPs concentrations in soil-applied manure; (2) Generate a modelling approach to identify groundwater vulnerability from VPs at different spatial scales (local to national); (3) Build a VPs transport model suitable for lowland catchments; (4) Quantify the presence of compounds associated with livestock husbandry in surface waters via sampling (i.e. verify the modelling approaches). The four objectives are connected, and could be identified as four transport phases, illustrating the VP route from livestock animals to water bodies, where the outputs of each previous phase feed the current one. The accomplishment of these objectives will contribute to the more complete picture on the presence of VPs in Dutch environment. Moreover, it will provide an insight into the factors that drive the movement of VPs through environment, particularly focusing on manure, soil, groundwater and surface water. Figure 1.2 illustrates the relationship between the chapters and the overall thesis structure.

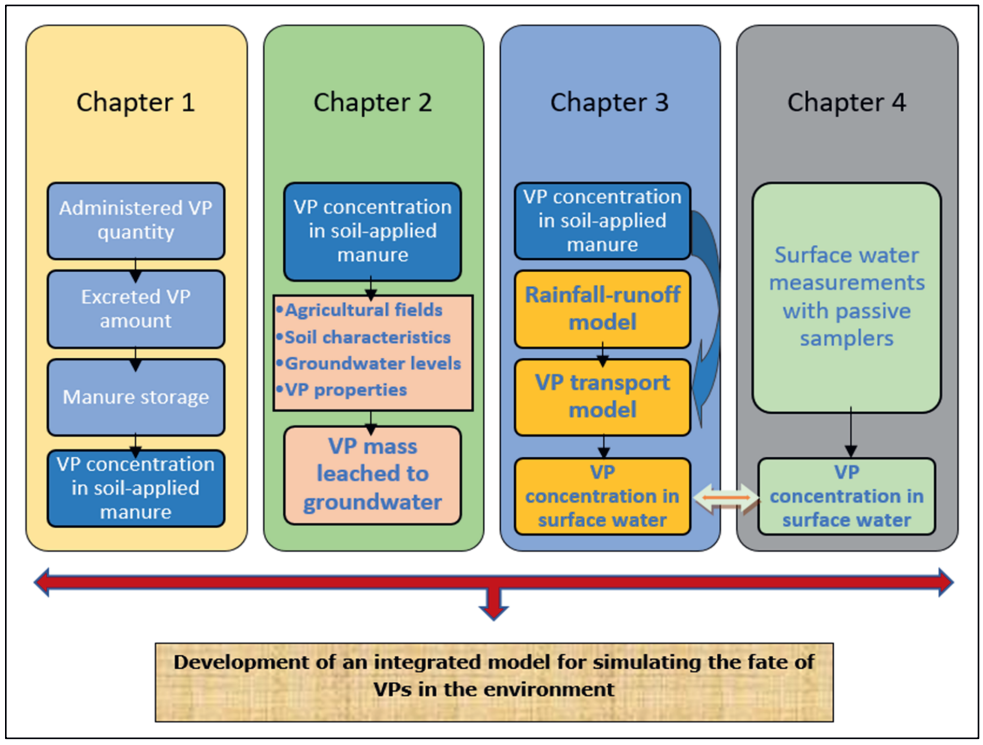


Figure 1.2: *Outline of the thesis.*

Even though usage data for antibiotic groups are publicly available in the Netherlands (published annually), insufficient information is available to identify the individual compounds and usage of the other VPs groups. This lack of quantitative information on administered VPs is rather crucial obstacle to properly assess the environmental fate of these compounds, being at the same time the initial input into the previously defined transport phases. Therefore, in the Chapter 2 of this thesis, we address the use of a number of VPs for the most relevant Dutch livestock sectors. In this chapter we also investigate substance properties, excretion rates and storage conditions, to predict the VPs concentrations in soil-applied manure. We further compare those predictions with available measurements, and we develop an indicator on the VPs residue potential in manure. This indicator could be used as a first tier assessment to identify if compounds could pose a risk to the environment, which is especially convenient considering the great number of active substances used in the Netherlands.

As indicated in the section 1.4, there were some earlier efforts to model the environmental fate of VPs in the Netherlands. However, those studies typically focused only on a very limited number of compounds, also highlighting the large uncertainty in the input data. Moreover, details relevant for georeferenced local situations (fields) were usually lacking, clearly limiting a closer identification of the

areas vulnerable to VPs pollution. For these reasons, we use the VPs soil-applied concentrations as estimated in the Chapter 2, to feed into the models described and applied in the Chapters 3 and 4 of this thesis. Chapter 3 describes a modelling approach to estimate the groundwater leaching potential of VPs at the field scale, for all fields in the Netherlands, by using the most detailed level of available data. Besides inputs in terms of soil-applied VPs concentrations and mass, this approach takes soil-hydraulic and soil-chemical properties, groundwater levels, and VPs properties into account. In addition, this chapter characterizes the relationship between different manure types and VPs leaching to groundwater. On the other hand, Chapter 4 illustrates a modelling approach to investigate the temporal dynamics of VPs in lowland surface waters of an agricultural catchment in the Netherlands. The approach combines an existing rainfall-runoff model with data on VPs concentrations in manure (Chapter 2), manure application patterns and VPs characteristics, to develop a parsimonious catchment-scale transport model that differentiates between quick flow routes and slower routes through the soil reservoir. The outputs of this model are then further compared with the measured VPs quantities in the surface water, the latter being discussed in details in the Chapter 5 of this thesis. Altogether, the aims of the Chapters 3 and 4 are to characterize the theoretical underpinnings, relate the available data with the developed models at different spatial scales, and discuss environmental pathways of VPs, in the context of Dutch environmental circumstances.

Although several sampling campaigns affirm the occurrence of VPs in surface water in the Netherlands, as indicated in the section 1.3, those measurements are still relatively scarce. This is especially the case with smaller streams that are not directly used as a source for drinking water but rather for agricultural activities (e.g. irrigation and groundwater level management). At the same time, those streams are directly exposed to VPs via runoff or drain pipe emission, being typically located near the fields on which manure is applied. Therefore, to complement the monitoring efforts on the presence of VPs, but also to partially validate some of the previously explained models, we deployed passive samplers in surface water on a number of locations in an agricultural area with intensive livestock activities. Next to a number of frequently used VPs, we also targeted naturally occurring hormones and disinfectants, resulting in 46 targeted compounds in total. The sampling strategy, compound details, chemical analysis, and results, are discussed in details in the Chapter 5.

The main findings and implications of this thesis are summarized and discussed in the Chapter 6, where also recommendations as well as potential challenges are provided for the direction of future scientific research and policymaking with respect to the occurrence of VPs in the environment.

Chapter 2

Emission Estimation and Prioritization of Veterinary Pharmaceuticals in Manure Slurries Applied to Soil

Based on:

Rakonjac N, van der Zee SEATM, Wipfler L, Roex E, Kros H. Emission estimation and prioritization of veterinary pharmaceuticals in manure slurries applied to soil. Science of The Total Environment 2022; 815: 152938

Abstract

Veterinary pharmaceuticals (VPs) are emitted into the environment and transfer to groundwater and surface water is diffuse and complex, whereas actual information on the fate is frequently limited. For 17 VPs of potential concern in the Netherlands, we assessed sources and emission due to animal slurry applications to soil. Hence, we examined the use of VPs in four livestock sectors in the Netherlands for 2015-2018, and quantified animal excretion rates and dissipation during slurry storage. For almost all VPs, administrated quantities to the animals during the period 2015-2018 decreased. VP concentrations during a storage period of six months could decrease between 10 and 98 % depending on the compound. Predicted concentrations of VPs in slurries after storage compared well with measured concentrations in the literature. Based on the storage model outcomes, we developed a residue indicator, that quantifies the potential for residues in applied slurry. This indicator agrees well with the most frequently detected VPs in the Dutch slurries, and is therefore useful to prioritize measures aiming at reducing VP emissions into the environment.

2.1 Introduction

Veterinary pharmaceuticals (VPs) are used worldwide to treat diseases and to protect the health of animals, where the type of used VP compounds depends on the animal sector and particular region (Berendsen et al., 2018). A major pathway by which VPs enter the environment is the excretion of urine and faeces from medicated animals and application of contaminated manure to agricultural land (Boxall et al., 2004; Crane et al., 2009). VP residues can reach and affect the quality of soil, groundwater and surface water (Kemper, 2008; Benotti et al., 2009; Lahr et al., 2018; Cycoń et al., 2019; Mooney et al., 2020; Zhang et al., 2021). Also, VP residues may end up in soil organisms and plants and be transferred into the food chain (Pan and Chu, 2017; El Agrebi et al., 2020).

Several comprehensive studies investigated the environmental fate and effects of veterinary medicines, from the regional or national context (e.g. Zhang et al., 2015), or a European (Kools et al., 2008) or global perspective (Sarmah et al., 2006). These studies concluded that information on VPs is available to identify environmental risks, but quantitative knowledge about administered VPs is very limited and in need of urgent attention. Similar conclusions can be derived from the European Medicines Agency guidelines (CVMP/VICH, 2005; EMEA/CVMP, 2008; EMEA/CVMP, 2016). Besides application rate and frequency, the fraction of administered active substances ending up in the environment also depends on the persistence during manure storage (Lahr et al., 2017). So far, some studies (Schlüsener et al., 2006; Kuchta and Cessna, 2009; Lamshöft et al., 2010; Berendsen et al., 2018) investigated VP dissipation during storage, but often those were focused on specific substances and particular manure type. On the other hand, number of studies provided insight into VP concentrations in different manures, as summarized in the reviews (Wohde et al., 2016a; Ghirardini et al., 2020).

In the Netherlands, animal manure from intensive livestock farming is spread onto arable land and grassland in considerable amounts, on average ca. 200 kg N ha^{-1} and 70 kg P_2O_5 ha^{-1} (amounting to 30 tons slurry ha^{-1}) (CBS(1), 2021). Over 95% of manure is applied as slurry and in the case of calves and pigs manure more than 70% is applied onto agricultural land untreated (CBS(2), 2021). How much and which VPs are applied to land with the slurries depends on the origin of slurry. Even though the antibiotic use in livestock farming in the Netherlands has been reduced by more than 60% over the last decade (Veldman et al., 2020), used quantities are still significant, especially in veal farming and to a lesser extent in the pig sector (SDa, 2020). Besides antibiotics, antiparasitics and hormones are used, and end up in manure (Lahr et al., 2014; Lahr et al., 2018). The relation between VP residues measured in manure with the active substance administration rates to animals in the Netherlands is

still hardly known, as only few studies (Montforts, 2006; Lahr and Van den Berg, 2009; Hoeksma et al., 2020; Wöhler et al., 2020) have elaborated this, and only for a limited number of compounds and animal types.

In this paper, we investigated the chain of processes that lead to VP concentrations in soil-applied slurry manure for a selection of VPs that were considered of potential concern and focusing on Dutch conditions. In addition, we aimed to develop a tool suitable for fast screening and prioritization of compounds, and our approach therefore did not focus on detailed modelling and sensitivity analyses. Underlying research questions were: (i) what was the use of VPs for the four most relevant livestock groups? (ii) how could we quantify the VP concentration in slurry based on substance property information, excretion rates (unmetabolized VP portion) and storage information? (iii) were predicted VP concentrations in agreement with measurements in the literature? (iv) how to translate insights of (i) and (ii) towards an indicator on the VP residue potential for stakeholders such as policy makers?

2.2 Methodology

We estimated the portion of used VPs which actually ends up in the slurry manure, both before and after the storage. For this purpose, we investigated the chain of processes in four different livestock groups: dairy cows, veal calves, fattening pigs and sows. Figure 2.1 schematically shows the various steps. Also, we focused on the behavior of 12 antibiotics, four antiparasitics and one hormone. We targeted VPs for this study based on three aspects. First, from reports (SDa, 2020; Veldman et al., 2020) that specify use of veterinary antibiotics in the Netherlands, the most commonly administered pharmacotherapeutic groups were selected. We identified tetracyclines, macrolides, trimethoprim/sulfonamides and penicillin groups as the most commonly administered in the veal calf sector. For dairy cows, the same groups were found to be dominant, where penicillin prevailed. For pigs (sows and fattening pigs), the same groups as in the veal calf sector, and additionally quinolones, were identified. In a second step, we screened recent Dutch studies (Lahr et al., 2014; Lahr et al., 2018; Lahr et al., 2019) with VP measured concentrations in slurry manure during or after storage to identify VPs that were persistent enough to reach soil when slurry manure is applied. Based on those inventories and taking into account the availability of VP environmental properties data from the literature, individual substances were prioritized (12) as described in Supplementary Material (SM, Section SM1). In addition to the antibiotics, six antiparasitics reported in Dutch slurry were selected, as well as one hormone (i.e. a hormone which is not naturally occurring). From these 19 VPs, a final selection was based on availability of use data, and two antiparasitics were disregarded as too few data were available. For this purpose, raw use data of VPs, for the period 2015-2018, were obtained

from Dutch Farm Accountancy Data Network (FADN) and processed. The selected VPs are shown in the Table 2.1, and further details are given in SM (Section SM1).

Excretion rates, manure production, storage duration and dissipation parameters were estimated based on literature. Manure storage duration and frequency of manure additions affect VP concentrations in storage and were analyzed based on previous investigation about the effects of application frequency on pesticide transport (Beltman et al., 1996) and by modifying approach developed for describing accumulation processes in soil root zone (Van der Zee et al., 2010). Predicted concentrations of VPs in calves and pigs slurry manure could be validated with data of two Dutch provinces: for Gelderland, modelling was compared with measurements from 2016 and 2017 (Lahr et al., 2018), and for North Brabant with data from 2013 (Lahr et al., 2014). To characterize the impact of VP use and substance properties on VP concentrations in a slurry manure, a VP residue indicator was developed.

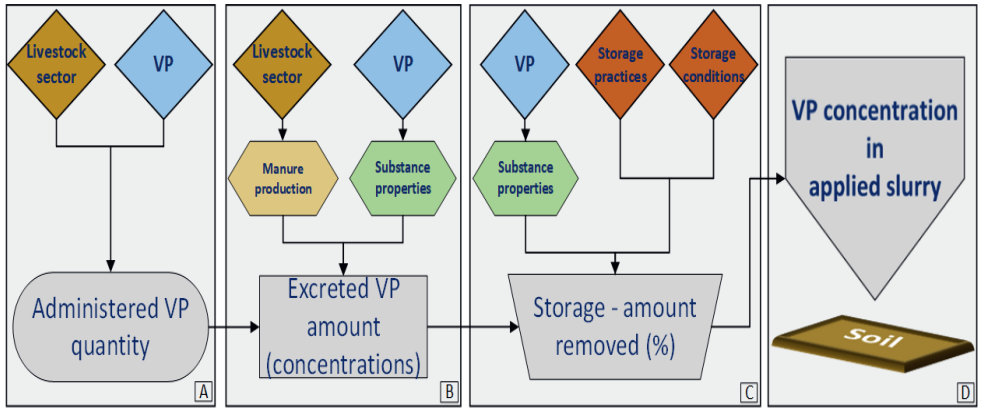


Figure 2.1: *Schematic representation of the process steps. (A) The administered VP amount depends upon the animal type and particular VP. (B) Portion of active substance which is excreted from animals into slurry depends on step A, quantity of produced slurry manure and VP excretion rates. (C) After excretion, VPs end up in the storage for slurry manure, where dissipation of VPs might occur. This process is influenced by storage practices, storage time, conditions and VP dissipation rates. (D) VP residues, present in the slurry after the storage period, have the potential to reach the soil when slurry is being spread on a land.*

Table 2.1: *Selected substances and their pharmacotherapeutic group, excretion rates and livestock sector.*

Group	Substance	Abbreviation	CAS no.	Sector of application		Excretion rate [%]	
				Cattle [a]	Pig [b]	Cattle[a]	Pig [b]
Antibiotics							
Tetracyclines	Tetracycline	TC	60-54-8	✓ [d]	×	80 [g]	×
	Oxytetracycline	OTC	79-57-2	✓	✓	23 [h]	60 [i]
	Doxycycline	DC	564-25-0	✓	✓	90 [j]	90 [k]
	Chlortetracycline	CTC	57-62-5	✓	×	75 [l]	×
Trimethoprim/ Sulfonamides	Trimethoprim	TMP	738-70-5	✓	✓	3 [c]	33.5 [m]
	Sulfadoxine	SDX	2447-57-6	✓	✓	65 [n]	52 [c]
	Sulfadiazine	SDZ	68-35-9	✓	✓	80 [c]	50 [o]
	Sulfamethoxazole	SMX	723-46-6	✓	✓	30 [p]	16 [q]
Macrolides	Tilmicosine	TIL	108050-54-0	✓	✓	90 [r]	80 [r]
	Tiamulin	TIA	55297-95-5	×	✓	×	84 [s]
	Tylosin	TYL	1401-69-0	✓	✓	30 [t]	6 [t]
Quinolones	Flumequine	FLQ	42835-25-6	✓ [e]	✓ [f]	5 [c]	5 [c]
Antiparasitics							
Avermectines	Ivermectin	IVM	70288-86-7	✓	✓	35 [u]	40 [v]
Benzimidazoles	Flubendazole	FLU	31430-15-6	×	✓	×	79 [w]
	Fenbendazole	FBZ	43210-67-9	✓	×	35 [c]	×
Pyrethroid	Permethrin	PERM	52645-53-1	✓	×	80 [c]	×
Hormones							
Corticosteroid	Dexamethasone	DEX	50-02-2	✓	✓	60 [c]	25 [x]

a Valid both for dairy cows and veal calves;

b Valid both for sows and fattening pigs;

c Estimated. Details are given in the SM4;

d TC is only administered intrauterine and used for the treatment of afterbirth cows, hence not applicable to veal calves;

e Not used for dairy cows, only for veal calves;

f Available use data only for sow category;

g Feinman and Matheson, 1978; ***h*** Arikan et al., 2007; ***i*** Mevius et al., 1986; ***j*** Shaw and Rubin, 1986; ***k*** Fernández et al., 2004; ***l*** Elmund et al., 1971; ***m*** Zhang et al., 2015; ***n*** Nielsen, 1973; ***o*** Nielsen et al., 1986; ***p*** Nouws et al., 1991a; ***q*** Nouws et al., 1991b ***r*** FAO/WHO, 1997; ***s*** Dreyfuss et al., 1979; ***t*** Lewicki, 2006; ***u*** Liebig et al., 2010; ***v*** Chiu et al., 1990; ***w*** Meuldermans et al., 1982; ***x*** Post et al., 2003.

2.2.1 Usage of VPs

In the Netherlands, the federation of the Dutch veterinary pharmaceutical industry (FIDIN) provides annual sales information for all antimicrobial veterinary medicinal products. However, these data do not distinguish between animal species, but only give the total sales for all animals, whereas actual administration can differ from the amounts sold, due to stockpiling and cross-border use (Veldman et al., 2020). On the other hand, MARAN (Veldman et al., 2020) and SDa (SDa, 2020) reports provide an overview of the used quantity of veterinary antibiotic groups in different livestock sectors on an annual basis. Still, used amounts of individual VPs in the Dutch livestock sector are not publicly disclosed. To improve estimates, we obtained additional information about the annual use of individual VPs at the farm level in the Netherlands, originating from the Dutch FADN system as collected by Wageningen Economic Research. This dataset contained purchase data of antimicrobial VPs, data about antiparasitics and hormones, and was based on 350 to 380 farms, depending on the survey-year and livestock sector. We considered VP quantities purchased at each farm as being used (irrespective of whether they are actually used). The details are given in the SM (Section SM2).

The FADN dataset did not cover the veal calf sector, where administered quantities of most antibiotics are structurally larger than for dairy cows (Veldman et al., 2020). Therefore, we decided to estimate the usage of individual VPs in veal calves by assuming constant ratios in defined daily doses (SDa, 2020; Veldman et al., 2020) between dairy cows and veal calves, for each antibiotic group. Then, for the selected individual antibiotics (Table 2.1), the (previously calculated) usage data of dairy cows were transposed into usage data for veal calves. The transformation factors (F_c), estimated VP usage, and details are given in the SM (Section SM3).

For antiparasitics and the hormone, such a conversion approach was unfeasible as their use in veal calves was not available. Therefore, for these substances we estimated use in veal calves by combining the FADN dataset with VP prescriptions (Dutch Veterinary Medicines Information Bank, 2021), as detailed in the SM (Section SM3).

2.2.2 Excretion of VPs

Of VPs administrated to animals, a significant percentage of VPs may be excreted via urine and faeces in its original form (unmetabolized) (Boxall et al., 2004; Kim et al., 2010). Several studies (Sarmah et al., 2006; Masse et al., 2014; Zhang et al., 2015) provided ranges of VP excretion rates that vary per livestock sector and active substance. However, the majority of these studies focused on antibiotics

while data about antiparasitics and hormones are scarce. Literature on VP excretion rates to manure (see Table 2.1 and Table S7), was searched, preferably for experimental data, and for individual VPs and animal type. We averaged if several values were reported, and in view of our focus on slurry manure, if rates for faeces and urine were found. When no data were available, an estimation was based on values reported for animal sector in general or structurally similar compounds (see Table 2.1 and SM4). From the portion of VP excreted and from the yearly production of slurry manure for a single animal (CBS, 2019), we calculated the initial concentration of VPs in the slurry manure, according to

$$C_{in} = \frac{U \times ER}{P \times 100} \quad (2.1)$$

where C_{in} represents the concentration in slurry manure prior to storage ([mg/ton]), U corresponds to VP yearly administered amount ([mg/animal per year]), ER is excretion rate ([%]) and P is the annual produced slurry manure ([ton/animal per year]).

2.2.3 Manure storage – dissipation model

Upon excretion, VPs end up in manure storage where they may dissipate by different processes (e.g. degradation/transformation, volatilization), that depend on environmental circumstances and physical-chemical properties of the VPs. Nationally regulated, manure slurry is stored for about 6 months (Lagerwerf et al., 2019; RVO, 2021) in the winter (from September to February). Half-lives (DT50) of antibiotics in stored manure have been experimentally determined for Dutch manure types (Berendsen et al., 2018), and half-lives for 11 antibiotics in slurry manure from this study were used. For the remaining six VPs values were taken from the literature as given in SM5.

Besides DT50 values and storage times, factors such as storage conditions (e.g. temperature) and manure handling may influence the VP concentration (Montforts, 2006). For dissipation kinetics, a first order rate law was assumed (Spaepen et al., 1997; Wang and Yates, 2008; Ray et al., 2017). We modelled dissipation by assuming that slurries are added to the storage basin stepwise. The frequency of manure additions may vary in practice, where continuous addition is one of the limiting cases (of high frequency) and where the unrealistic situation of instantaneous addition of the full yearly quantity is the other limit. The periodic addition of manure to the storage depot imply that dissipation always follows the same 1st order pattern, but the available time for dissipation differs between earlier and later additions. Moreover, each time when new manure is added, dilution of VP already present in the storage occurs. Referring for details of the original derivations to the earlier two papers (Beltman et al., 1996; Van der Zee et al., 2010), the governing modified expressions are

$$C_{f,tb} = \frac{C_{in}(t=0)}{n} \times \frac{\exp(-\mu \times \Delta t) \times [1-(\exp(-\mu \times \Delta t))^{n}]}{1-\exp(-\mu \times \Delta t)} \quad (2.2)$$

$$C_{f,ta} = \frac{C_{in}(t=0)}{n} \times \frac{1-[\exp(-\mu \times \Delta t)]^{n}}{1-\exp(-\mu \times \Delta t)} \quad (2.3)$$

where $C_{f,tb}$ and $C_{f,ta}$ represent the final VP concentration in slurry manure at the end of storage period ([mg/ton]), C_{in} (*t=0*) is the initial concentration ([mg/ton]) prior to storage from *eq.(2.1)*, n denotes how many times manure has been added into the storage ([-]) during the entire storage period, μ is the dissipation constant ([day^{-1}]) and equals $\mu=\frac{\ln(2)}{DT50}$, and Δt is the application interval ([day]). The extra term compared with the original equation (Beltman et al., 1996; Van der Zee et al., 2010), accounts for the mentioned dilution effect. Whereas *eq.(2.2)* gives the concentration just before new manure is placed into the storage (t_b), *eq.(2.3)* gives the concentration immediately after new manure is brought into storage (t_a). Accordingly, they represent the minimum and maximum concentrations of the sawtooth concentration pattern in time (Beltman et al., 1996; Van der Zee et al., 2010).

2.2.4 VP residue indicator

Besides the fraction of VPs ending up in soil-applied slurries, also the national coverage (e.g. the number of farms involved) affect the environmental urgency. Hence, a VP residue indicator (*R*), was developed that informs on the VP residue potential in the Netherlands for dairy cows and pigs. This indicator is confined to these two animal sectors as for veal calves administered VP quantities were unavailable. The indicator is based on the VP excretion rate (ER), concentration change during storage (C_f/C_{in}), but also on the percentage of farms where individual VP was reported administered (F_u). Thus, F_u characterizes how widespread a certain substance was used and therefore its influence on VPs appearance in Dutch soil-applied slurry. The VP residue indicator, *R* ([-]), is given by:

$$R = \frac{ER}{100} \times \frac{C_f(t_a)}{C_{in}} \times \frac{F_u}{100} \quad (2.4)$$

All three terms in *eq.(2.4)* are dimensionless and range from zero to one. R equal to *zero* implies that this VP residue will not be found in the soil-applied slurry, whereas for R equal to *one* on all farms all administered amounts are fully excreted, and also no dissipation in storage occurs.

2.3 Results and Discussion

2.3.1 Usage and excretion of selected VPs

The number of investigated farms varied each year, and ranged from 230 to 260 for dairy cows, for sows from 50 to 60, and for fattening pigs from 50 to 70. In case of dairy cow farms, the representative sample covered around 1.5% of farms in the Netherlands, whereas for pigs this was around 4% (Lahr et al., 2019). For over 95% of the farms in the dataset, at least one substance of our interest was reported as purchased in that respecting year. Since each farm location was identified at the province level, we could observe that the biggest variety of purchased VPs corresponded with the provinces with the highest farm density: see SM (Section SM2).

The dataset revealed that four out of 17 selected VPs were exclusively used to treat dairy cows, three only in the pig sector (fattening pigs or sows), and 10 VPs were used in all involved livestock sectors. As purchase data for veal calves were unavailable but estimated, we assumed that all VPs applied for dairy cows (except Tetracycline) were also used for veal calves. This claim was for investigated VPs based on the prescription data (Dutch Veterinary Medicines Information Bank, 2021). Selected substances and their main characteristics are given in Table 2.1.

The VPs purchased on most of the dairy cow farms during the analyzed period were OTC (87%), TMP (86%), SDX (68%) and DEX (61%). In the case of sow farms, these were TMP (80%), OTC (74%) and FLU (64%). For fattening pigs the dominant ones were OTC (62%), TYL (52%) and DC (51%). The complete overview of average F_u-values and standard deviations for the period 2015-2018 is shown in Figure 2.2.

As Figure 2.2 reveals, the primarily purchased VPs on the dairy cow farms were antibiotics, and antiparasitics only on less than 5%. This is rather different for pigs, where the antibiotics prevailed, but two of the investigated antiparasitics (IVM, FLU) were applied on a substantial number of farms. The hormone (DEX) was administered on more than half of dairy cow and sow farms, and on about 20% of the fattening pig farms. Worth mentioning was the considerable use of TMP, originated from its combined application with the antibiotics from the sulfonamide group. Based on the prescription data (Dutch Veterinary Medicines Information Bank, 2021), the aforesaid mixture contained around 20% of TMP while the rest was reserved for, in this study, SDX, SDZ or SMX. Additional observations about Figure 2.2 are given in the SM (Section SM6).

Regarding administered quantities (*U in [mg per animal per year]*), based on average values for the period 2015-2018, in a dairy cow sector the VPs FBZ, CTC and the mixture TMP/SDX were prevalent

(Figure 2.3). For sows, the main VPs were DC and TMP/SMX (Figure S2-B), and for the fattening pigs DC and TYL (Figure S2-C). Figure 2.3 gives an overview on the distribution of use data in dairy cow sector for the period 2015-2018 with respect to VPs reported in the Table 2.1. The situation for the other two sectors is illustrated in the SM (Section SM6).

The administered amounts over the years revealed a clear trend of reduction in use of TMP/SDZ, TYL, and FBZ for dairy cows (Figure S3-A), DC, TMP/SDZ, and TMP/SDX for sows (Figure S3-B), and TMP/SDZ for fattening pigs (Figure S3-C). Administration of VPs OTC, PERM and DEX remained pretty constant in dairy cows (Figure S3-A/A1), as well as for DEX in fattening pigs (Figure S3-C1). For other VPs no clear patterns were seen. However, the overall distribution for the entire analyzed period (2015-2018) showed that almost all administered quantities in 2018 were lower than in 2015. For antibiotics this is consistent with the official Dutch reports (SDa, 2020; Veldman et al., 2020). Exceptions and details are indicated in the SM (Section SM7).

Used quantities of antibiotics in the veal calf sector were estimated based on the conversion factors (Table S5) and use data of dairy cows (Figure 2.3). Some bias may occur as for example FLQ is not used for dairy cows, while in calves it is used to treat respiratory and digestion tract infections. The use of the mixture TMP/SDX may be lower in veal calves than dairy cows (being preferentially used for lactating animals). Research by Lahr et al. (2019) can be interpreted likewise. Derived usage for veal calves and details are shown in the Table S5.

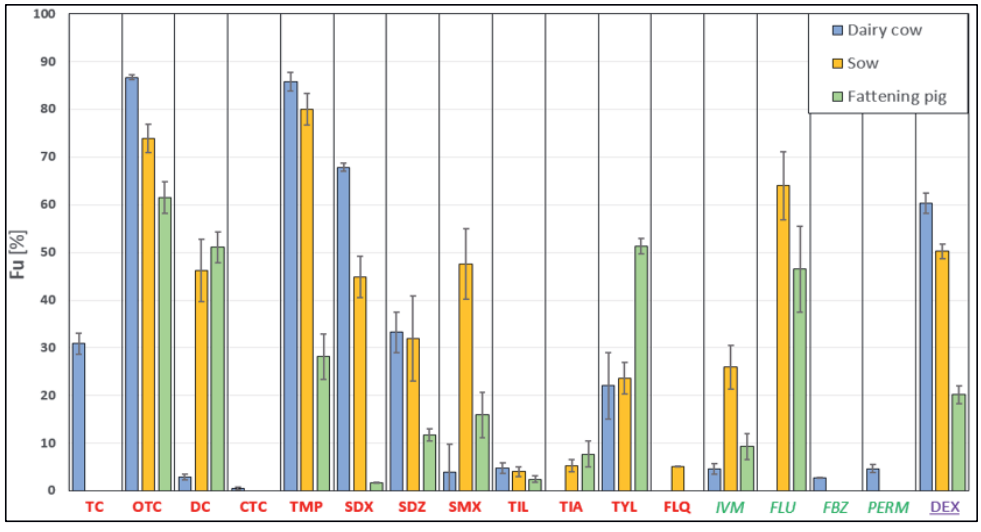


Figure 2.2: *Part (F_u in %) of analysed farms on which VP was administered (refers to the percentage of farms on which particular VP is reported as being purchased) for three livestock sectors (dairy cow, sow and fattening pig). F_u values are averaged for the period 2015-2018, and error bars denote standard deviations. Antibiotic names are in bold red, antiparasitics are in italic green, and hormone is in underline purple.*

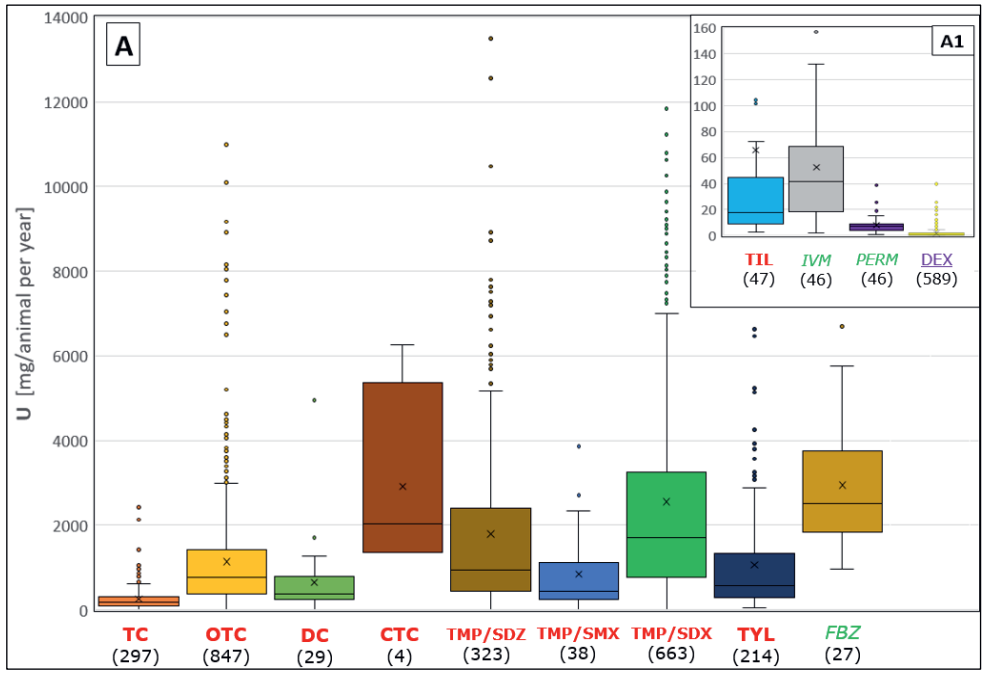


Figure 2.3: *Distribution of VP use data for the period 2015-2018, illustrated with Box and Whisker Plot. Numbers in legend indicate sample size. (A) VP use in dairy cow sector; inset (A1) shows VPs with administered quantities below 160 mg/animal per year. Antibiotic names are in bold red, antiparasitics are in italic green, and hormone is in underline purple. VP use in sow and fattening pig sectors is shown in Figure S2-B/C, where details about Box and Whisker Plot and sample size are also given.*

2.3.2 VP concentration in the slurry manure

Concentrations of VPs in the slurry, prior to and after the storage, were calculated based on the average annual VP use per animal for the period 2015-2018 (Figure 2.3 and S2, and Table S5). For the same period and considering four investigated animal sectors, also the average annual quantities of produced slurry manure in the Netherlands were estimated (CBS, 2019). Figure 2.4 provides an overview on the predicted VPs concentrations using the DT50 values in the storage as specified in SM (Section SM5). The concentrations concerned those prior to storage, Cin (mg/ton) and after 6 month of storage, Cf (mg/ton), for dairy cows/veal calves and sows/pigs, respectively.

As Figure 2.4-A divulges, initial concentrations in dairy cow slurry were below 80 mg/ton for all investigated antibiotics, and for the hormone and antiparasitics (except FBZ) smaller than 1 mg/ton. The initial concentrations in veal calf slurry were significantly larger due to higher use (Table S5) and a lower manure production. Concentrations after storage greatly depended on substance DT50, which

was assumed to be equal for the two cattle categories and therefore had the same dissipation pattern. During the storage period of six months, FBZ and DEX concentrations reduced less than 20%, and SMX, TIL, TYL and PERM over 90%. For other analyzed VPs, this was between 50% and 85%. In the pig sector (Figure 2.4-B), reduction between sows and fattening pig slurry was similar, with the exception of SDX, TIL and TYL, where again DT50 was the same for both animal types with respect to individual VP. For pigs, all investigated tetracyclines and sulfonamides concentrations reduced for more than 87%, while FLU and DEX less than 15%. Remaining VPs had concentration reductions between 42% and 78%.

The results indicate that the lowest VP-total mass ends up on the soil if dairy cow slurry coming from storage is applied: 12 out of 14 investigated VPs used in dairy cows had calculated concentrations lower than 10 mg/ton. This finding, and general distribution of VP concentrations within four investigated slurries, seems comparable with studies done in other countries (Zhao et al., 2010; Ghirardini et al., 2020; Li et al., 2021). Some VP groups with predicted high concentrations in the slurries (e.g. tetracyclines), after being applied on the soils are found in the upper soil layers due to their low mobility and sorptive behavior (Gros et al., 2019). The group of sulfonamides, even though extensively used, showed small concentrations when applied in manure slurry to soil. That this group cannot be disregarded is caused by their high mobility in soil, that favors their leaching to groundwater (Aust et al., 2010; Kivits et al., 2018). Furthermore, particular focus should be on the behavior of FLU, as our results indicated its high concentration in pig slurries, and it is known that this VP can be detected at various soil depths (Gros et al., 2019). In general, VP concentrations in soil and groundwater are influenced, among others, by additional adsorption, degradation, and transport processes, which was out of the scope of this study.

To calculate VP concentrations at the end of the storage period, we used *eq.(2.3)* and made an assumption that manure has been added to the storage every day during a six months period (*n=180, Δt=1d*). The sensitivity of the stored manure concentration to the DT50 is illustrated in Figure S4 which shows concentration decline during storage for VPs with a DT50 varied from 5 to 333 d. As Figure S4 reveals, if different VPs enter the storage with the same initial concentrations and at the same moment, by further following identical manure addition patterns (n, Δt), the influence of DT50 on final concentration is considerable, i.e. after six months the ratio of concentrations between a substance with a DT50 of 333d and the one of 5d is almost 22. On the other hand, with periodic applications (Figure S5), the VP concentration present in the storage at a particular moment ($T_{storage}$) can vary depending on the frequency of manure additions up to that point. Nevertheless, animals produce manure each day and even if that manure is not immediately added to the storage, we can assume that VPs start their dissipation process after being excreted. Thus, the simplification of daily manure

additions and de-emphasizing the sawtooth pattern seems reasonable. Note that besides stored slurry, dairy cows excrete manure directly on the land during pasture season excluding dissipation in storage. This pathway represents about 10% of the total produced manure by dairy cows (CBS, 2019), consequently these soil-applied amounts are less significant. As residues of VPs in manure from grazing animals could affect dung fauna (e.g. IVM) (Lahr et al., 2019), further investigation of that pathway may be required.

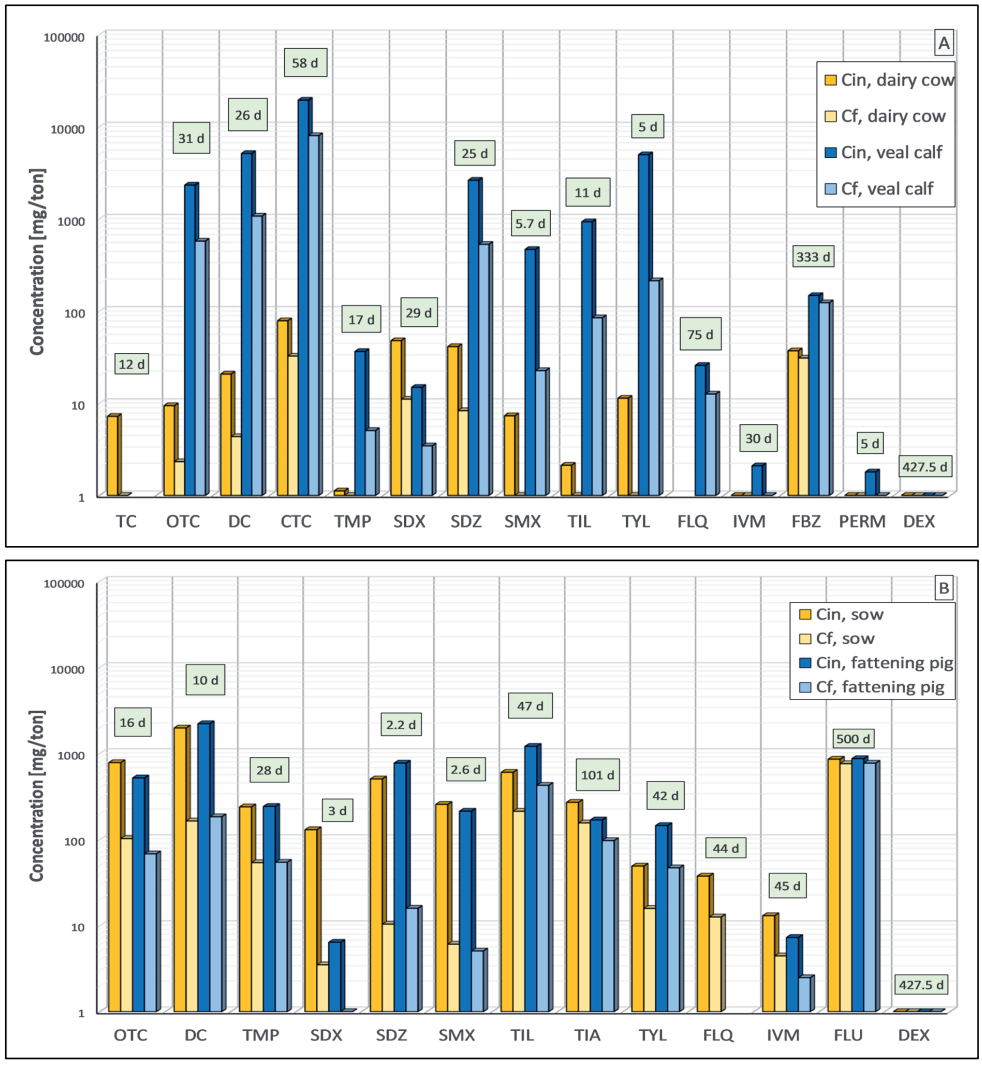


Figure 2.4: *Concentrations of VPs in slurry manure prior to storage (Cin) and after 6 months of storage (Cf) (log scale), including substance half-live in the storage (DT50 in days) displayed in the green text box. (A) In cattle sector for 15 different VPs. (B) In pig sector for 13 VPs. Cin is calculated with eq.(2.1) and Cf with eq.(2.3). Quantities of produced slurry manure (P) were estimated as 28 (dairy cow), 3.5 (veal calf), 4.5 (sow) and 1 (fattening pig) ton/animal per year.*

2.3.3 Results validation

Berendsen et al. (2015) investigated concentrations found in animal faeces at 20 randomly selected pig and calve farms in the Netherlands in 2014. The faeces were taken from the animal gut from animals selected in the slaughter phase. VPs investigated both in our study and in the mentioned research were OTC, DC, SDX, SDZ and TIL for the cattle sector. In general, quantities found in the calve faeces were considerably lower (except for OTC) than our predicted prior-storage concentrations in slurry manure. In the case of pigs our estimations for OTC, DC and TYL were within the measured range, whereas for SDZ and TIA our predictions were above measured values. Table 2.2 provides an overview on the predicted and measured VPs concentrations. The differences might be caused by a lower usage of particular VPs in 2014 (not included in our study period) compared to the one from 2015-2018, but this is less probable since for the antibiotics we observed a reduction trend in usage over the years. A more plausible explanation is the VP elimination route through urine, which was incorporated in our predicted concentrations via ER, yet not considered in the aforementioned study (Berendsen et al., 2015). In addition, samples in Berendsen et al. (2015) were taken from the slaughterhouse, implying that last VP administration might have been weeks before, and therefore concentrations were expected to be lower than our predicted initial concentrations.

Table 2.2: *Comparison between VP concentrations measured in faeces (Berendsen et al., 2015) and predicted prior to storage VP concentrations in slurry manure.*

Calves				**Pigs**			
VP	Number of farms [a]	Measured range [mg/ton]	Predicted concentration [b] [mg/ton]	VP	Number of farms [a]	Measured range [mg/ton]	Predicted concentration [b, c] [mg/ton]
OTC	17	4 - 21000	ca. 2300	OTC	8	4 -1500	ca. 750
DC	11	5 - 177	ca. 5100	DC	9	2 - 4500[d]	ca. 2000
SDX	1	1 - 5	ca. 15	SDZ	6	1 -216	ca. 500
SDZ	12	1 - 81	ca. 2600	TYL	6	2 - 516[e]	ca. 50
TIL	8	1 - 218	ca. 900	TIA	2	1 - 4	ca. 250

a Number of farms measured range is based on.

b From Figure 2.4.

c Assumed concentration in sow slurry.

d At one farm the reported maximum was 95000. We considered this as an outlier.

e At one farm the reported maximum was 7700. We considered this as an outlier.

ca. Circa (=about).

Predicted VP concentrations in slurry manure after storage, for veal calves and pigs, were compared with the measurements done in 2017 in slurry manure in the province of Gelderland (Lahr et al., 2018). We assumed that concentrations in that study resulted from VP use in 2016 and 2017, hence we used average administered quantities of VPs over those years in the Netherlands (Figure S3), and considered only those found in the slurry at farms which actually reported those VPs as used, as detailed in Lahr et al (2018). This maximized the chance that measured VP amounts were a consequence of actual usage. The measured and calculated concentrations in slurry are shown in Figure 2.5. In another study, Lahr et al. (2014) provided VP quantities found in 2013 in fattening pigs slurries in storage tanks of processing installations in the province of North Brabant, and also shown in Figure 2.5. As no VP use data of 2013 were available, these points in Figure 2.5 used national use data from 2015 instead of 2013, which made the comparison more an impression. In all cases, we also estimated the quantities of produced slurry manure with respect to animal type and relevant year (CBS, 2019).

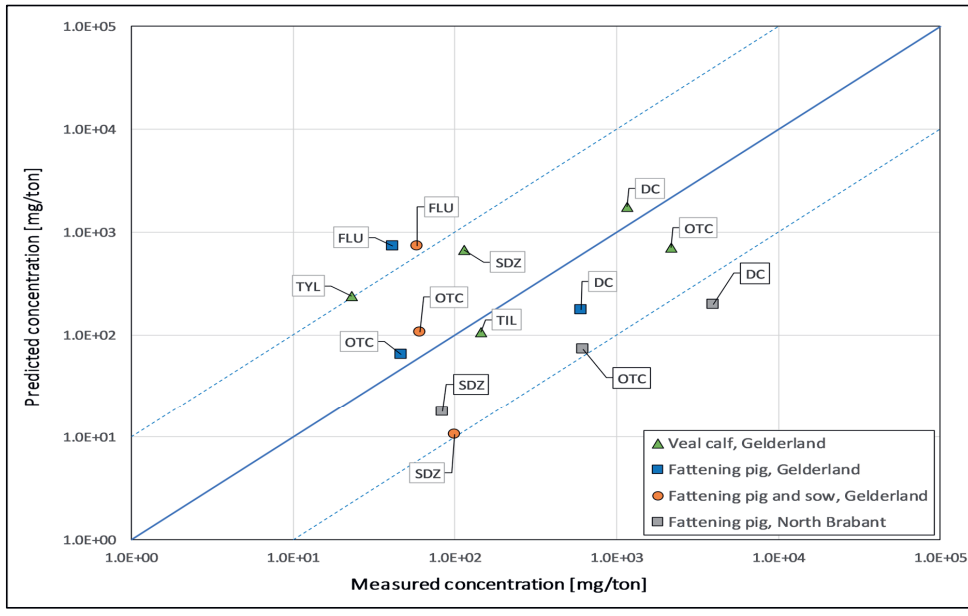


Figure 2.5: *Predicted and measured (fresh weight) concentrations in slurry manure. The solid line marks the ratio of 1:1, dotted lines differ a factor 10 from 1:1. Four cases* concern: in Gelderland measurements and estimations for 2016/2017, in North Brabant combined measurements for 2013 and estimations for 2015. When the concentration of a particular VP was measured in more than one farm of the same type, an average concentration was taken.*

**For the case when measurements were done in a combined slurry (fattening pigs and sows), estimates from both sectors were aggregated according to the portion of produced slurry (80% sow, 20% fattening pig).*

From totally 14 combinations of measured and predicted concentrations, 11 were within the 10-fold deviation from the ideal ratio of 1:1. Outside this range were only FLU (Gelderland) and DC (North Brabant). For FLU, our predicted concentration was around 15 times larger than measured, both for fattening pigs and the combined case. This could possibly be explained by the fact that DT50 of FLU was roughly estimated, as detailed in SM5. For DC, the predicted concentration at North Brabant was around 20 times lower than the measured one, even though the agreement for fattening pigs in Gelderland was good. We attribute this to our approximation of use in North Brabant. In view of the trends mentioned before, the use of DC in 2013 was likely higher than in 2015, which was possibly amplified at particular farms from where the slurry originated. As also for other two VPs in North Brabant our predictions were lower than measured quantities, this reasoning seems plausible. As two cases (TYL-veal calf, SDZ-fattening pig and sow) were based on single measurements, their reliability is in need of improvement.

As Figure 2.5 reveals, our estimates of VP usage in the veal calf sector resulted in predicted VP concentrations in the after-storage slurry that corresponded fairly well to measurements. In general, our predictions for hormone and antiparasitics (except FBZ) suggested that concentrations in the veal calf slurries after the storage were below the detection limits (Lahr et al., 2018), which for IVM was shown to be not always the case (Wohde et al., 2016b; Lahr et al., 2019). The possibly underestimated IVM administered to veal calves and its toxicity for manure organisms (Liebig et al., 2010), warrants closer examination.

Besides considering national VP usage, for veal calves in Gelderland (2016/2017) we also calculated after-storage concentrations with use data aggregated from the dairy cow farms located only in the Gelderland province. Despite the accuracy lost by regional aggregation, this gave a good (TYL, SDZ, OTC) or reasonable (DC) agreement with the national analysis (Figure S6).

2.3.4 Residue indicator

We calculated R (*eq.(2.4)*) for all VPs for dairy cows, sows and fattening pigs (Table S9). Based on R, and with respect to predicted after-storage concentrations (Figure 2.4), we performed a ranking in all three livestock categories, as shown in Table 2.3.

Table 2.3: *VP ranking based on residue indicator (R indicated in parentheses), with respect to predicted after-storage concentration for the period 2015-2018.*

Predicted concentration [mg/ton] - classes	Dairy cow	Sow	Fattening pig
High (>100)	/	1. FLU (0.448)	1. FLU (0.325)
	/	2. OTC (0.058)	2. DC (0.038)
	/	3. DC (0.035)	3. TIL (0.007)
Medium (>10 - 100)	1. FBZ (0.008)	1. TMP (0.06)	1. OTC (0.048)
	2. CTC (0.002)	2. TYL (0.005)	2. TIA (0.037)
	/	3. SDZ (0.003)	3. TMP (0.021)
Low (>1-10)	1. SDX (0.102)	1. IVM (0.035)	1. IVM (0.013)
	2. SDZ (0.054)	2. SDX (0.006)	2. SMX (0.001)
	3. OTC (0.049)	3. SMX (0.002)	/
Very low (≤1)	1. DEX (0.314)	1. DEX (0.109)	1. DEX (0.044)
	2. TC (0.024)	/	2. SDX (0.0002)
	3. TIL (0.004)	/	/

VPs with highest potential (highest R) of being present in the after-storage slurries at dairy cow farms in the Netherlands were DEX, SDX, SDZ and OTC. However, since their predicted concentrations in these slurries were very low to low (Figure 2.4-A), these VPs might have escaped measurement in view of detection limits (Lahr et al., 2018). On the other hand, ranking VPs based on R for sow farms indicated that FLU, DEX, OTC, TMP, DC and IVM were most probable to occur in slurry. For fattening pig slurry, the most probable to occur were FLU, OTC, DEX and DC. To arrive at F_u (to calculate R) for the veal calf sector, we used observations by Lahr et al (2018). That study revealed that four out of five investigated veal calf farms reported administration of OTC, DC and TIL and three out of five reported SDZ and IVM. This small sample resulted in F_u of 80% for OTC, DC and TIL, and 60% for SDZ and IVM, and we obtained R-values of 0.15 (DC), 0.1 (SDZ), 0.07 (TIL), 0.05 (OTC) and 0.05 (IVM). In addition, for those VPs (except IVM) our predicted concentrations in the after-storage veal calf slurry were in the range medium to high (Figure 2.4-A).

In summary, based on the residue indicators, we predicted that OTC was the most frequently soil-applied VP in the Netherlands, as it came out as prioritized in all investigated slurries, followed by DEX, DC, SDZ, FLU and IVM. These findings are coherent with the reported frequencies of detection in the earlier Dutch studies which targeted VPs in the slurry manure (Lahr et al., 2019), except for DEX, that

was not considered in those earlier studies. According to our predictions, DEX seems to have a high potential to be widely present in all investigated slurries (Table 2.3), although used DT50 and ER are subject to uncertainty (Table S7). In addition, for antibiotics our results seem comparable with studies done in other countries, as summarized by Wohde et al. (2016a) and Ghirardini et al. (2020), which also highlighted OTC, DC and SDZ as one of the most frequently detected VPs. Those studies indicated wide occurrence of CTC, whereas this was not expected in the Netherlands as only few farms reported administration of this VP.

Note that R is only indicating the potential risk with respect to produced manure. For the actual risk the spatial distribution of the applied manure should be also taken into account. This depends on the manure produced on farms, the available area of agricultural land, the manure transport from farm to farm, manure processing and export, and the admissible nitrogen and phosphorus applications rates being regulated by the EU Nitrates Directive. This aspect will be addressed in a next paper.

2.3.5 Uncertainties

For the steps in Figure 2.1, some assumptions have been made. To calculate VP concentration changes during storage, we disregarded if storage conditions as temperature varied from place to place. Implicitly, this variation may have been accounted for, as literature data on storage dissipation mostly concerned different Dutch manure types. For some VPs (e.g. DEX), such an implicit correction was not done. To obtain Figure 2.4, we used average yearly administered VP quantities despite that slurries at individual farms may deviate from those average values. Similar reasoning could also apply to the VP measured quantities, as they are known to deviate per farm (Lahr et al., 2014, 2018).

Based on the estimated concentrations in manure, the conversion between administered VP quantities in dairy cows to veal calves using factors given in Table S5 performed quite well. For at least one antibiotic from each group, predicted concentrations compared to measurements with a deviation<10-fold. In cases, where this conversion would not work, that was clearly mentioned (e.g. SDX and FLQ). However, our conversion of use data for the hormone and antiparasitics could not be validated, and there were indications that for the latter we underestimated the usage in the veal calf sector (e.g. IVM). Most reasonable explanation is that some of the medicines containing antiparasitic drugs are not recommended for use in animals producing milk for human consumption. Therefore, the assumption that veal calves receive the same number of doses annually as dairy cows is probably not valid for all antiparasitics and an individual approach is required. To completely explore this issue, an insight into the dairy cow farms that reported those VPs as used is necessary, but also a deeper

investigation on prescription data and withdrawal periods (in milk and meat). All of that was out of the scope in this paper.

Another important hypothesis related to the assumed VP excretion rates. In general very little is known about these parameters and their variations between animal types. Knowledge about VP excretion in specific animal (e.g. veal calf) is mostly based on the studies done more than 20 years ago, hence a lot of VPs are not even considered. Frequently, focus is either on excretion through urine, or faeces, whereas data related to both fractions are extremely limited. In addition, we focused only on the excretion of unmetabolized portion of VPs, whereas in reality some metabolites may be of greater interest (e.g. when metabolite is more toxic than the parent compound).

2.4 Conclusion

Given the uncertainty associated with the input data, which also differed between livestock sectors, our modelling approach showed estimated VPs emissions in manure and prioritization in VPs, which were in reasonable agreement with monitoring results of VPs. For dairy cow, sow and fattening pig sector, VP usage was investigated at the national scale, while using national datasets on farmer uses. For veal calves VP usage was approximated based on the administered VP quantities in dairy cows. Based on more than 40 different literature sources, we observed that available data on VP excretion and dissipation in manure show a large spreading, if available at all. This lack of accurate information is an important source of uncertainty in predicting VP emission to soil. Our results also showed that emissions of VPs to the environment varied per livestock sector due to differences in VP usage and excretion, and manure production. This aspect might be very relevant for identifying regions of potential risk and for spatial prioritization. Our modelling results showed further that the most commonly administered VPs are frequently not the ones ending up on the soil in high concentrations. Also, a VP residue indicator was developed to prioritize the potential for VP residues in soil-applied slurry manure at a national level. This indicator is based on VP excretion potential, VP behavior in the storage and number of farms where VP is reported as administered. Since influence of VP quantities administered to animals is indirectly excluded, this indicator requires less information and could be used as a first tier assessment to identify if compounds could pose a risk to the environment. This is especially convenient as there are nearly 900 active substances used as VPs in the Netherlands, whereas only a small portion of them is studied in detail.

Supplementary Material

SM1. Substance selection

Selection of Veterinary Pharmaceuticals (VPs) was done in three steps with respect to four animal categories. The overall focus was on antibiotics, antiparasitics and hormones.

Step 1. Dutch annual reports (SDa, 2020; Veldman et al., 2020) about VP use

These reports do not provide data on usage of individual VPs, but only on the antibiotic pharmacotherapeutic groups. Therefore, we identified the most commonly administered groups for further consideration:

Table S1. *Relevant pharmacotherapeutic groups with respect to animal category.*

Animal category	*Pharmacotherapeutic groups* [a]				
Veal calf	Tetracyclines	Macrolides	Trimethoprim/Sulfonamides	Penicillin	
Dairy cow	Tetracyclines	Macrolides	Trimethoprim/Sulfonamides	Penicillin	
Pig (sow and fattening pig)	Tetracyclines	Macrolides	Trimethoprim/Sulfonamides	Penicillin	Quinolones

a β-lactams are known to be rarely measured in the environment due to their instantaneous hydrolysis, hence penicillin group is not further considered in this paper. Details are given in the SM6.

Step 2. Dutch studies (Lahr et al., 2014, 2018, 2019) with VP measured concentrations in slurry

These studies confirm the presence of more than 30 different VPs in Dutch manure. From these 30 VPs, antibiotics were selected that belong to the groups given in Table S1. We selected only those antibiotics of which environmental properties (e.g. DT50, ER) were known or possible to estimate. The latter was the main criterium for the selection of antiparasitics, next to the detection in the Dutch slurries. Other important substances that were also found in manure are hormones, but most of them are either forbidden for usage and/or are (also) a result of natural biological processes. Those are disregarded in this study, but indicated in Table S2.

Table S2. *Hormones found in manure in the Netherlands.*

Substance	*CAS no.*
17 beta estradiol	50-28-2
17 alpha estradiol	57-91-0
Estrone	53-16-7
Progesterone	57-83-0
Androstendion	63-05-8
Beta testosterone	58-22-0

As an outcome of first two steps, 19 VPs were identified. For those, we investigated if the information on usage is available in the FADN dataset (step 3).

Step 3. Available data on annually administered quantities – FADN dataset

This step eliminated antiparasitic Fipronil (Cas no. 120068-37-3) and Toltrazuril (Cas no. 69004-03-1) from further consideration. Based on Dutch Veterinary Medicines Information Bank (2021), Fipronil is characterized as insecticide and not used for the animal categories of our interest, but widely used for the treatment of fleas and ticks on cats and dogs. The same source indicates that Toltrazuril has certain application as a coccidiostat drug in cattle and pig sectors. Note that besides Toltrazuril, also its sulfone metabolite - Ponazuril, is occasionally detected in Dutch manure (Lahr et al., 2018).

SM2. Use data structure (FADN dataset)

The acquired FADN dataset for the three livestock sectors is based on 350 to 380 farms, depending on the survey-year, as detailed in the Table S3.

Table S3. *Number of farms for the period 2015-2018 with respect to three animal categories.*

	Year				Total number of farms in the Netherlands (2017) [a]
Animal category	2015	2016	2017	2018	
Dairy cow	253	235	252	237	18247
Sow	56	52	60	57	1004
Fattening pig	60	63	68	57	1865

a (Lahr et al., 2019)

To calculate the VP administered amounts, the following information was extracted from the FADN dataset:

(1) *Annual mass of individual VP purchased at each farm to treat animals belonging to a specific livestock sector on that farm [mg/year];*

(2) *Number of animals per farm.*

VP yearly administered quantities per animal ([mg/animal per year]) on each farm were obtained according to

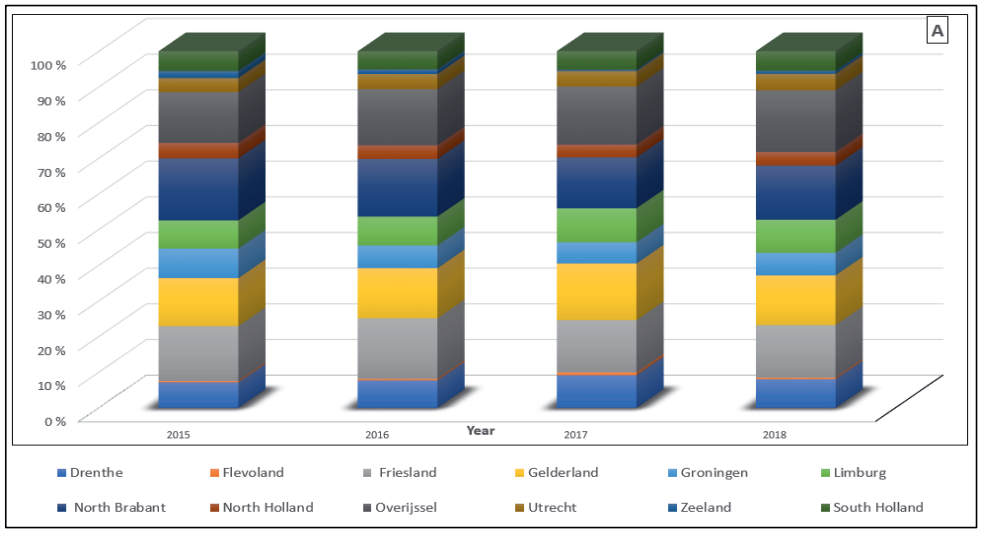
$$U = \frac{(1)}{(2)} \quad \text{(S2.1)}$$

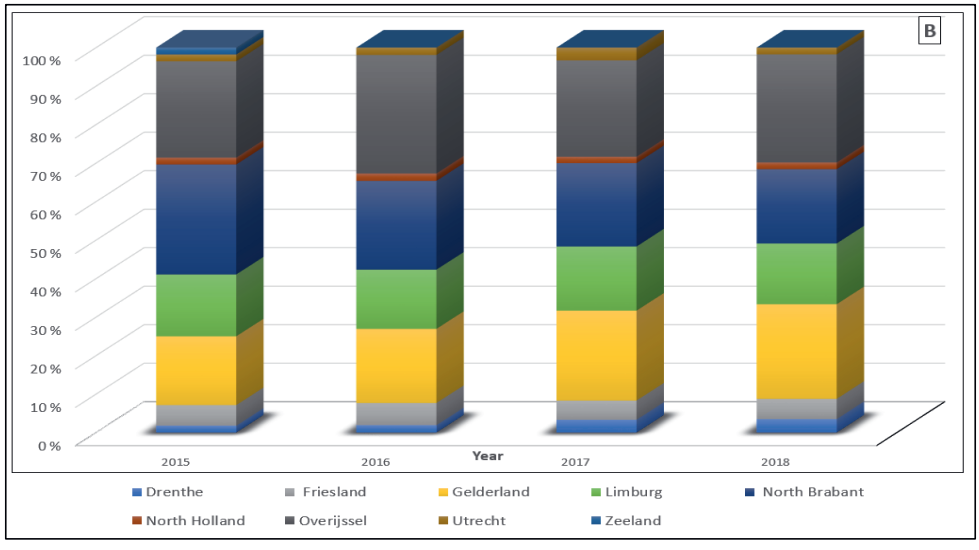
To estimate the national averages, previously calculated administered VP quantities from all farms were aggregated, as illustrated in Figure 2.3 and S2. Note that we considered VP quantities purchased at each farm as being used, which might not be always the case.

In addition, we extracted and processed:

- *Names of the administered drugs which contain the VP of our interest as an active ingredient*. This was used for comparison and cross check with Dutch Veterinary Medicines Information Bank (2021).

- *Farm location per province*, was used for providing an insight into the spatial distribution of investigated farms and variations in administered VPs, as illustrated with Figure S1 and Table S4.





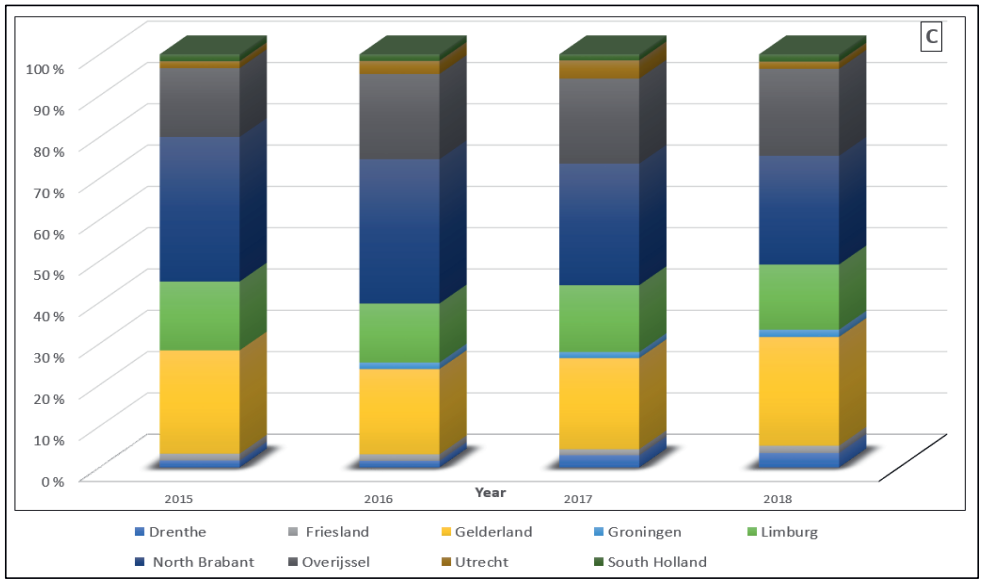


Figure S1. *Portion of investigated farms per particular Dutch province (12). (A) Dairy cow farms – located in all 12 provinces. (B) Sow farms – located in 9 provinces. (C) Fattening pig farms – located in 9 provinces.*

Spatial distribution of investigated farms is fully coherent with the farm densities reported by Lahr et al. (2019), implying that representative sample proportionally covers the territory of the Netherlands.

Table S4. *VPs administered in the Dutch provinces, with respect to three animal categories and four different years.*

Province	**Year**	**Reported VPs**		
		Dairy cow	**Sow**	**Fattening pig**
Drenthe	2015	TC, OTC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	FLU	OTC
	2016	TC, OTC, TMP, SDZ, SDX, TYL, IVM, PERM, DEX	DC, FLU	OTC, FLU
	2017	TC, OTC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDX, FLU, DEX	OTC, FLU
	2018	TC, OTC, TMP, SDZ, SDX, SMX, TIL, TYL, IVM, PERM, DEX	OTC, FLU, DEX	OTC, DC, TYL, FLU
Flevoland	2015	OTC, TMP, SDZ		
	2016	OTC, TMP, SDX		
	2017	OTC, TMP, SDZ, SDX, TIL, DEX		
	2018	OTC		

Table S4. *Continued*

Friesland	2015	TC, OTC, DC, CTC, TMP, SDZ, SDX, TIL, TYL, IVM, DEX	OTC, TMP, SMX, FLU, DEX	TIA, FLU
	2016	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, IVM, DEX	OTC, TMP, SMX, IVM, FLU, DEX	TIA, TYL, FLU
	2017	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, IVM, DEX	OTC, DC, TMP, SDZ, SDX, FLU, DEX	DC, TIA, FLU
	2018	TC, OTC, DC, TMP, SDZ, SDX, SMX, TIL, TYL, DEX	OTC, DC, TMP, SDZ, SMX, FLU, DEX	OTC, TIA, TYL, FLU
Gelderland	2015	TC, OTC, DC, CTC, TMP, SDZ, SDX, SMX, TIL, TYL, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, TIA, TYL, FLU, DEX
	2016	TC, OTC, CTC, TMP, SDZ, SDX, SMX, TYL, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIA, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, TIL, TYL, FLU, DEX
	2017	TC, OTC, DC, CTC, TMP, SDZ, SDX, SMX, TYL, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TYL, IVM, FLU, DEX	OTC, DC, TMP, SMX, TIA, TYL, FLU, DEX
	2018	TC, OTC, TMP, SDZ, SDX, SMX, TYL, IVM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TYL, IVM, FLU, DEX	OTC, DC, TMP, SMX, TIA, TYL, FLU, DEX
Groningen	2015	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, DEX		
	2016	TC, OTC, TMP, SDZ, SDX, TIL, TYL, DEX		OTC, DC, TYL, IVM
	2017	TC, OTC, TMP, SDZ, SDX, TYL, DEX		OTC, DC, TYL, IVM
	2018	TC, OTC, TMP, SDZ, SDX, SMX, TIL, TYL, DEX		OTC, DC, TYL, FLU
Limburg	2015	TC, OTC, TMP, SDZ, SDX, TIL, TYL, FBZ, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIL, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, SDX, TIA, TYL, FLU, DEX
	2016	TC, OTC, TMP, SDZ, SDX, TIL, TYL, FBZ, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIL, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TYL, FLU, DEX
	2017	TC, OTC, TMP, SDZ, SDX, TIL, TYL, FBZ, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TYL, FLQ, IVM, FLU, DEX	OTC, DC, TMP, SMX, TYL, IVM, FLU, DEX
	2018	TC, OTC, TMP, SDZ, SDX, SMX, TIL, TYL, FBZ, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TYL, FLQ, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TIA, TYL, IVM, FLU, DEX
North Brabant	2015	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIL, TIA, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TIA, TYL, FLU, DEX
	2016	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIL, TIA, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, SDX, TIL, TIA, TYL, FLU, DEX
	2017	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIL, TIA, TYL, FLQ, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TIA, TYL, FLU, DEX
	2018	TC, OTC, DC, TMP, SDZ, SDX, SMX, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIL, TYL, FLQ, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TIL, TIA, TYL, IVM, FLU, DEX

Table S4. *Continued*

North Holland	2015	OTC, TMP, SDZ, SDX, TYL, IVM, PERM, DEX	OTC, TMP, SMX, FLU	
	2016	TC, OTC, TMP, SDZ, SDX, TYL, DEX	OTC, FLU	
	2017	OTC, TMP, SDZ, SDX, TYL, PERM, DEX	OTC, TYL, FLU	
	2018	OTC, TMP, SDZ, SDX, SMX, TIL, TYL, DEX	FLU	
Overijssel	2015	TC, OTC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TYL, IVM, FLU, DEX
	2016	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIL, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TYL, IVM, FLU
	2017	TC, OTC, DC, TMP, SDZ, SDX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TIA, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SMX, TYL, IVM, FLU, DEX
	2018	TC, OTC, DC, TMP, SDZ, SDX, SMX, TIL, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, TYL, IVM, FLU, DEX	OTC, DC, TMP, SDZ, SDX, TIA, TYL, IVM, FLU, DEX
Utrecht	2015	TC, OTC, TMP, SDX, TYL, IVM, DEX	OTC, DC, TMP, SDZ, TYL	
	2016	TC, OTC, TMP, SDZ, SDX, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, FLU	OTC, DC, TMP, SMX, TYL, DEX
	2017	TC, OTC, TMP, SDZ, SDX, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SMX, TYL, FLU, DEX	OTC, DC, TYL, IVM, FLU, DEX
	2018	TC, OTC, TMP, SDZ, SDX, SMX, TYL, IVM, PERM, DEX	OTC, DC, TMP, SDZ, SDX, SMX, FLU	FLU
Zeeland	2015	TC, OTC, TMP, SDZ, SDX, DEX	OTC	
	2016	TC, OTC, TMP, SDZ, SDX, DEX		
	2017	OTC, TMP, SDZ, DEX		
	2018	OTC, TMP, SDZ, DEX		
South Holland	2015	TC, OTC, TMP, SDZ, SDX, SMX, TYL, DEX		OTC, FLU
	2016	TC, OTC, TMP, SDZ, SDX, TIL, TYL, PERM, DEX		OTC, TYL, FLU
	2017	TC, OTC, TMP, SDZ, SDX, TYL, PERM, DEX		OTC, TYL, FLU
	2018	TC, OTC, DC, TMP, SDZ, SDX, SMX, DEX		OTC, FLU

When a VP is not indicated in the particular province/year/animal category, this means that no farm from the dataset reported the VP as purchased, or that the type of farm was not investigated in that specific province (e.g. South Holland – sow category). On the other hand, if a VP is listed in the Table

S4, at least one farm located in a certain province reported that VP as purchased in the survey year. The variety of reported VPs depends on the sample size (Figure S1) and farm density (Lahr et al., 2019).

SM3. Conversion of use data – veal calves

Usage of antibiotics in the veal calf sector was estimated in accordance with the defined daily doses (i.e. DDDANAT). As detailed in the MARAN (Veldman et al., 2020) and SDa (2020) reports, this parameter is derived by first calculating the total number of treatable kilograms within a particular livestock sector for a specific year, and then dividing this number by the average number of kilograms of animal present within the livestock sector concerned. This unit of measurement is used to determine the amount of antibiotics administered within a particular livestock sector, irrespective of the types of livestock farms included in the livestock sector concerned. Even though not its main purpose, we used DDDANAT to obtain the antibiotic usage ratios between dairy cows and veal calves (Table S5), and to translate known administered quantities for dairy cows (FADN dataset) into that for veal calves. Note that Dutch farm statistics distinguishes between two veal types – white and red, and that VP usage may differ between them.

Table S5. *Conversion factors and estimated VP usage in the Dutch veal calf sector for the period 2015-2018.*

Group	Conversion factor F_c	VP	Usage [g/animal per year]
Tetracyclines		TC	0 [a]
	31	OTC	32 – 39 [b]
		DC	18 – 22 [b]
		CTC	81 – 99 [b, c]
Trimethoprim/ Sulfonamides	8	TMP/SDZ	13 – 16 [b, d]
		TMP/SMX	6 – 7 [b, d]
		TMP/SDX	0.1 [d, e]
Macrolides	55	TIL	3 – 4 [b]
		TYL	52 – 64 [b]
Quinolones		FLQ	1.8 [e]
Antiparasitics		IVM	0.02 – 0.03 [b, c]
		FBZ	1.3 – 1.6 [b, c]
		PERM	0.007 – 0.01 [b, c]
Hormones		DEX	0.0005 – 0.0006 [b, c]

a TC is only administered intrauterine and used for the treatment of afterbirth cows.

b Estimated range is based on the mean VP usage in a dairy cow sector (Figure 2.3) and percentage error (+-10%). Percentage error is a measurement of the discrepancy between an observed and a true, or accepted value, and equals $\frac{|X_{true} - X_{observed}|}{X_{true}} \times 100$

c Significant deviations possible.

d 20% is TMP, 80% is Sulfonamide antibiotic. Total usage of TMP considers contribution from all three mixtures.

e Estimated based on Lahr et al. (2019)

In order to estimate the usage of antiparasitics (IVM, FBZ and PERM) and hormone (DEX) in the veal calf sector, we converted the amounts used in the dairy cow sector (FADN dataset) to the one in veal calf based on the prescription data (Dutch Veterinary Medicines Information Bank, 2021). For a particular administered drug, prescriptions mention the usual VP amount given to dairy cows with each dose (in mass per animal). The drug dose was determined based on the standard weight of dairy cow, and converted to administered VP using the VP concentration of the drugs. VP amount given with one dose and the total per year result in the number of doses needed. For veal calves, the same number of doses but quantity adjusted for their weight gave the national used amounts as shown in Table S5. For this purpose, we assumed average weight of dairy cow as 600kg, and the one of veal calf as 172kg (Veldman et al., 2020). Conversion details are shown in the Table S6.

Table S6. *Drugs administered to dairy cows (FADN dataset) and their prescribed doses.*

VP	**Administered drug (FADN dataset)** [a]	**One full dose (based on weight)** [e]		**Number of doses administered per dairy cow in one year** [c]
		Dairy cow	Veal calf	
IVM	Noromectin [b]	100 mg	40 mg	0.5
FBZ	Panacur sr bolus [b]	24 g	12 g	0.125
PERM	Auriplak, ear tag for cattle [d]			
DEX	Dexa-ject	36 mg	10.5 mg	0.05

a Drug reported as administered to dairy cows. When several drugs are reported, the most common one was taken.
b This particular drug is not recommended for use in animals producing milk for human consumption. Still, it is reported as administered to dairy cows.
c Calculated by dividing the average yearly administered quantity (Figure 2.3) and prescribed dose.
d Used for protection against flies, so the dosage goes per ear tag/animal. Therefore, for veal calves we assumed the same administered quantity as in dairy cows.
e Dutch Veterinary Medicines Information Bank, 2021

Since we assumed the same number of yearly doses between dairy cows and veal calves, the administered quantities in the latter were obtained by multiplying the number of calculated doses with the VP amount in one dose (e.g. DEX -> 0.05*10.5). Note that drugs which contain IVM and FBZ as an active ingredients (Table S6) are not recommend for use in animals producing milk for human consumption. This could lead to underestimation of calculated usage in veal calves.

SM4. Estimated excretion rates

Excretion rates were estimated for six VPs in the cattle sector and for two in the pig, as detailed in the Table S7.

Table S7. *Estimated excretion rates (ER).*

VP	Animal sector	Estimated ER [%]	References used for estimation
TMP	Cattle	3	Alexander and Collett, 1975; Nielsen and Rasmussen, 1975a
SDZ	Cattle	80	Nouws et al., 1987; Duijkeren et al., 1994; Halling-Sørensen et al., 2001; Anderson et al., 2012
FLQ	Cattle/Pig	5	Harrison et al., 1986; Mevius et al., 1990; Villa et al., 2005
FBZ	Cattle	35	Prichard et al., 1981; Short et al., 1987; Short et al., 1988; Hennessy et al., 1993
PERM	Cattle	80	Pope, 2009; INCHEM, 2021
DEX	Cattle	60	Courtheyn et al., 1994; Vincenti et al., 2009; Vanhaecke et al., 2011
SDX	Pig	52	Nielsen and Rasmussen, 1975b; Halling-Sørensen et al., 2001; Qiu et al., 2016

To the best of our knowledge, ER of TMP involving both urine and faeces for cattle sector has not been reported in the literature. However, a study done by Nielsen and Rasmussen (1975a) showed that TMP was extensively metabolised in pigs, goats and cows with, respectively, 15%, 2% and 3% of parent compound excreted unchanged in urine. On the other hand, Alexander and Collett (1975) investigated behaviour of TMP in the horses and revealed that unchanged compound is detected in extremely small amounts in the faeces and around 10% in the urine. Considering all beforementioned, we assumed that unchanged TMP is being excreted mainly through urine, hence we assigned ER of 3% to dairy cows/veal calves.

Halling-Sorensen et al. (2001), based on both parent compound and metabolites, reported ER of 90% for SDZ in cattle. A few studies (Nouws et al., 1987; Duijkeren et al., 1994; Anderson et al., 2012) indicated that SDZ is barely metabolized in cattle and excreted mainly as a parent drug. Based on all mentioned studies, we assumed that around 80% of SDZ is being excreted in unchanged form in dairy cows/veal calves.

Research done by Mevius et al. (1990), showed that 3.2–6.5% of FLQ is excreted unchanged in the urine of veal calves. To the best of our knowledge, information on excreted portions in faeces of veal calves is not reported in the literature, hence we assumed ER of 5% for this animal type. Since we were unable to find any information about excretion in sows, we assumed the value from veal calves. However, few studies (Harrison et al., 1986; Villa et al., 2005) indicated that pharmacokinetics of FLQ varies in different animal types, therefore deviations from our assumed ER are possible.

In the case of FBZ, several studies (Prichard et al., 1981; Short et al., 1987; Short et al., 1988; Hennessy et al., 1993) reported different ER in cattle, thus we assumed the average value of 35%.

Regarding PERM, few studies (Pope, 2009; INCHEM, 2021) reported that in cattle around 80% is being excreted into faeces and urine as a parent compound.

According to Vanhaecke et al. (2011), faecal elimination of DEX in the calves proved to be quite rapid, but notably lower than urine excretion. This is in agreement with the studies by Courtheyn et al. (1994) and Vincenti et al. (2009), which revealed that DEX is mainly excreted in cattle urine as unmodified parent drug and that the amount of excreted concentration in faeces is only 2% of the one measured in urine. Therefore, we assumed that dominant excretion route of DEX in cattle is through urine and assigned an average ER from the studies mentioned above.

To the best of our knowledge, ER of SDX for pigs has not been reported in the literature. For this reason, to SDX we assigned an average ER based on the excreted amounts in pigs reported for other Sulfonamide antibiotics (Nielsen and Rasmussen, 1975b; Halling-Sørensen et al., 2001; Qiu et al., 2016). This estimated excretion is also comparable to the one reported for cattle.

SM5. Estimated half-lives (DT50) in stored manure

Dissipation rates of 11 antibiotics were obtained from Berendsen et al. (2018), while for the remaining VPs (6) we roughly estimated DT50 values based on different literature, as detailed in the Table S8.

Table S8. *DT50 in (stored) manure.*

VP	DT50 [days]		References used for estimation
	Cattle	Pig	
TMP	17	28	Menz et al., 2019; Hoeksma et al., 2020; VSDB, 2021
IVM	30	45	Pope, 2009; Wohde et al., 2016; VSDB, 2021
FLU		500	Kreuzig et al., 2007
FBZ	333		Kreuzig et al., 2007
PERM	5		Doyle et al., 1981; Pope, 2009; Hénault-Ethier, 2016; Ong et al., 2016; VSDB,2021
DEX	427.5	427.5	Combalbert et al., 2010; Combalbert et al., 2012; Zhang et al., 2019

Kreuzig et al. (2007) reported that due to slow degradation of FLU and FBZ in pig manure, a storage period of six months is not considered to substantially reduce the environmental exposure. In particular, they said that during the storage period of 100 days, concentration of FLU and FBZ reduced

for around 10 and 15%. Accordingly, we estimated that time needed to dissipate for 50% is 500 (FLU) and 333 (FBZ) days. For FBZ, we assumed that this is also the case in cattle manure.

Pope (2009) stated that pyrethroids (e.g. PERM) are only slightly-to-moderately persistent in soils, but that no data are available about their persistence in manure. DT50 in soil goes up to 40 days (VSDB, 2021), but by adding cattle manure into the soil there is an increase in the breakdown of PERM by near 100% (depending on the amount of added manure) (Doyle et al., 1981). Research by Ong et al. (2016) showed that DT50 of another pyrethroid insecticide, Cypermethrin, in poultry manure is lower than four days. Since pyrethroids are considered to have similar physic-chemical properties that affect their persistence and fate in the environment (Hénault-Ethier, 2016), we could assume alike behaviour also for PERM. Considering all beforementioned, we concluded that PERM is generally not persistent in manure and therefore assigned a DT50 of five days. However, note that assigned dissipation rate not necessarily relates to the stored manure and that it may vary under different environmental conditions.

Even though not specifically related to DEX, few studies (Combalbert et al., 2010; Combalbert et al., 2012; Zhang et al., 2019) indicated that only a minor degradation of steroid hormones (e.g. DEX) is expected during manure storage period of six months. Based on those studies, we roughly estimated DT50 for DEX somewhere in the range of 400-455 days for both cattle and pig manure.

In addition to the VPs reported in Table S8, DT50 of TYL in cattle manure has been estimated based on the values reported in few studies (De Liguoro et al., 2003; Scott Teeter and Meyerhoff, 2003; VSDB, 2021), whereas DT50 in pig manure has been taken from Berendsen et al. (2018).

SM6. VP administration

Comparison of Fu of VPs used in all investigated livestock sectors reveals a substantial differences for DC and SDX (Figure 2.2). DC is hardly used for dairy cows (on <3% of farms) but common for pigs (around 50% of farms), whereas SDX is negligible for fattening pigs (<2% of farms) but frequent for dairy cows (68% of farms) and sows (45% of farms). For DC and SDX, we compared the administered medicines and their brand names with the data from Dutch Veterinary Medicines Information Bank (2021) and found that some medicines which contain DC (e.g. Doxycycline 500 mg / g powder) are not recommended for use in animals producing milk for human consumption. As for SDX, the medicine reported as administered to fattening pigs is DOFATRIM-JECT (mixture TMP/SDX) and its applicability in the pig sector is quite broad (e.g. treatment of arthritis). Based on the regulations for DOFATRIM-JECT, no special warnings or side effects are indicated for fattening pigs, hence seldom usage of SDX in that particular sector requires further investigation. Furthermore, even though not listed in the Table 2.1, it is worth to mention that according to the analysed dataset, the compound Amoxicillin, alone or

in combination with Clavulanic acid/Colistin, has been extensively used to treat animals. Nonetheless, some studies (Berendsen et al., 2015; Zhang et al., 2015) indicated that β-lactams such as Amoxicillin are rarely found in the environment due to their instantaneous hydrolysis, which agrees with reported measurements in the Netherlands (Lahr et al., 2019), where no Amoxicillin was found in manure, soil or water. Hence, Amoxicillin is not further considered in this paper.

Distribution of the VP use data for sows and fattening pigs is given in Figure S2.

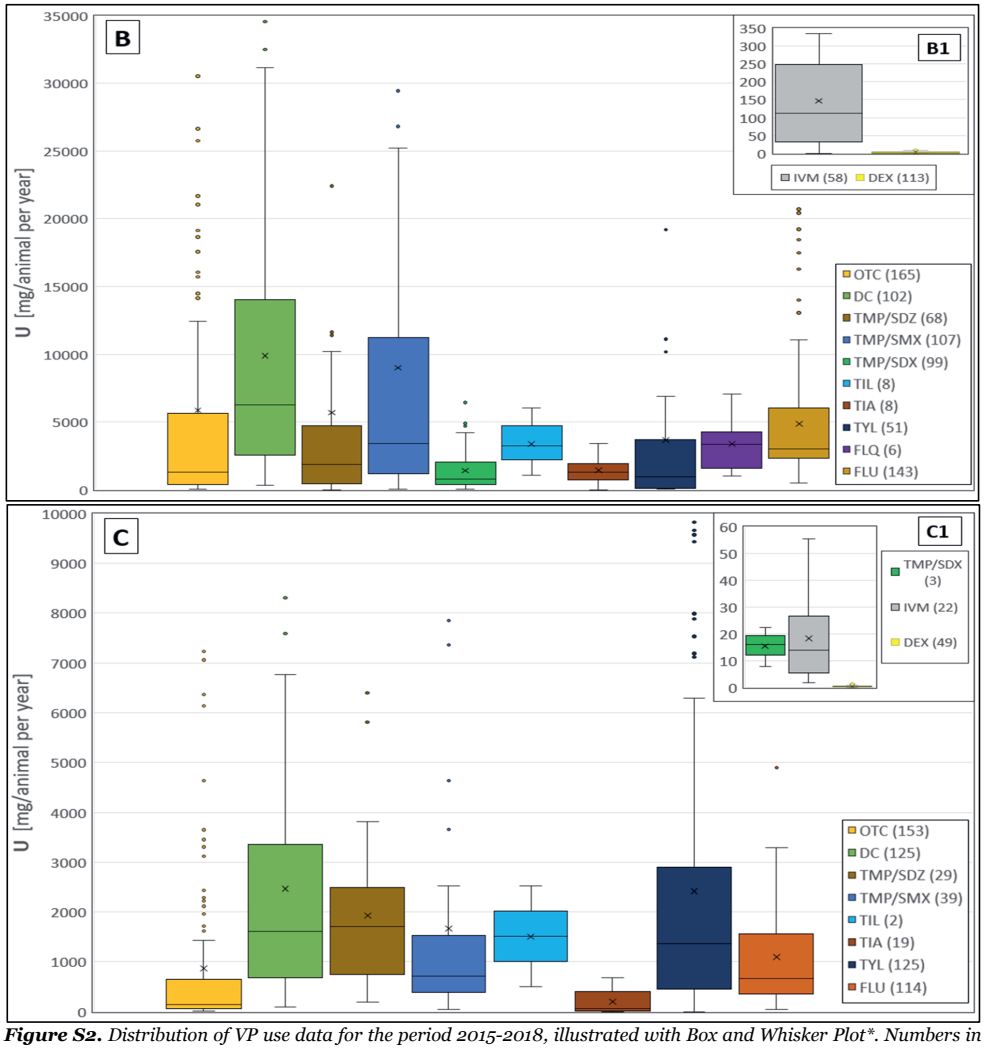


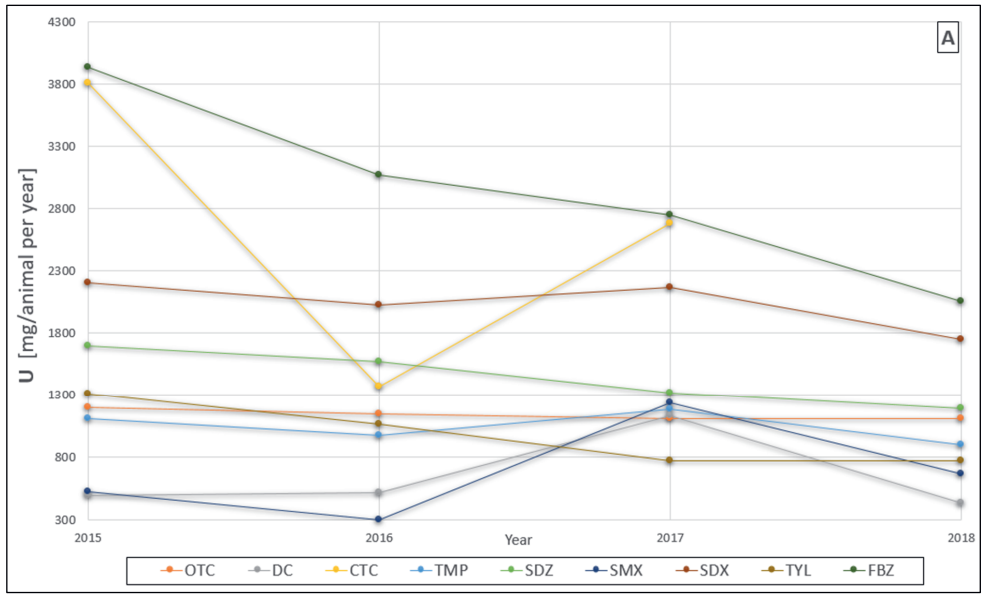
Figure S2. *Distribution of VP use data for the period 2015-2018, illustrated with Box and Whisker Plot*. Numbers in legend indicate sample size**. (B) VP use in the sow sector; inset (B1) for two VPs with administered quantities below 350 mg/animal per year. (C) VP use in the fattening pig sector, where (C1) illustrates VPs whose administered quantities are below 60 mg/animal per year.*

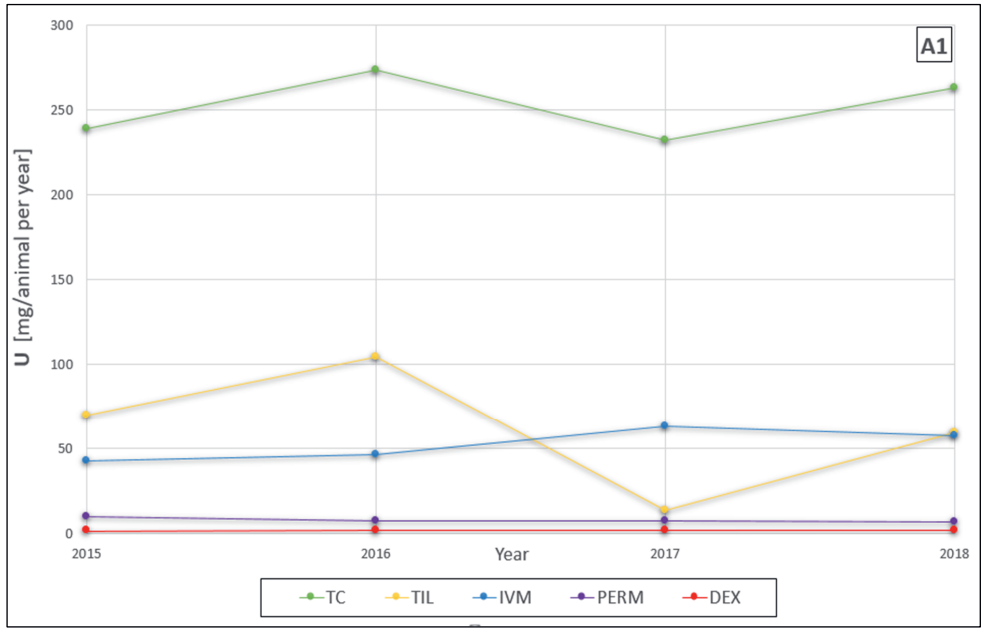
**A Box and Whisker Plot shows the minimum value, first quartile, median, third quartile and maximum value of a data set. The line inside the box represents the median, whereas x in the box represents the mean. The bottom line of the box*

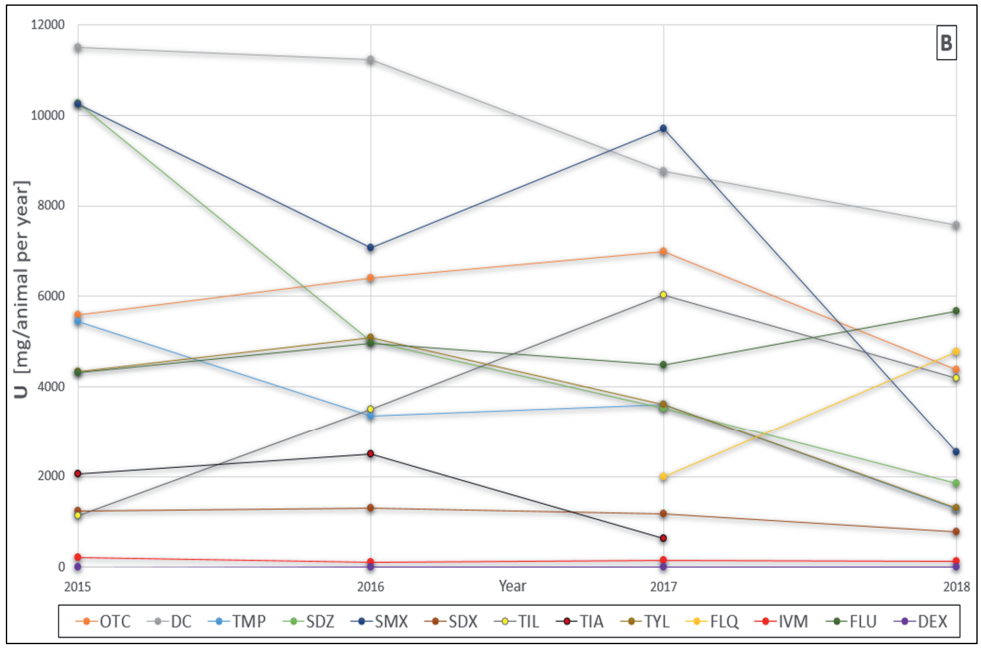
represents the median of the bottom half or 1st quartile. The top line of the box represents the median of the top half or 3rd quartile. The whiskers (error bars) extend from the ends of the box to the minimum and maximum value. Points outside whiskers are considered as an outliers.

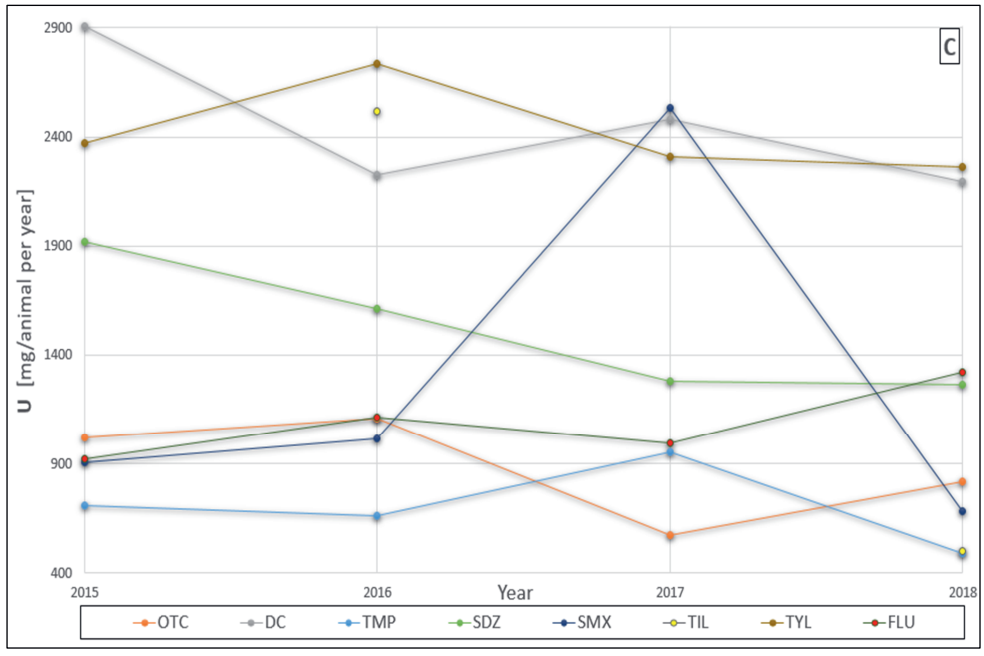
***When the same farm reports VP use for more than one year, the input from that particular farm is counted in each corresponding year. Thus, the numbers in the legend do not indicate the total number of considered farms, but the total number of available VP use values.*

SM7. VP administered quantities - trends






B
12000
10000
8000
6000
4000
2000
0
U [mg/animal per year]
2015
2016
2017
2018
Year
OTC
DC
TMP
SDZ
SMX
SDX
TIL
TIA
TYL
FLQ
IVM
FLU
DEX


C
2900
2400
1900
1400
900
400
U [mg/animal per year]
2015
2016
2017
2018
Year
OTC
DC
TMP
SDZ
SMX
TIL
TYL
FLU

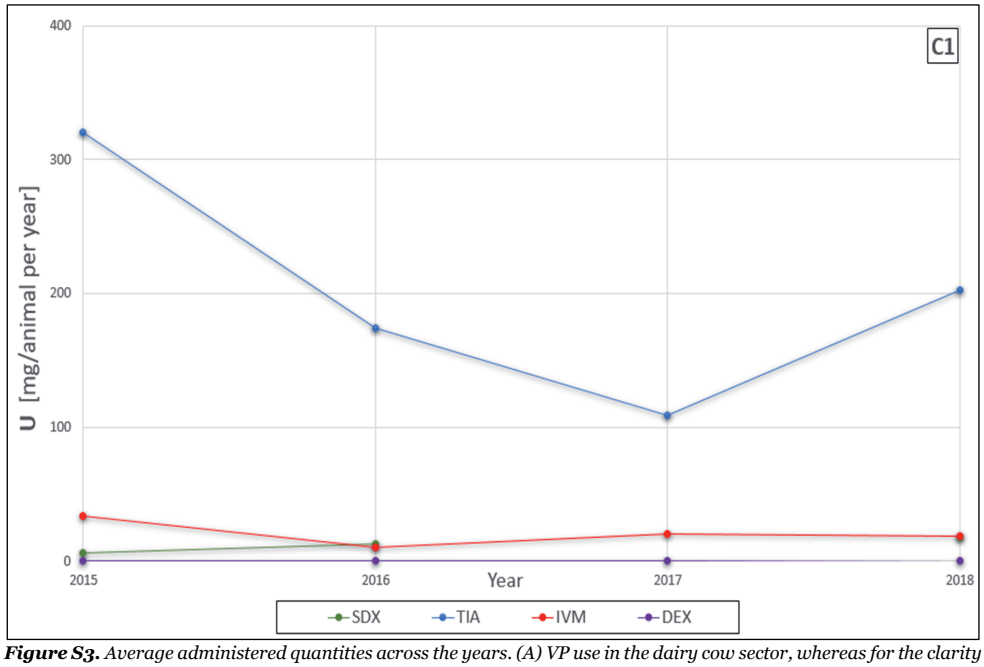


Figure S3. *Average administered quantities across the years. (A) VP use in the dairy cow sector, whereas for the clarity reasons (A1) shows VPs for which administered quantities are below 300 mg/animal per year. (B) VP use in the sow sector. (C) VP use in the fattening pig sector, where (C1) illustrates VPs whose administered quantities are below 400 mg/animal per year.*

As Figure S3 reveals, exceptions where administered quantities in 2018 are not lower than in 2015 are TMP/SMX and IVM for dairy cows, with 27% and 35% higher use in 2018 than in 2015; TIL, FLU and DEX for sows, with 270%, 32% and 57% higher use in 2018 than in 2015; TMP/SDX and FLU for fattening pigs, with 188% and 44% higher use in 2018 than in 2015. Partly these exceptions may be caused by the relatively small farm sample sizes for these VPs (TMP/SMX, TIL, TMP/SDX and IVM, see Figure 2.2). Since observations for FLU and DEX are based on a considerable sample size (Figure 2.2), an increase in use for those VPs is convincing. Furthermore, although Figure S3-A suggests significant use of CTC, the data are from merely four farms hence reliability is low.

SM8. Storage modelling - figures

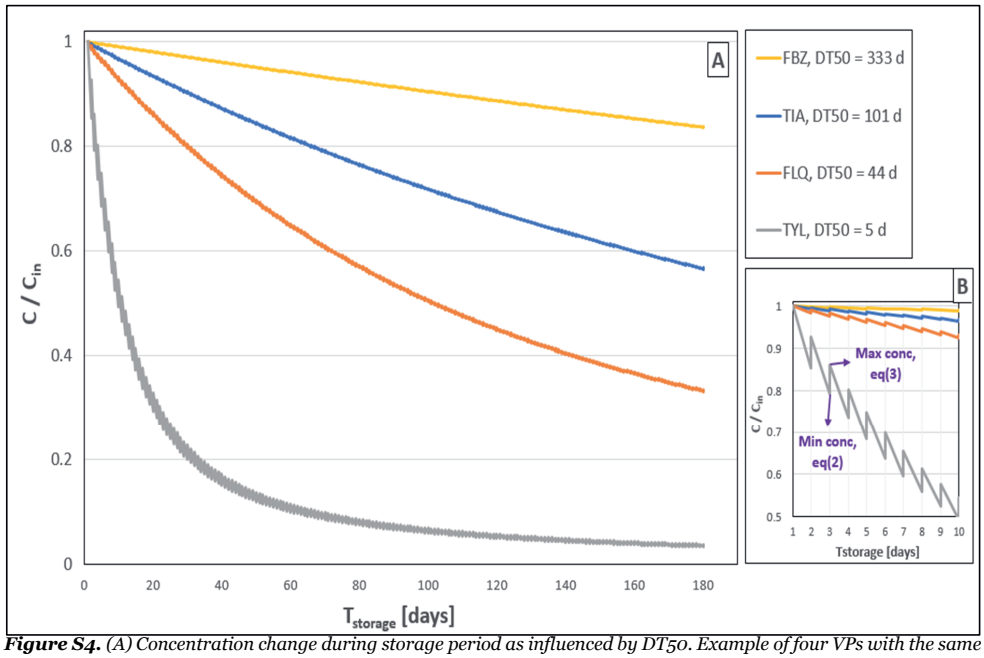


Figure S4. *(A) Concentration change during storage period as influenced by DT50. Example of four VPs with the same initial (prior to storage) concentration – FBZ and TYL in cattle manure, TIA and FLQ in pig manure. Inset (B): Illustration of points associated with the moment just before new manure is placed into the storage (eq.(2.2)) and immediately after placement (eq.(2.3)).*

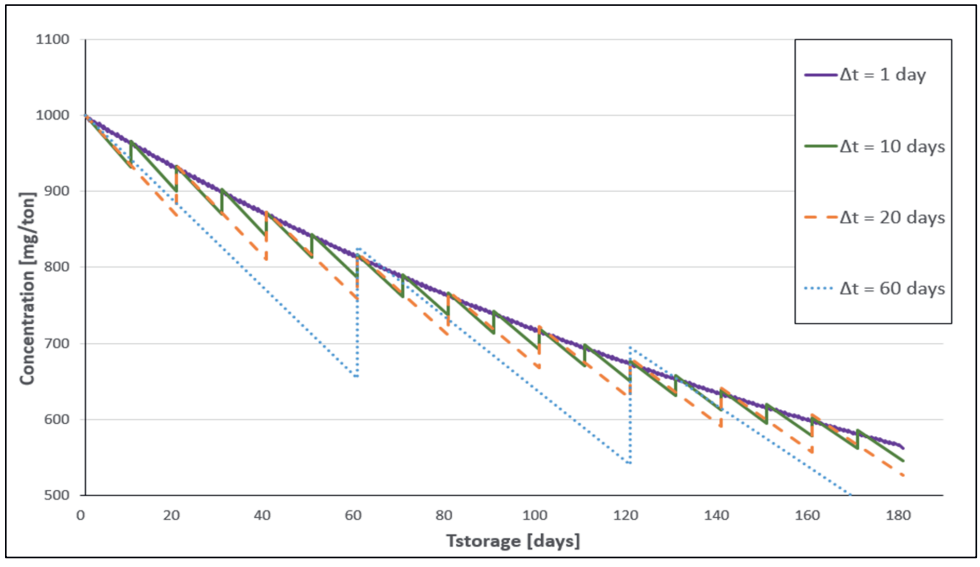


Figure S5. *Concentration distribution in the case of periodic manure additions into the storage. Illustration of the sawtooth patterns for the case of TIA in the pig manure (DT50=101 d), with assumed Cin of 1000mg/ton.*

SM9. Results validation

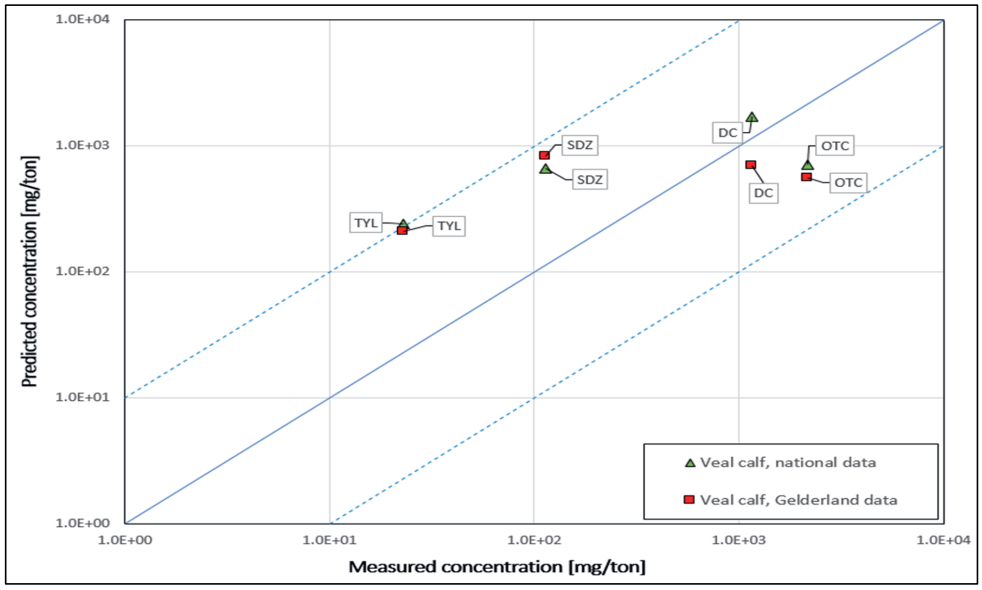


Figure S6. *Predicted and measured concentrations in the slurry. The continuous line marks the ideal ratio of 1:1, dotted lines indicate a 10-fold deviation. Predicted concentrations are based on the average national administered quantities (green triangle) and average administered quantities on the farms located in the province of Gelderland (red square). Both situations refer to period 2016/2017. Note that TIL was not compared, as no dairy cow farm reported it as administered in the Gelderland for the investigated period.*

SM10. Residue Indicator (R)

Table S9. *Calculated R for all investigated VPs with respect to three animal categories.*

VP	Dairy cow	Sow	Fattening pig
TC	0.024		
OTC	0.049	0.058	0.048
DC	0.006	0.035	0.038
CTC	0.002		
TMP	0.004	0.060	0.021
SDX	0.102	0.006	0.0002
SDZ	0.054	0.003	0.001
SMX	0.001	0.002	0.001
TIL	0.004	0.011	0.007
TIA		0.026	0.037
TYL	0.003	0.005	0.010
FLQ		0.001	
IVM	0.004	0.035	0.013
FLU		0.448	0.325
FBZ	0.008		
PERM	0.002		
DEX	0.314	0.109	0.044

Chapter 3

An Analytical Framework on the Leaching Potential of Veterinary Pharmaceuticals: A Case Study for the Netherlands

Based on:

Rakonjac N, van der Zee SEATM, Wipfler L, Roex E, Urbina CAF, Borgers LH, et al. An analytical framework on the leaching potential of veterinary pharmaceuticals: A case study for the Netherlands. Science of The Total Environment 2023; 859: 160310.

Abstract

Veterinary pharmaceuticals (VPs) residues may end up on the soil via manure, and from there can be transported to groundwater due to leaching. In this study an analytical framework to estimate the leaching potential of VPs at the national scale is presented. This approach takes soil-applied VPs concentrations, soil-hydraulic and soil-chemical properties, groundwater levels, sorption and degradation of VPs into account. For six commonly soil-applied VPs in the Netherlands, we assess quantities leached to groundwater and their spatial distribution, as well as the relative importance of processes that drive leaching. Our results for VPs Oxytetracycline, Doxycycline, and Ivermectin indicate that maximum quantities that may leach to groundwater are very low, i.e. << 1 ug/ha, hence spatial differences are not investigated. For VPs Sulfadiazine and Flubendazole we identify a few regions that are potentially prone to leaching, with leached quantities higher than 1ug/ha. Leaching patterns of these two VPs are dominated by soil properties and groundwater levels rather than soil-applied quantities. For Dexamethasone, even though applied on the soil in much lower concentrations compared to other investigated VPs, spatially widespread leaching to groundwater is found, with leached quantities higher than 1ug/ha. Due to the leaching affinity of Dexamethasone, variations in the soil-applied amounts have significant influence on the quantities leached to groundwater. Dexamethasone is highlighted as important for the future environmental risk assessment efforts. This study has shown that the leaching potential of VPs is not determined by one single parameter, but by a combination of parameters. This combination also depends on the compound investigated.

3.1 Introduction

Veterinary pharmaceuticals (VPs) are used to treat or prevent diseases of animals. Which specific VP is used varies per disease, animal sector and region (Berendsen et al., 2018). A prominent way by which VPs enter the environment is the excretion of urine and faeces from medicated animals and application of contaminated manure to agricultural land (Boxall et al., 2004). Thereafter, VPs may reach the groundwater due to leaching (Blackwell et al., 2009) and possibly affect water quality (Ostermann et al., 2013). Considering that groundwater is an important source of drinking water in Europe, and already high pollution pressures exerted by man-made chemicals on groundwater bodies (Tiktak et al., 2006; Stuart et al., 2012; Sanchez-Gonzalez et al., 2013; Rasheed et al., 2019; Wanner, 2021), identifying and quantifying VPs in groundwater is important. Many studies have confirmed presence of various VPs in groundwater (Lapworth et al., 2012; Mooney et al., 2020; Mooney et al., 2021), while some investigated environmental conditions that are relevant for VP leaching to groundwater (Pan and Chu, 2017; Gros et al., 2021). VP leaching to groundwater is mostly quantified through experiments and monitoring (Popova et al., 2013; Spielmeyer et al., 2017; Spielmeyer et al., 2020), whereas only few studies explored VPs leaching to groundwater through modelling (Di Guardo and Finizio, 2017). The lack of latter approaches represents a limitation in estimating the VPs pollution levels at e.g. national scales (Wohler et al., 2021). The advantage of a mechanistic modelling approach is that it is applicable to various environmental conditions and different types of VPs, whereas experimental efforts provide results for particular situations only. In addition, a model which estimates VPs leaching to groundwater could be useful for legislators and policy makers to identify environmental conditions and VP types that provide the highest environmental leaching potential in soil and groundwater systems. Therefore, modelling approaches could represent an important asset in determining the risk to the environment for these compounds.

In the Netherlands, manure from intensive livestock farming is spread onto arable land and grassland in considerable amounts. Consequently, together with Belgium, Netherlands shapes one of the highest nitrogen input regions in Europe (de Vries et al., 2021). Besides, applied manure may contain a broad range of VPs of different quantities, as detailed in Rakonjac et al. (2022). Dutch groundwater quality is systematically monitored and results affirm occurrence of VPs at diverse locations (Loon et al., 2020). In addition, scientific studies confirm the presence of VPs in groundwater of different ages and at different depths (Kivits et al., 2018). However, information concerning the VP origin, travel time through the unsaturated zone, and impact of spatial variable conditions (e.g. soil characteristics) on the VP transport is highly lacking, thereby hampering a proper risk assessment of this group of compounds. To the best of our knowledge, so far only two Dutch studies (Lahr and van den Berg, 2009;

Hoeksma et al., 2020) investigated spatially distributed modelling to evaluate the VP leaching to groundwater. In both cases, a pesticide-targeted model GeoPEARL (Tiktak et al., 2002) was used and the obtained results provided an aggregated national overview, whereas the details relevant for georeferenced local situations (fields) were not provided. This lack of local scale information limits the identification of groundwater vulnerable areas to VPs. Furthermore, both mentioned studies highlighted the large uncertainty in the input data, particularly in the estimated VP load. For these reasons, there is a need for an improved approach that will integrate the most detailed level of available data, but also takes into account the time processing efficiency of the calculations. The latter is especially relevant considering the number of VPs used in the Netherlands and yearly variations in their used quantities (Rakonjac et al., 2022).

Processes and conditions that affect VP leaching are similar to those involved for the leaching of many pesticides. Therefore, in this paper, we further elaborate on the analytical framework of van der Zee and Boesten (1991) concerning pesticide leaching to groundwater to estimate the VPs leaching towards phreatic groundwater. To quantify annual VPs loads, we use the VPs concentration in soil-applied slurry manure as derived in Rakonjac et al. (2022) and combine these with spatial allocation of manure soil-applied amounts which are given by the (national) model INITIATOR (Kros et al., 2019). Besides VP loads, we take into account local soil-hydraulic and soil-chemical properties, local groundwater levels, and VP environmental properties, to feed into the analytical model of van der Zee and Boesten (1991). Our approach is applied at a national scale to the Netherlands and provides spatially distributed quantification of VP leaching to groundwater at the spatial resolution of the field. According to our knowledge, this is the first VP-targeted approach to combine the abovementioned input data at local scale, resulting a time-efficient national scale model for assessing VP leaching to groundwater. Additional objective of this study is to provide suggestions on how to extend the analytical model with soil layering, and identify national hotspot (vulnerable) leaching locations, while considering soil and crop characteristics. This study further aims to determine the relative importance of various processes affecting VP leaching, and to distinguish them between the investigated VPs. Additionally, to address the influence of VP origin, we investigate the impacts of the VP loadings on leaching and the contribution of the different manure types. The framework proposed in this paper can be used to identify groundwater vulnerability from VPs at different spatial scales (local to national), thereby providing an important asset in environmental risk assessment of VPs.

3.2 Methodology

3.2.1 Leached fraction: Conceptual model

To assess spatially distributed leaching for every field in the Netherlands we assume that each field can be represented by a hypothetical equivalent soil column. This column consists of several soil layers of varying thickness, and with an upper boundary at the soil surface, where the VP via manure is introduced (i.e. VP load), and a lower boundary at groundwater level, as illustrated in Figure 3.1. Soil-hydraulic properties and organic matter content may vary per layer. The groundwater level is spatially variable and is assumed to be constant over time. After VP application on the soil surface, all layers above the phreatic water level contribute to the leaching. If the groundwater level is located within a particular soil layer (Figure 3.1 - left) only the part above groundwater level is taken into account to calculate the leaching. If groundwater level is located below the lowest (known) layer, we extend that layer and its properties till the phreatic water level (Figure 3.1 - right).

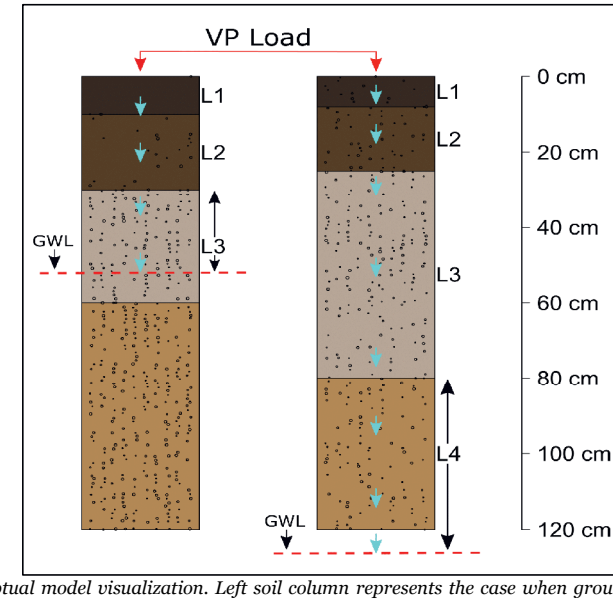


Figure 3.1: *Conceptual model visualization. Left soil column represents the case when groundwater level (GWL) is located within soil layer, while right soil column illustrates situation when GWL is below the lowest known soil layer. In both cases, soil layer thicknesses relevant for VP leaching are corrected (L3 and L4).*

Referring for details of the derivations to van der Zee and Boesten (1991), we assume that the VP leached fraction of each soil layer, F [-], is defined as the exponent of (minus) the transformation rate times the residence time in the layer i, i.e.

$$F_i = \exp\left(-\frac{\mu_i * R_i * L_i}{v_i}\right) \quad (3.1)$$

where μ represents the first-order transformation rate of VP in the soil [T^{-1}], R is the retardation factor [-], L corresponds to the thickness of the soil layer [L], and v is the pore water velocity [$L\ T^{-1}$]. Note that *eq.(3.1)* is based on the concept that both dissolved and adsorbed solute can degrade, where R reflects the solute travel time dependence on retarding processes such as sorption. A large R indicates that VP transport to groundwater will be retarded and VPs therefore reside in the soil for a longer time. R of a soil layer *i* is defined in the model as

$$R_i = 1 + \frac{\rho_i}{\theta_i} * f_{om,i} * K_{om,i} \quad (3.2)$$

where ρ represents the dry bulk density of the soil [$M\ L^{-3}$], θ is volumetric water fraction [$L^3\ L^{-3}$], f_{om} is mass fraction of organic matter [$M\ M^{-1}$], and K_{om} is coefficient for distribution over organic matter and water [$L^3\ M^{-1}$], which defines the sorption capacity of a VP. To calculate R and subsequently F, an estimation on pore water velocity, v, is needed. To pursue flow of water analytically, we assume a steady state situations, driven by excess precipitation. For this purpose, we recognise water flow rate as a specific discharge q [$L^3\ L^{-2}\ T^{-1}$] and calculate rates depending on annual precipitation, evaporation, and transpiration, which are obtained from the Royal Netherlands Meteorological Institute (KNMI). For the top soil layer, where root water uptake gradually diminishes the flow rate with increasing depth, we assume q driven by precipitation minus evaporation. Below the top layer, we account for the loss of transpiration water and the flow rate is therefore equal to precipitation minus evapotranspiration, and smaller than for the top layer. This implies that we assume that the crop transpiration water is taken from the upper layer.

For both the top soil and layers below, we then apply the Staring series approach which is used for deriving the standard soil-hydraulic characteristics (Heinen et al., 2020). Heinen et al. (2020) map soil types in the Netherlands and provide standard soil-hydraulic properties for 36 unique building blocks: 18 top soils and 18 subsoils. Each building block is described, among other things, with an average water-retention capacity and hydraulic conductivity. Then, depending on the soil layer and its properties we assume the previously calculated q to be equal to the hydraulic conductivity and from that we calculate hydraulic heads via Mualem equation (Mualem, 1976). We further use those as an input into van Genuchten equation (van Genuchten, 1980) to estimate the time-averaged volumetric water fraction θ. Finally, we calculate pore water velocities based on the *eq.(3.3)*. Details and equations about the above mentioned water flow estimation procedure are revealed in the Supplementary Material (see SM1).

$$v_i = \frac{q_i}{\theta_i} \tag{3.3}$$

In addition to sorption, dissipation due to e.g. microbial degradation or other degradation processes has a significant impact on the leached fraction. Degradation kinetics might differ between the VPs and soil types (Berendsen et al., 2021), hence we use a pragmatic approach and assume first order degradation kinetics. Literature data on VPs half-lives in the soil, τ_0 [T], differs depending on e.g. the source (i.e. country/region), soil type and conditions (Cycon et al., 2019). We use VPs half-lives found in the literature and consider them as representative for the first (top) soil layers (*eq.(3.4)*), but we are aware that field conditions may vary or have a gradient in depth (e.g. temperature) (van den Berg et al., 2016). To partly encompass for differences in biological activity, we assume that μ in the soil is proportional to organic matter content, and correct μ for the lower layers as given with *eq.(3.4 and 3.5)*. This correction is also proposed for pesticides by Boesten and van der Linden (1991) and van der Zee and Boesten (1991).

$$\mu_1 = \frac{\ln(2)}{\tau_0} \tag{3.4}$$

$$\mu_i = \mu_1 * \left(\frac{f_{om,i}}{f_{om,1}} \right) \tag{3.5}$$

In line with van der Zee and Boesten (1991), we propose that leached fractions from all soil layers above the groundwater level affect the total VP leached fraction, F_{total} [-], of a hypothetical soil column. In a nutshell, when the VP fraction leached from a layer enters the next layer, it may further dissipate but partly also transport to the layers below. Together, they form a series of leaching layers as given in *eq.(3.6)*. This allows to include the impact per soil layer of which the properties vary.

$$F_{total} = \prod_{i=1} F_i \tag{3.6}$$

By coupling F_{total} with the soil-applied VP load per field (A [M L^{-2}]), we calculate the total VP leached quantity (T [M L^{-2}]) for each field, as given by

$$T = F_{total} * A \tag{3.7}$$

Details regarding A are provided in Section 3.2.3.

3.2.2 Parameterization of the spatially distributed model

Spatially distributed data is taken from available datasets combined with geographic maps. Sources and adjustments are discussed hereafter. To spatially identify individual fields on which manure is applied, we use a geographic data shapefile from Dutch Crop Parcels Database (BRP, 2022), for the year 2017. The spatial scale of used BRP data is 1:25000. For obtaining soil properties, we use Soil Physical Unit Map (Wosten et al. (2013), spatial scale 1:50000) which provides the spatial distribution of different soil physical units in the Netherlands. Each unit has a schematic representation of the soil profile with affiliated soil layers (up to 7 and a soil depth of 120cm). Soil-hydraulic and soil-chemical characteristics are associated with the individual soil layers on the basis of the Staring series approach. To estimate Dutch groundwater levels, we use a publicly available map with 19 groundwater level classes (spatial scale 1:50000) describing the average fluctuations of the groundwater level, and classes of highest and lowest levels (Knotters et al., 2018). However, the public (free) version of the mentioned map covers only 95% of the BRP identified fields, particularly missing the fields located in higher and hilly areas of Limburg province and Veluwe region (5%). For these regions we extrapolate the map (via the Raster Calculator tool in ArcGIS Pro) resulting in a good comparison with other publicly available data about Dutch groundwater levels. More details can be found in the SM2 of this chapter. Since our approach assumes a constant groundwater level per field, for each groundwater level class we derive the average of both the highest and lowest ranges and assume their mean value as a representative one. Finally, we identified for each field from BRP, the soil type and the averaged groundwater level. Identification was done based on the largest fraction of soil type/groundwater level class per specific field. Based on the groundwater level and the soil type, soil-hydraulic and soil-chemical properties were derived for each field and per layer. Details and technical (software) procedures are provided in the SM3 of this chapter.

3.2.3 Estimation of VP loadings

The spatial distribution of VP loadings is significantly influenced by local manure application patterns. These local patterns depend on the amount and type of manure produced on nearby farms, the available area of agricultural land, the manure transport from farm to farm and optional manure processing and export. Manure application is confided by admissible nitrogen and phosphorus applications rates being regulated by the EU Nitrates Directive (Kros et al., 2019; de Vries et al., 2021). With regard to the Nitrates Directive 91/676/EEC (EC, 1991) the Netherlands as a whole is assigned as a vulnerable zone implying a strict maximum threshold for the use of animal manure of 170 kg N ha^{-1}.

However, the EC has granted a derogation for the Netherlands implying a maximum application rate for dairy farm (with more than 80% grassland) ranging from 230 to 250 kg N ha^{-1}, depending on soil type and region. This results in considerable amounts of applied animal manure, on average ca. 200 kg N ha^{-1} and 70 kg P2O5 ha^{-1}. Nationally regulated, manure slurry is applied on the grassland between February and August, while on the arable land this is between February and September (RVO, 2022).

Distribution of manure in the Netherlands is calculated with the INITIATOR model (Kros et al., 2019). This model takes into account manure production on the individual farm, the manure sales outside the Netherlands and the manure utilization capacity, given the applicable N and P application standards. This makes it possible to distinguish between the effects of the generic (national) and the area-oriented (provincial) policy on the reduction of N deposition. The model is used to substantiate and evaluate Dutch manure and ammonia policy. INITIATOR simulates manure applied mass distribution [M L^{-2}] at the individual farm level, and we use predictions of the year 2017. We assume that this applied mass is equally distributed over all the fields associated with individual farms. For the most commonly soil-applied Dutch manure slurries (dairy cow, veal calf, pig), Rakonjac et al. (2022) estimated the VPs residue concentrations based on VPs usage, animal metabolism and manure storage practices, and prioritized these substances according to their residue potential (i.e. residue indicator). Based on that indicator, we select the VPs Oxytetracycline, Doxycycline, Sulfadiazine, Flubendazole, Ivermectin, and Dexamethasone for consideration in this paper. According to Rakonjac et al. (2022) those are the most frequently soil-applied VPs in the Netherlands, and they are prioritized in different slurries, as detailed in the Table 3.1. The mentioned study provided nationally averaged VPs soil-applied concentrations and not spatially distributed concentrations. As a first approximation of potential leaching of VPs we assume that those VPs concentrations are spatially constant and introduced everywhere in the Netherlands when being applied in manure types in which they are prioritized. To match with the available INITIATOR/BRP data, we use VPs concentrations for the year 2017. Accordingly, by multiplying the estimated VP concentration in a particular slurry manure type (Rakonjac et al., 2022) with the amount of corresponding slurry manure applied on each field in the Netherlands, we estimate the VP load distributed on the fields with each of the slurry manure types. Finally, the total soil-applied VP load on the field level (A, used in *eq.(3.7)*) is obtained by summing the VP quantities present in all slurries applied on the particular field. National maps with soil-applied VP loads are given in SM4 of this chapter.

Table 3.1: *Selected VPs, VP type, their properties, and concentrations in manure.*

VP	Type	Cas no.	K_{om} [L/kg]	τ_0 [d]	C_{manure} [mg/ton][a]			
					Veal calf	Dairy cow	Dairy cow grazing	Pig [b]
Oxytetracycline	Antibiotic	79-57-2	869	8.5	555.8	2.2	8.9	106.6
Doxycycline	Antibiotic	564-25-0	1207	10	1887.3	7.5	35.7	153.5
Sulfadiazine	Antibiotic	68-35-9	2.4	0.8	486.7	7.5	37	9.1
Flubendazole	Antiparasitic	31430-15-6	650	89	0	0	0	697.2
Ivermectin	Antiparasitic	70288-86-7	8207	112	0.6	0.2	0.8	4.1
Dexamethasone	Hormone	50-02-2	139	120	0.09	0.036	0.042	0.11

[a] Grey numbers indicate that this VP is not prioritized in the particular manure type, and therefore not considered in our study. Prioritization is based on findings by Rakonjac et al. (2022) for the year 2017.

[b] Refers to a combined pig slurry (fattening pigs and sows).

We estimate VPs properties (i.e. K_{om} and τ_0) for antibiotics based on the study done by Berendsen et al. (2021), who investigated two typical Dutch soils (sand and clay). Since our approach assumes one single value of K_{om} and τ_0 per substance in the top soils for the whole of the Netherlands, we average the reported values between two soils. For the targeted antibiotics, this approach is reasonable because measured values are quite similar (Berendsen et al., 2021). For other targeted VPs, due to data availability, we use different literature sources to estimate the VP properties. For Flubendazole we use K_{om} and τ_0 as in van der Linden et al. (2017), who reported an estimate based on the QSAR calculations. For K_{om} and τ_0 of Ivermectin we refer to the Veterinary Substances DataBase (VSDB) (Lewis et al., 2016). For Dexamethasone we use K_{om} as reported in the VSDB (Lewis et al., 2016), while we estimate τ_0 with the EPI Suite™ (EPA, 2022). Note that the VP sorption parameter is frequently provided in the literature as an organic carbon-water partition coefficient (K_{OC}), which is converted to K_{om} by multiplying with 0.58 (van den Berg et al., 2016).

3.3 Results and Discussion

3.3.1 Leached quantities

After allocating a soil type, a groundwater level, and data from the INITIATOR model to each field of the BRP, in total 1099456 fields were available for analyses. We excluded part of the fields from further consideration: nature areas, fields that overlap with objects such as buildings, and fields that mismatch in the input maps. The latter is due to soil data originating from 2012, whereas manure data is from

2017, and therefore some discrepancies in identified fields are possible (e.g. reclamation of land close to sea). Among the available fields, 62.1% are maize fields, 11% are grassland fields, 5.3% are fields used for grazing, 4.6% are fields with summer barley, while the rest (17%) are spread on 22 different crops as defined in BRP.

We calculated the total VP leached quantity, T, for all combinations of substances and manure types as indicated in the Table 3.1. Our calculations for Oxytetracycline, Doxycycline, and Ivermectin show that the maximum T that one field can have is 1.96E-38 mg/ha, 3.02E-52 mg/ha, and 3.77E-33 mg/ha, respectively, which is very low. These results suggest that combinations of properties and applied amounts for these particular VPs are not causing any relevant leaching. On the other hand, results for Sulfadiazine, Flubendazole, and Dexamethasone indicate some leaching at several locations in the Netherlands, with maximum T being 0.072 mg/ha, 2.91 mg/ha, and 0.63 mg/ha, respectively. However, distribution of T for Sulfadiazine and Flubendazole shows that the majority of the fields (>98%) have T lower than 1 pg/ha, which we considered as the lower threshold for our mapping. For Dexamethasone this is significantly different with only 38% of the fields having T lower than 1 pg/ha. Leaching maps of these three VPs are given in Figure 3.2 and 3.3, while T distributions are revealed in the SM5.

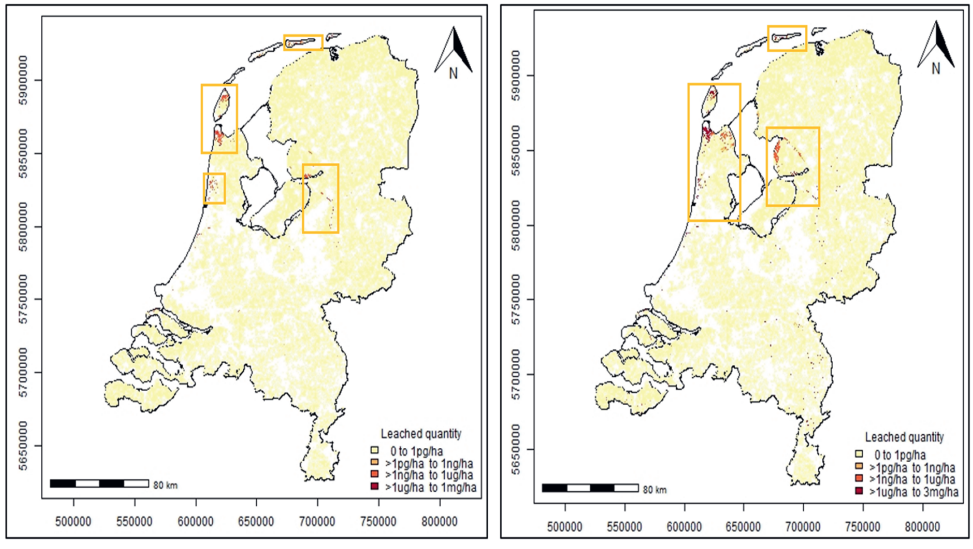


Figure 3.2: *Sulfadiazine (left) and Flubendazole (right) leaching maps. Coordinates are in UTM Northing system. Areas highlighted with the orange rectangles are the ones where fields with maximum leaching are located (>1ng/ha). These are generally fields with sandy soil types.*

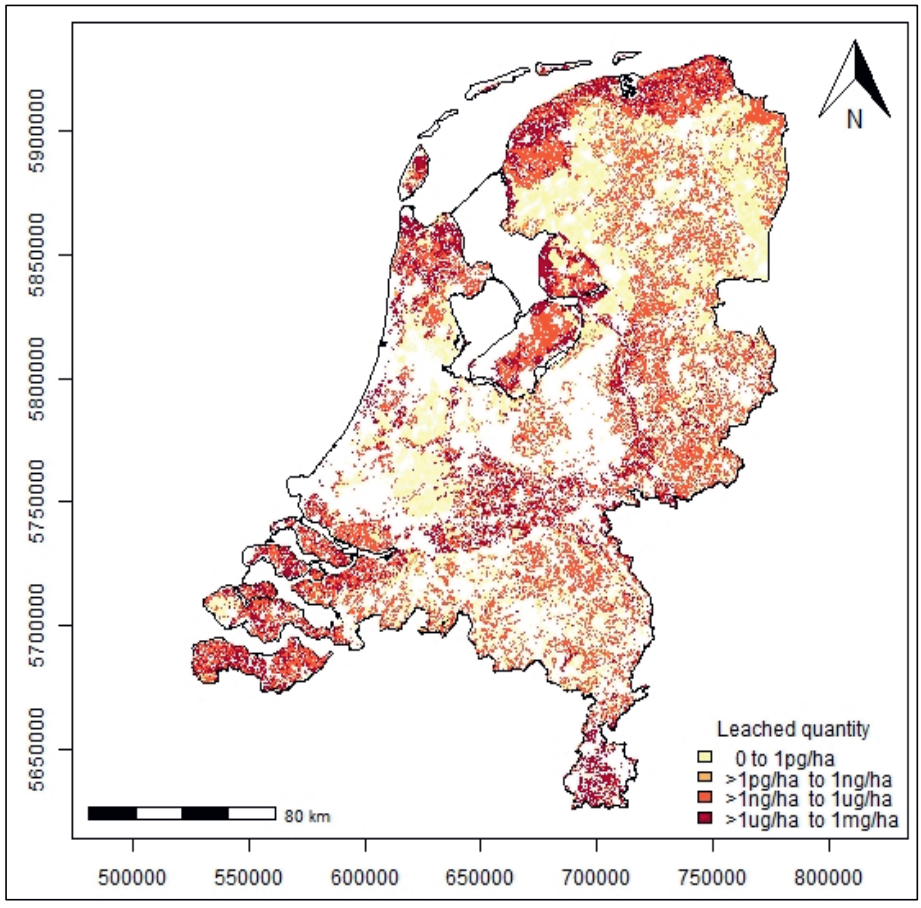


Figure 3.3: *Dexamethasone leaching map. Coordinates - UTM Northing.*

As Figure 3.2 reveals, for both Sulfadiazine and Flubendazole, fields with leached quantities larger than 1 pg/ha are located at the northern tip of Dutch province North Holland, on two Dutch islands, near the town of Lisse, and around river IJssel. In addition, in the case of Flubendazole some of these fields are located at the northern part of province Flevoland, and occasionally at some other regions.

For Sulfadiazine, all fields with leached quantities belonging to the highest defined category (T>1ug/ha) have a moderately fine sandy soil type. Typical profile of this soil type, as defined by Wosten et al. (2013), is: top soil layer of 5cm (f_{om}= 0.04, ρ= 1.43 kg/L), 1st subsoil layer of 45cm (f_{om}= 0.002, ρ= 1.67 kg/L), and 2nd deeper subsoil layer of 70cm (f_{om}= 0.002, ρ= 1.67 kg/L). The average groundwater depth at these fields is 65cm (median is 66cm), meaning that groundwater level is typically located somewhere in the deeper subsoil layer.

For Flubendazole, 58% of the fields with T>1ug/ha are located on moderately fine sandy soil, while 41% are on (low loam)-sandy soil and 1% on coarse sandy soil. Typical soil profile of (low loam)-sandy soil is: top soil layer of 6cm (f_{om}= 0.03, ρ= 1.47 kg/L), 1st subsoil layer of 4cm (f_{om}= 0.012, ρ= 1.63 kg/L), 2nd subsoil layer of 5cm (f_{om}= 0.018, ρ= 1.6 kg/L), and 3rd subsoil layer of 105cm (f_{om}= 0.002, ρ= 1.68 kg/L). Average groundwater depth at fields with T>1ug/ha on (low loam)-sandy soil is 137cm (median is 145cm), meaning that groundwater level is typically below the deepest known subsoil layer. For fields on moderately fine sandy soil, average groundwater depth is 88cm (median is 81cm). Typical soil profile of coarse sandy soil is represented with one soil layer of 120cm (f_{om}= 0.003, ρ= 1.54 kg/L), while average groundwater depth at fields with T>1ug/ha is 73cm (median is 67cm).

Crop types among fields with T>1ug/ha, for both Sulfadiazine and Flubendazole, are similar to the national crop type distribution, where maize is the most frequent crop covering 69% of Sulfadiazine fields and 31% of Flubendazole. Except for floriculture, which is quite common among these fields with 17% for Sulfadiazine and 22% for Flubendazole and not so frequently observed at the national level (has only a national share of <2%). Reason for this is that in the Netherlands soils adjacent to dunes are frequently transformed into fields for bulb cultivation. These soils are very prone to leaching due to sandy texture and low organic matter content, resulting in higher VP mobility, rapid drainage, and less sorption. Typically, these fields have shallow groundwater level (around 60cm), which is sometimes kept constant to optimize the conditions for bulb cultivations. Note that we use average groundwater levels for calculations, whereas seasonal variations could result in even shallower levels and therefore pose higher risk for leaching. Furthermore, fields used for bulb cultivation are also noted as fields of concern for pesticide leaching to groundwater in the Netherlands (de Snoo and Vijver, 2012; Swartjes et al., 2016).

Unlike for the two previously discussed VPs, leached quantities of Dexamethasone higher than 1pg/ha are distributed all over the Netherlands (681655 fields, Figure 3.3). The hotspot leaching locations (where T>1ug/ha) identified for Sulfadiazine and Flubendazole are also present for Dexamethasone but with addition of the northern parts of the provinces Friesland and Groningen, the province Zeeland, parts of Gelderland province, and southern Limburg. 21% of fields with T>1ug/ha are situated on sandy soils, 66% on clay soils, and 13% on loamy soils, representing in total 36 different soil types as defined by Wosten et al. (2013) and detailed in SM6. In this case, we do not investigate groundwater depths for each of the 36 soil types but rather refer to groundwater map (see SM2) for regional impressions. Crop types distribution among fields with T>1ug/ha follows the national pattern where maize is the most spread (55%), followed by grassland (10%) and summer barley (7%). Due to the much larger number of considered fields, the ones used for bulb cultivation are less common than for other two

VPs, with presence of 3.5%. Note that the differences between crop coverages among the three mentioned VPs are affected by the manure/crop application patterns, where e.g. Flubendazole is applied only in pig manure.

3.3.2 Discussion on parameters

Even though both compounds exhibit significantly different properties and concentrations in soil-applied manure (Table 3.1), calculations for Sulfadiazine and Flubendazole result in similar leaching patterns at the national scale. Sulfadiazine with its low K_{om} tends to be very mobile, but because of the low τ_0 it does not persist in the environment long enough to display widely spread leaching. On the other hand, Flubendazole is >100 times more persistent in the soil compared to Sulfadiazine, but it also has 270 times higher sorption coefficient. This means that it will reside in the soil for a longer time and therefore allows for degradation to occur.

For Sulfadiazine, leaching occurs mostly in fields with moderately fine sandy soil. Here, the top soil layer retards the leaching process for around 1.5 times (R≈1.5), while the 1st and 2nd subsoil layers retard for around 1.1 times. This seems reasonable because the top layer contains more organic matter, as explained earlier. Pore water velocity, v, in the three mentioned soil layers is estimated between 0.5 and 0.8 cm/day (top soil). Note that in reality due to spatial variability of water flow there might be a large number of different velocities, as addressed by van der Zee and Boesten (1991). The associated residence time of Sulfadiazine in the top soil layers of fields with moderately fine sandy soil is around 10 days, while in the deeper layers it could go up to 150 days. Residence times in the deeper soil layers are significantly influenced by the groundwater level which determines relevant soil layer thickness (Figure 3.1).

When Flubendazole has a T>1ug/ha, depending on the soil layer, R ranges between 12 and 156 for fields with moderately fine sandy soil, between 60 and 120 for fields with (low loam)-sandy soil, and is around 28 for fields with coarse sandy soil. The highest R values correspond to the top soil layers. The range of v values is larger compared to Sulfadiazine, ranging from 0.5 to 1.8 cm/day, where higher values correspond mostly to fields on coarse sandy soil. Residence times are between 30 and 2000 days, depending on the soil type and soil layer. This substantial increase in residence times compared to Sulfadiazine is in line with the larger sorption coefficient, K_{om}, in the case of Flubendazole (Table 3.1). This also indicates that the leaching processes of Oxytetracycline, Doxycycline, and Ivermectin allow for (almost) complete degradation before reaching groundwater.

Dexamethasone is applied on the soil in much lower concentrations compared to other investigated VPs (Table 3.1), but it frequently shows fields with T >1ug/ha. This implies that the VP properties (K_{om} and τ_0) combined with the soil properties influence leaching to a larger extent rather than applied quantities. To further explore this for Dexamethasone, but also Sulfadiazine and Flubendazole, we analysed the values, patterns and distributions of T, F_{total} and A, based on all 1099456 fields.

Figure 3.4 shows that Sulfadiazine ends up on the soil with around 2.5 times larger quantities than Flubendazole and around 4500 times larger quantities than Dexamethasone (x-axis middle figure). Still, it has the lowest T among the three (y-axis middle figure). The explanation for this is that the range of F_{total} values is around 100 times smaller compared to Flubendazole, and around 21000 times smaller compared to Dexamethasone (x-axis left figure). Figure 3.4 further shows (right vertical) that for all three VPs the largest quantities (A) are not applied on the fields with largest F_{total}, which is certainly something that reduces the possible leached mass. This means that the fields that are associated with a high leaching potential do not receive a high amount of Sulfadiazine, Flubendazole, or Dexamethasone.

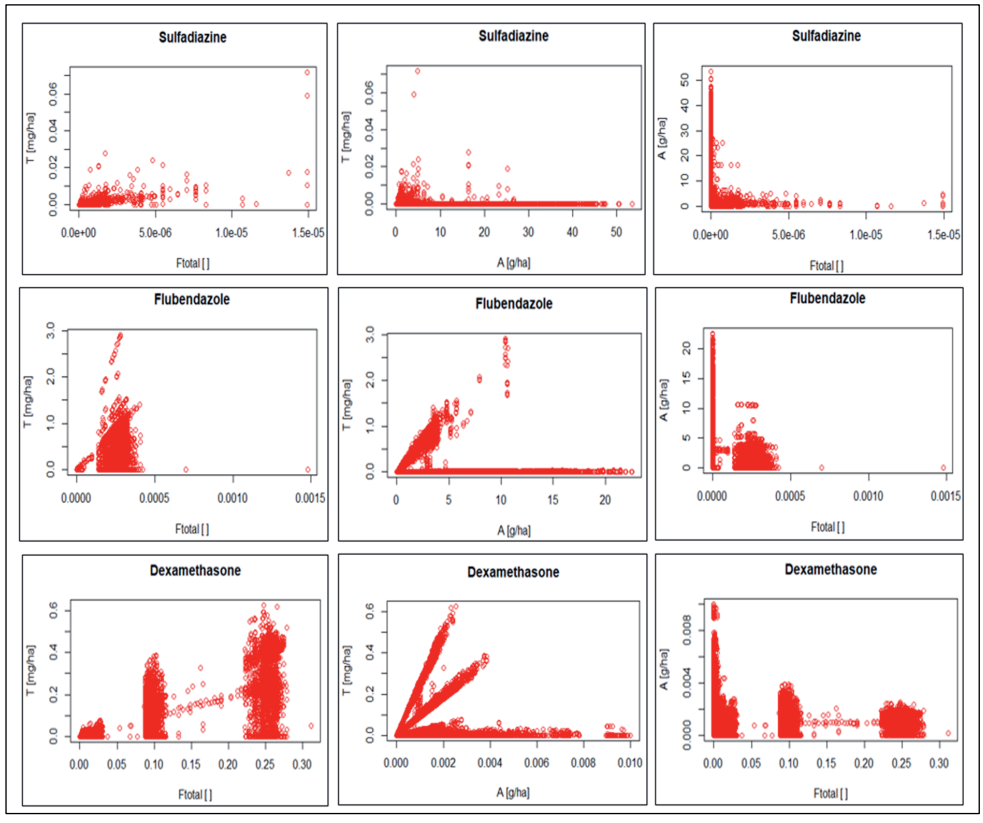


Figure 3.4: *Leached VP amount per field (T) compared to total VP leached fraction per field (F_{total}), T compared to applied VP amount per field (A), and A compared to F_{total}. Each dot represents one field of different size.*

Only in the case of Sulfadiazine, the field with the largest F_{total} is the one with the largest T (top-left figure). For leaching which is dominated by soil and substance properties above applied amounts, we expected to observe this also for the other substances. However, note that Flubendazole and Dexamethasone are not applied (A=0) on the field with largest F_{total} (right vertical). Different leaching behavior between soil types is most notable with Dexamethasone, particularly on the figure T vs F_{total}. Here, we observe three data accumulation zones, where the two on the right, with F_{total} around 0.10 and 0.25, group fields on moderately fine sandy soil and (low loam)-sandy soil, respectively. The same accumulation zones are visible on the figure T vs A, where previously mentioned F_{total} values define the slope of the two linear zones. The reason for this clustering is caused by the different soil type parameters used to calculate F_{total}, where the ones related to the two mentioned soils are clearly in favor of relatively high leaching potential.

In our calculations we assumed Dexamethasone to be used in dairy cows and pigs only, and also this VP is considered to be used in all farms in the Netherlands. We assess the influence of manure application types on T as shown in Figure 3.5. The impact of neglecting veal calves in the calculations for Dexamethasone is shown by comparing the red line (original calculation) and the blue dotted line (while including veal calves). A minor increase in A is indicated if veal calf manure is added. We therefore compare the hypothetical veal calve included A values with T. The results of this calculation can be found in the SM7. In principle, observed patterns are the same as without this manure type, but since applied amounts increased there is also an increase in leached quantities. This makes sense, because Dexamethasone is prone to leaching due to its properties, and therefore sensitive to changes in applied amounts. Note that this is not expected for other investigated VPs, because of the low leaching potential.

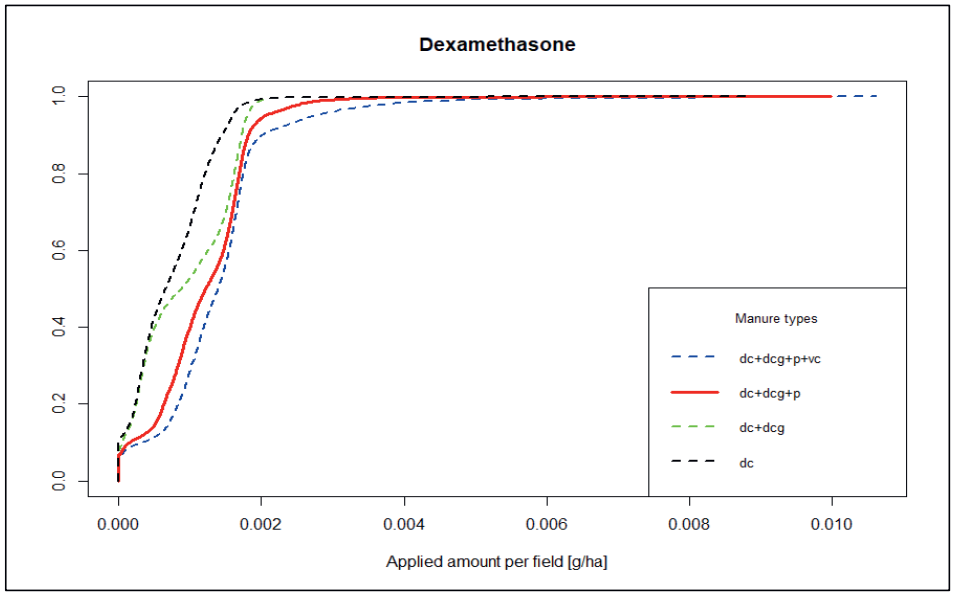


Figure 3.5: *Ascending cumulative distribution of applied amounts of Dexamethasone per field (A). dc-dairy cow, dcg-dairy cow grazing, p-pig, vc-veal calf. In this paper we considered application of Dexamethasone via dairy cows and pigs only. Veal calves were considered to have a minor contribution (see also Table 3.1). Our assumed case is represented with the red line.*

3.3.3 Uncertainties

To investigate leaching at the national scale, a number of assumptions have been made. To simulate VP transformation rate in the soil we disregarded the temperature dependence, which is considered to be one of the environmental factors with relatively high influence on pesticide transformation rates in the soil (van den Berg et al., 2016). The transformation rate of a pesticide increases sharply as the temperature increases. For calculations we used VP transformation rates from literature, usually experimentally determined for soils on a room temperature (20-25 °C). Soil temperatures in the Netherlands are known to vary with depth, per season, per region, per year, and are usually lower than 20 °C (Jacobs et al., 2011). This could imply that our assumed VPs transformation rates were overestimated and result in even higher leached fractions than calculated. However, considering that most VPs end up on the soil during spring and summer, when soil warms up, the eventual influence of temperature differences is minimized. Still, we recommend this to be explored in future work. Besides transformation rates, model parameters characterizing sorption are shown to have a significant influence on pesticide leaching to groundwater (Urbina et al., 2020), which we also observe for VPs in our model. However, for VPs these parameters vary a lot in the literature. Ideally, they should be

estimated with respect to the soil type and environmental conditions, which is hardly available information.

In general, measured soil physical properties show already substantial in-field variation and heterogeneity. In addition, due to land management (ploughing, riding with tractors etc.) and weather conditions (heavy rainfall, droughts) soil structural changes might evolve affecting flow and transport processes. In particular top soils are prone to time-variant changes (compaction, soil crusting, development of shrinkage cracks in clay, peat and loamy soil, occurrence of macropores due to earthworm activity, water repellence, preferential flow etc.). This might result in substantial uncertainties.

Another important assumption is that all soil-applied yearly VP load is available for leaching. In general, this is probably not the case because part of the VP load might be affected by rainfall events and transported to surface water via fast flow routes (e.g. runoff, field drains). If this is the case, then our calculated leached quantities could be overestimated, particularly for VPs prone to leaching and due to their sensitivity on applied amounts, as discussed earlier for Dexamethasone.

Further, we assume that VPs are introduced per particular manure type everywhere in the Netherlands, which is probably not the case in reality. This assumption is a consequence of the available data on VP concentrations in manure, which are based on national averages. Considering our results, certain regions are more prone to leaching than others, hence assessing leaching with field specific input data might be relevant. Similarly, we assume national averages of precipitation and evapotranspiration to calculate water flow. This could be improved by taking into account regional weather data such as KNMI weather stations, and spatially connecting those with the appropriate regions. Assumption on constant groundwater levels is briefly explained earlier, but it is worth mentioning that eventual fluctuations in levels might be more relevant when looking at multiyear VP applications on soil. Some of the VP residues might persist in soil layers and be affected by those groundwater level fluctuations.

Note that the calculated leached mass (T) is an indication of potential groundwater concentrations, because of a number of assumptions which had to be made in order to create a national overview, and which are valid for both VP leached mass and VP concentration in groundwater. To assess whether the leached mass is objectively high and if it results in high groundwater concentration, it should be compared to the water flux evaluated at a certain depth in soil, which depends on the precipitation, soil/crop type, and local water management practices.

3.4 Conclusion

In this paper we quantified spatially distributed leaching of VPs to groundwater at the national scale, investigated the impacts of factors relevant for leaching, and identified locations vulnerable to leaching. Our model results showed that for the VPs Oxytetracycline, Doxycycline, and Ivermectin the maximum mass leached to groundwater is very low, i.e. << 1 ug/ha. These three VPs were prioritized as one of the most frequently soil-applied VPs in the Netherlands (Rakonjac et al., 2022a), hence information on their very low leaching potential could be used in identifying their environmental pathways and impacts. For the VPs Sulfadiazine and Flubendazole we identified a few hotspot regions (Figure 3.2) where the modelled quantities leached to groundwater were relatively high. These fields are located on sandy soils and have maize and floriculture as the most common crop types. Considering the leaching tendency, identified regions could be prioritized for future groundwater monitoring when targeting the VPs with similar environmental properties as Sulfadiazine and Flubendazole. On the other hand, our results for Dexamethasone indicated a widespread spatial distribution of fields where quantities leached to groundwater were relatively high (Figure 3.3). Besides sandy soils, some of these fields also have clay and loamy soils. Based on the findings from an earlier study (Rakonjac et al., 2022a), soil in the Netherlands is highly exposed to the application of Dexamethasone via different manure types. Therefore, being prioritized as spatially widespread in the two consecutive steps, i.e. application on the soil and leaching to groundwater, Dexamethasone warrants a closer examination when performing an environmental risk assessment of VPs.

Our results showed that the spatial patterns of VP leaching to groundwater were affected by many processes, and that the relative importance of those processes differed between the investigated VPs. When comparing the influence of the soil-applied VP masses (A) and estimated leached fractions (F_{total}) on the spatial distribution of calculated leached quantities (T), it was found that leaching patterns of Sulfadiazine and Flubendazole were dominated by F_{total} more than by A. For Dexamethasone this was also the case, but due to its high leaching potential, leaching patterns were found to be sensitive to changes in applied amounts. This implies that for assessing the factors with the highest influence on VPs leaching, a substance individual approach is probably the best option. Still, some generalization is possible regarding the leaching potential of VPs, as discussed in the following.

Even though we did not perform a targeted sensitivity analysis on the VP leaching potential with respect to degradation (τ_0) and sorption (K_{om}) parameters, indications from our study seem to be completely in agreement with the guidelines on groundwater exposure assessment proposed by the Organisation for Economic Co-operation and Development (OECD, 2013). Based on the experience

with the PELMO model (Klein et al., 2000), which simulates the vertical movement of pesticides in soil, OECD advises that substances with a K_{om} < 290 L/kg and a τ_0 > 21 d in soil may leach to groundwater and that the assessment of groundwater exposure must be performed. The only VP in our study which satisfies the mentioned thresholds is Dexamethasone (Table 3.1), and based on our results we also prioritized it for the leaching risk assessment compared to other investigated VPs. According to the OECD guidelines, substances with a K_{om} > 290 L/kg and τ_0 < 21 d are not likely to leach to groundwater, which is indeed also an observation from our study (Oxytetracycline and Doxycycline). For the intermediate cases, when a substance has a K_{om} < 290 L/kg or a τ_0 > 21 d, there are no specific OECD guidelines. In this case, for VPs we observed that some minor leaching could happen at the vulnerable (hotspot) regions, so location specific groundwater leaching assessment might still be needed.

Supplementary Material

SM1. Water flow estimation

Step 1.

Top soil: q = P – E

Other soil layers: q = P – E – T

where q is specific discharge [$L^3\ L^{-2}\ T^{-1}$], P is precipitation [$L\ T^{-1}$], E is soil evaporation [$L\ T^{-1}$], and T is transpiration [$L\ T^{-1}$].

Step 2.

Depending on the soil layer, calculated q is assumed to be equal to the hydraulic conductivity (K [$L\ T^{-1}$]), hence Mualem equation

$$K(h) = K_s \frac{((1 + |\alpha h|^n)^m - |\alpha h|^{n-1})^2}{(1 + |\alpha h|^n)^{m(\lambda+2)}}$$

is transformed to

$$q(h) = K_s \frac{((1 + |\alpha h|^n)^m - |\alpha h|^{n-1})^2}{(1 + |\alpha h|^n)^{m(\lambda+2)}}$$

where n, m, and λ are dimensionless shape parameters, α is a shape parameter [L^{-1}], h is a pressure head [L], and K_s is saturated hydraulic conductivity [$L\ T^{-1}$]. From the transformed equation, h has been calculated.

Step 3.

Van Genuchten equation is used to estimate time-averaged volumetric water fraction θ

$$\theta = \theta_r + \frac{(\theta_s - \theta_r)}{(1 + |\alpha h|^n)^m}$$

where θ_r is the residual volumetric water content, and θ_s is saturated volumetric water content.

q and θ are further used to calculate pore water velocity.

SM2. Groundwater map

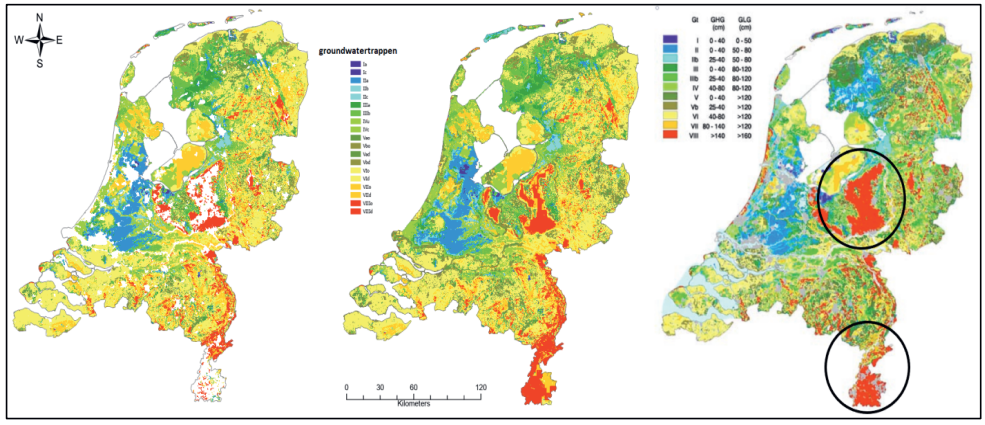


Figure S1. *Left - public (free) map (Knotters et al., 2018) used as an input; Middle – extrapolated map; Right - Map created by Steur et al. (1991). Black circles are extrapolated parts of Limburg province and Veluwe region.*

SM3. Technical (software) details

Table S1. *Tools used in ArcGIS Pro*

Tool name	Description	Link to ArcGIS tool description	Purpose
Dissolve	Aggregates features based on specified attributes.	https://pro.arcgis.com/en/pro-app/latest/tool-reference/data-management/dissolve.htm	To create research areas necessary for extrapolating groundwater map.
Mosaic	Merges multiple existing raster datasets or mosaic datasets into an existing raster dataset.	https://pro.arcgis.com/en/pro-app/latest/tool-reference/data-management/mosaic.htm	To combine the extrapolated groundwater map of Limburg and the rest of the Netherlands.
Intersect	Computes a geometric intersection of the input features. Features or portions of features that overlap in all layers or feature classes will be written to the output feature class.	https://pro.arcgis.com/en/pro-app/latest/tool-reference/analysis/intersect.htm	To assign groundwater level, soil type, soil-hydraulic and soil-chemical properties to each field and soil layer.
Summarize within	Overlays a polygon layer with another layer to summarize the number of points, length of the lines, or area of the polygons within each polygon, and calculate attribute field statistics about those features within the polygons.	https://pro.arcgis.com/en/pro-app/latest/tool-reference/analysis/summarize-within.htm	To assign groundwater level, soil type, soil-hydraulic and soil-chemical properties to each field and soil layer.
Raster to polygon	Converts a raster dataset to polygon features.	https://pro.arcgis.com/en/pro-app/latest/tool-reference/conversion/raster-to-polygon.htm	To convert the groundwater raster to polygons necessary to link average groundwater level to fields.

SM4. Soil-applied VP loads

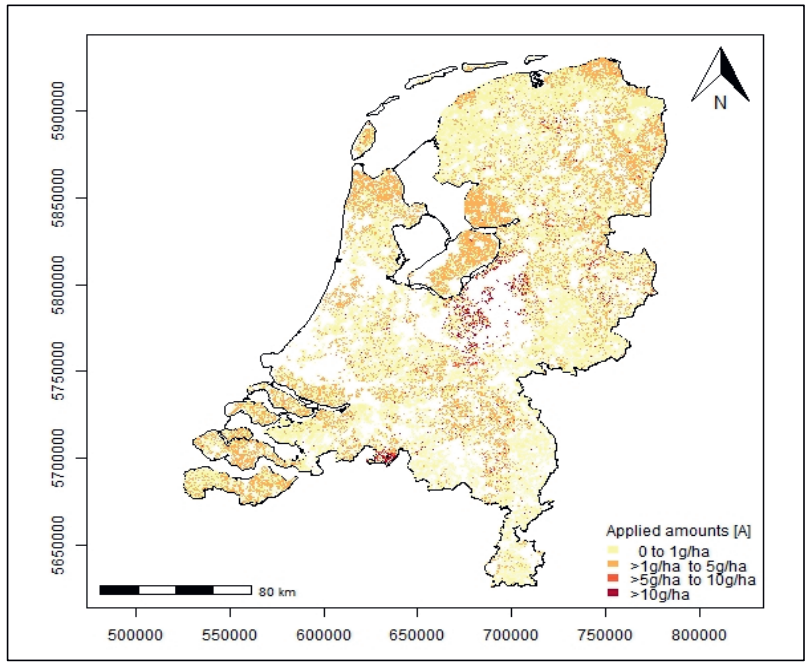


Figure S2a. *National scale overview of the soil-applied masses of Sulfadiazine*

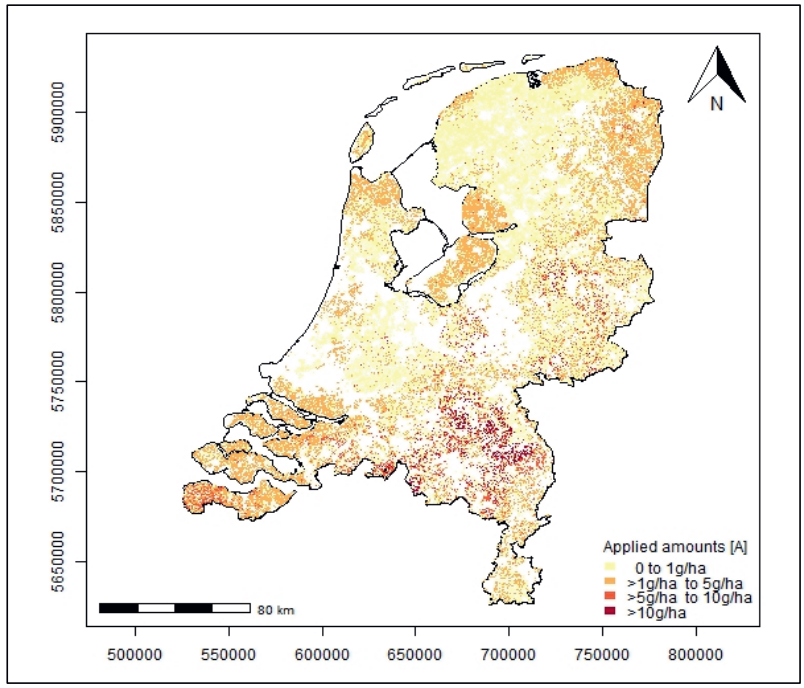


Figure S2b. *National scale overview of the soil-applied masses of Flubendazole*

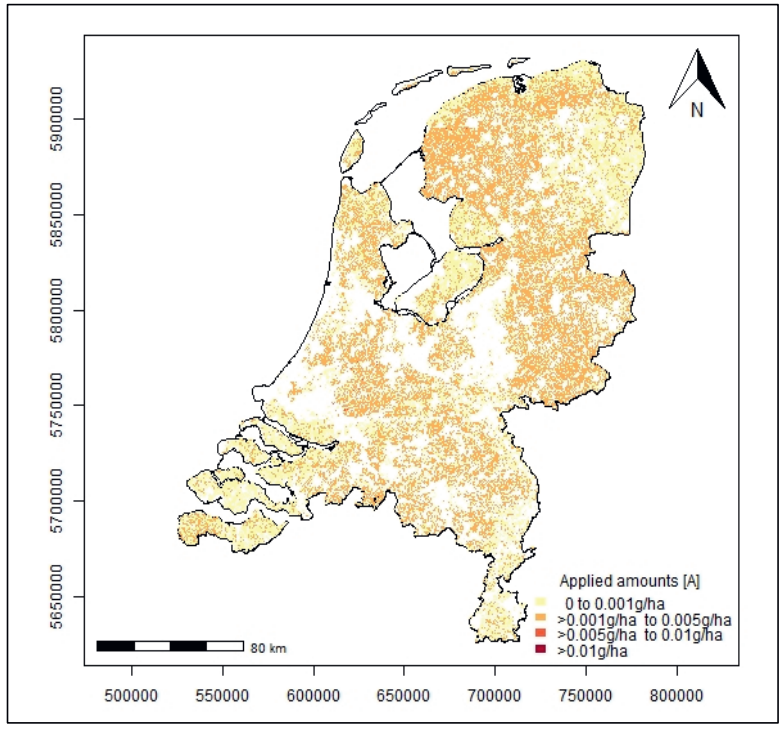


Figure S2c. *National scale overview of the soil-applied masses of Dexamethasone*

SM5. Distribution of leached masses (T)

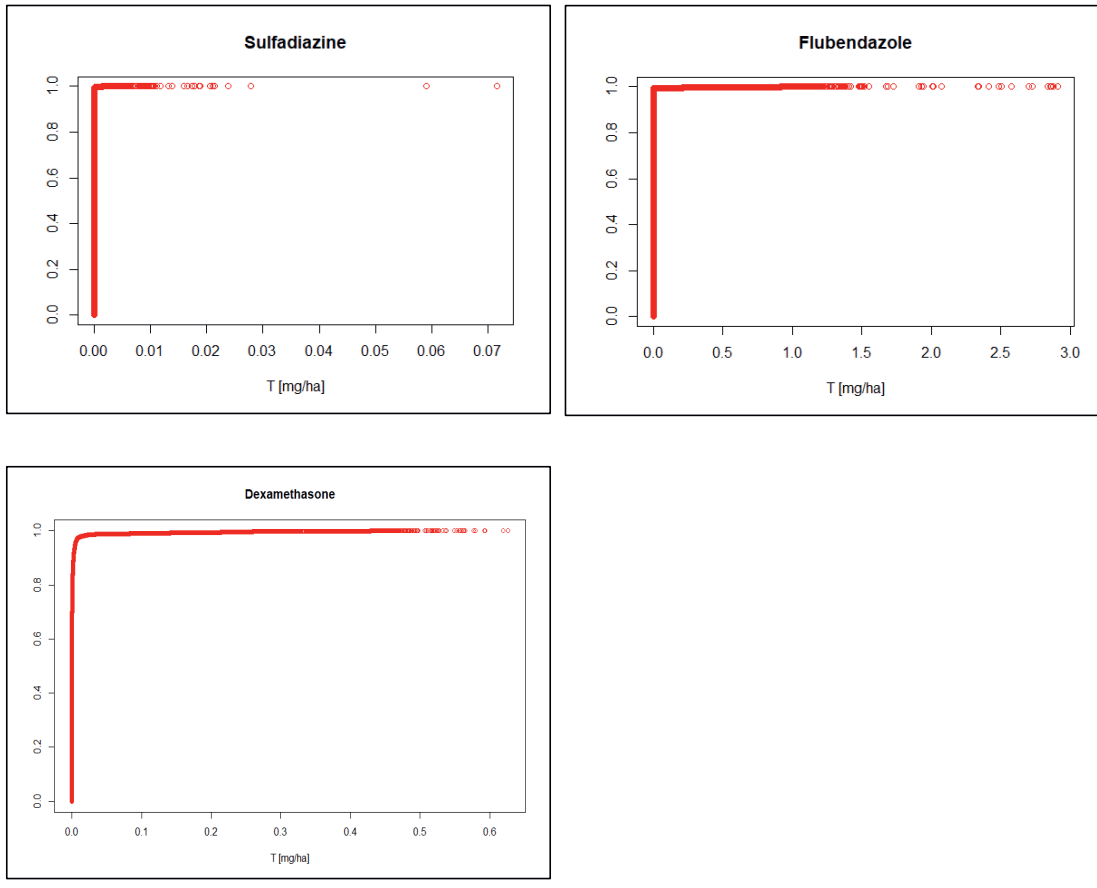


Figure S3. *Ascending cumulative distribution of leached quantities for Sulfadiazine, Flubendazole, and Dexamethasone.*

SM6. Soil types for fields with leached quantities of Dexamethasone larger than 1ug/ha

Table S2. *Distribution of soil types for fields with T>1ug/ha of Dexamethasone*

BOFEK code [a]	Number of fields	%
302	6571	3.370
303	2887	1.480
309	6	0.003
313	1570	0.805
314	834	0.428
316	5818	2.983
321	300	0.154
323	5732	2.939
324	7365	3.777
325	4142	2.124
326	160	0.082
327	4871	2.498
402	683	0.350
406	775	0.397
407	3	0.002
408	14888	7.634
409	2087	1.070
410	10794	5.535
411	2415	1.238
412	3512	1.801
413	1366	0.700
414	844	0.433
415	1279	0.656
416	29999	15.383
417	553	0.284
418	18187	9.326
419	13942	7.149
421	14841	7.610
422	13309	6.825
501	447	0.229
502	2484	1.274
503	217	0.111
504	1837	0.942
505	1260	0.646
506	1318	0.676
507	17714	9.084

[a] Defined in Wosten et al. (2013)

SM7. A compared to T for Dexamethasone when veal calf manure is added

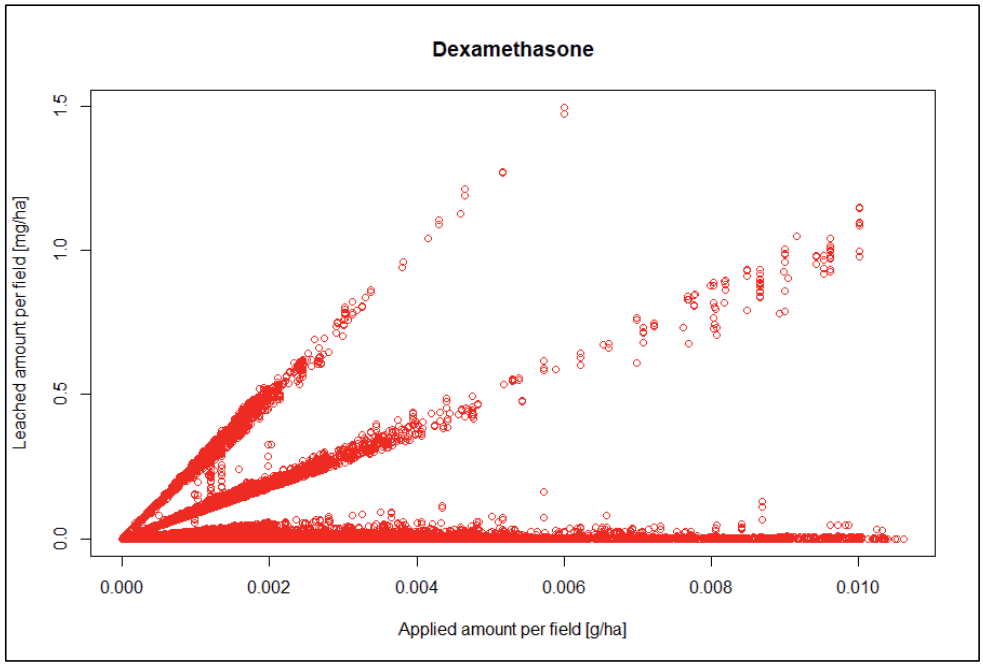


Figure S4. *Applied amounts (A) of Dexamethasone when concentration in veal calf manure is considered compared to the corresponding leached amounts (T) per field.*

Chapter 4

Transport of Veterinary Pharmaceuticals in Lowland Catchments: a Lumped Modelling Approach

Based on:

Rakonjac, N., Rinaldo, A., Ritsema, C. J., Benettin, P. Transport of Veterinary Pharmaceuticals in Lowland Catchments: a Lumped Modelling Approach. *To be submitted to Water Resources Research.*

Abstract

Livestock animals are commonly treated with veterinary pharmaceuticals (VPs), and their residues often enter the environment through manure applied to soil. A portion of these residues may be further transported to surface water through intricate transport mechanisms. The purpose of this study is to examine the temporal dynamics of VPs in lowland surface waters of an agricultural catchment in the Netherlands, utilizing information on VPs concentrations in manure and surface water measurements. To accomplish this goal, we first calculate the amounts of two VPs applied in the catchment of interest and analyze the local manure application practices to establish the temporal distribution of VP application. We then employ an established rainfall-runoff model to simulate the hydrological patterns of the chosen catchment. To assess the accuracy of this model in reproducing runoff, we compare its results to discharge observation data. Furthermore, we use the computed fluxes generated by this model to develop a simple catchment-scale transport model for VPs that distinguishes between fast and slow transport routes. The transport model considers all the reaction processes experienced by the VPs during their transfer to the stream network through a basic first-order reaction. Our results suggest that the hydrological model and its associated parameters are capable of accurately replicating catchment discharge patterns over a prolonged period. The predictions of VP transport model were found to have insufficient fit to the surface water measurements. The modeled transit time dynamics were deemed realistic, but the modeled VP concentrations were considerably higher than the measurements. This suggests that either the first-order decay approach does not accurately represent reactive and sorption processes, or direct comparison of simulated and measured VP concentrations may not be suitable. Nevertheless, the transport model still yielded valuable observations, such as the impact of manure application timing on the temporal distribution of VP occurrence in a stream.

4.1 Introduction

The escalating demand for water supplies and the release of contaminants from multiple sources are leading to deterioration of water quality. The proliferation of water pollution is a growing global issue, impacting both aquatic ecosystems and human well-being (Geissen et al., 2015). Pharmaceuticals are one of the contaminants that are commonly detected in surface waters (Wilkinson et al., 2022). A substantial body of global data (Umwelt Bundesamt) has confirmed that, in particular, veterinary pharmaceuticals (VPs) are frequently found in surface waters. The complex and diffuse hydrogeochemical dynamics of VPs, along with their potential negative impacts on the environment, have raised significant concerns.

The primary pathway for VPs to enter the environment is through the excretion of urine and faeces from medicated animals, followed by the application of contaminated manure to agricultural land (Boxall et al., 2004a). While some recent studies (Spielmeyer et al., 2017; Albero et al., 2018; Kivits et al., 2018; Hang et al., 2021; Huygens et al., 2022; Rakonjac et al., 2023) have investigated the fate of VPs in soil and their movement towards groundwater, limited attention has been given to the transport of VPs to and within the stream network. Bailey (2015) has provided a comprehensive analysis of the transport mechanisms of VPs from agricultural fields to surface water. In principle, this transport route is indirect, difficult to quantify, and can lead to pollution of surface water systems over long distances downstream of the application areas. The contamination level of surface water is impacted by several factors, including hydrology, physical processes occurring during transport, the application method of manure (such as spreading, injection, or incorporation), and the distance to surface water. Additionally, the VPs' transport availability is strongly influenced by their chemical properties.

Although most studies on the transport of VPs to surface water have focused on field-scale issues (Kay et al., 2005; Stoob et al., 2007; Dolliver and Gupta, 2008; Barrios et al., 2020), with only a few exploring catchment-scale transport (Burke et al., 2016; Hanamoto et al., 2021; Zhao and Lung, 2022), the number of available studies investigating VPs transport in lowland catchments, such as those found in the Netherlands, is particularly limited. These catchments are characterized by a network of slow-moving water bodies and extensive agricultural land use, with a complex balance between precipitation, evaporation, runoff, and subsurface water storage (van der Velde et al., 2010; Benettin et al., 2013). In such catchments, soil-applied VPs can be transported via surface runoff, drainpipes or soil cracks, and phreatic groundwater, which can then drain into surface water. The timescales associated with these flowpaths can vary by orders of magnitude (van der Velde et al., 2010), making the overall solute transport process from the catchment to the river network extremely complex. It is

reasonable to expect that VPs observed in a stream come from a combination of sources whose contributions are variable in space and time. In particular, we can think of two main end-member sources of flow and transport: the quick preferential flowpaths, which can rapidly mobilize solutes in a few hours/days after a storm event, and groundwater, which is typically the major and persistent contribution to streamflow and whose characteristic timescale is often larger than a year. The interplay between these variable water sources and the resulting variability in water transit times have been identified as a significant factor in the variability of water quality (Hrachowitz et al., 2016).

To the best of our knowledge, Wöhler et al. (2021) is the only study that has specifically investigated the transport of VPs in Dutch lowland catchments. However, it highlighted noteworthy uncertainties regarding the soil-applied VPs masses and manure application patterns. In addition, the study solely investigated overland transport and did not take into account subsurface flow, which could be of significant relevance for overall VPs transport behavior (Mehrtens et al., 2020). We also acknowledge some ongoing efforts on this topic in the Netherlands, such as the study by Bregoli et al. (2023), but complete results are not yet available.

As indicated previously, the transport of VPs to and within the stream network is a complex issue that requires reliable catchment-scale VP input, measurements of VPs in streamflow and a meaningful transport model that can reproduce the variability in streamflow generation sources. In this paper, we use information on VPs concentrations in manure (Rakonjac et al., 2022; Rakonjac et al., 2023) and VPs quantities measured in surface water (Chapter 5) to investigate the temporal dynamics of VPs in lowland surface waters of an agricultural catchment in the Netherlands. We build on the WALRUS rainfall-runoff model (Brauer et al., 2014), which was shown to be appropriate for lowland environments, and develop a parsimonious catchment-scale transport model that differentiates between quick flow routes and slower routes through the soil reservoir. We use our VP data and the transport model to: (i) characterize water transit times in agricultural lowland catchments subjected to VP application; and (ii) test whether modeling reactive and sorption processes as a simple first-order reaction controlled by the VP's half-life (DT50) is compatible with observations of VPs in a real-world catchment.

4.2 Materials and Methods

4.2.1 Study area and selected VPs

The study area is a rural catchment situated in the central region of the Netherlands. The catchment has a total area of approximately 26 km^2 and is situated in a valley formed during the Saale period (150 ka BC). The catchment is characterized by intensive livestock breeding practices, which may result in elevated levels of emitted VPs. Notably, the absence of upstream discharges from wastewater treatment plants has minimized the impact of human pharmaceuticals. Within this catchment, there are approximately 1500 agricultural fields where manure is applied, and many of these fields are located in close proximity to water streams, as illustrated in Figure 4.1. The shallow subsurface is composed primarily of sandy deposits of peri-glacial and coastal origin. In fact, around 98% of all fields where manure (i.e. VPs) is applied in this catchment are on sandy soils. Maize is the dominant crop in this area, covering approximately 70% of the fields, followed by grasslands with approximately 20% coverage (Kros et al., 2019).

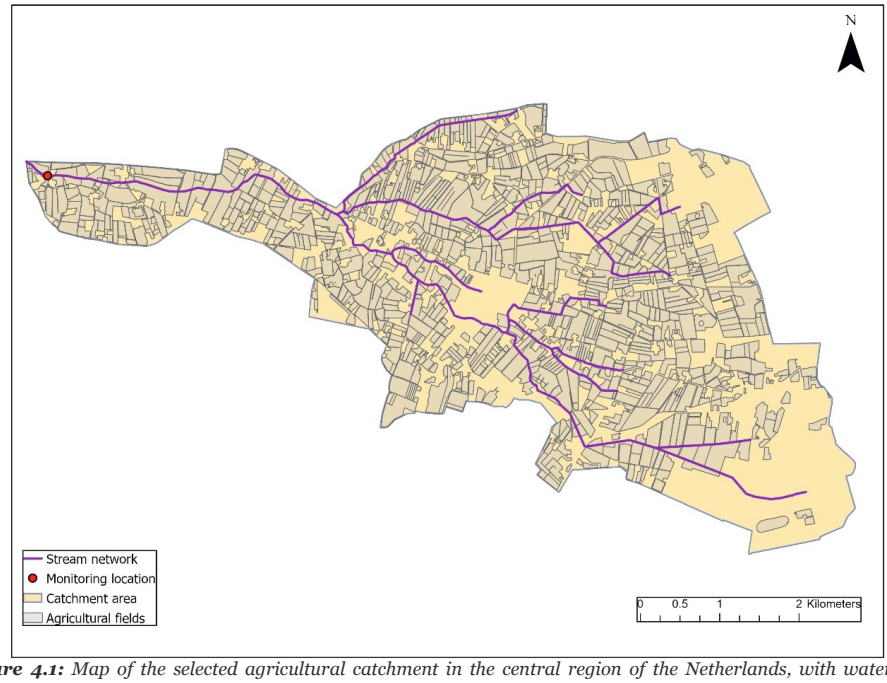


Figure 4.1: *Map of the selected agricultural catchment in the central region of the Netherlands, with water flow direction from E to W.*

We select VPs for investigation based on twofold criteria. Firstly, we consider the known quantity of VPs applied to the soil (as documented by Rakonjac et al. (2023)). Secondly, we examine the VPs for

which quantities can be reliably measured at the monitoring location shown in Figure 4.1 and described in Chapter 5. By doing so, we ensure that we have consistent input and output data. The intersection of these criteria yields two VPs, namely Sulfadiazine (Cas no. 68-35-9) and Flubendazole (Cas no. 31430-15-6), which are from different groups of VPs, antibiotics and antiparasitics, respectively. Additionally, these two VPs indicate different combinations of livestock sectors, as Sulfadiazine is present in various manure types (e.g. dairy cow, veal calf), while Flubendazole is only present in pig manure. Moreover, Sulfadiazine is a highly mobile compound, while Flubendazole is relatively persistent in the environment. The combination of these factors makes these two VPs representative of different VP transport conditions.

To examine local manure application patterns, we assume the combination of sandy soil and maize, which is present on the majority of agricultural fields in the area, as representative of the whole catchment. As a result, over 95% of yearly (slurry) manure loads are typically applied to the soil between mid-March and the beginning of June (Kros et al., 2019; de Vries et al., 2023). It should be noted that this practice may vary slightly between farms, but it is consistent with the Dutch national regulations for manure application (RVO, 2022).

It is further important to acknowledge the temporal scale of the available data in this study. The quantities of VPs applied to the soil are based on the average annual quantities for the period 2015-2018, while manure application patterns are based on 2017. On the other hand, the quantities of VPs at the monitoring location were quantified in 2020. However, it is worth mentioning that our earlier research (Rakonjac et al., 2022) suggests that the first two factors are not expected to change significantly during the considered period and may also be representative for 2020.

4.2.2 Hydrometeorological data

The hydrologic model in this study relies on data inputs for precipitation (P) and potential evapotranspiration (ET_{pot}) obtained from the Royal Netherlands Meteorological Institute (KNMI) covering the period 2018 - 2022. This period is selected to coincide partially with the VPs surface water measurements (Chapter 5) and include the available discharge measurements (2020-2022) at the monitoring location shown in Figure 4.1, which will be used to validate the model's performance. In a nutshell, gridded files of daily precipitation data measured at approximately 300 locations across the Netherlands and daily Makkink evaporation data based on 7 to 35 automatic weather stations from the KNMI (Hiemstra and Sluiter, 2011) were collected for the period of interest and subsequently refined to concentrate on the particular catchment. For more information on data compatibility and

interpretation, please refer to Imhoff et al. (2022). The year 2021 (see Figure 4.2) has the typical features of a Dutch lowland catchment: Rainfall is frequent and evenly distributed throughout the year, totaling about 700-800 mm/y; potential evapotranspiration may exceed 4 mm/d in June to August; streamflow has marked seasonality with very low flows in the summer and reacts quickly (within a day) to most storm events.

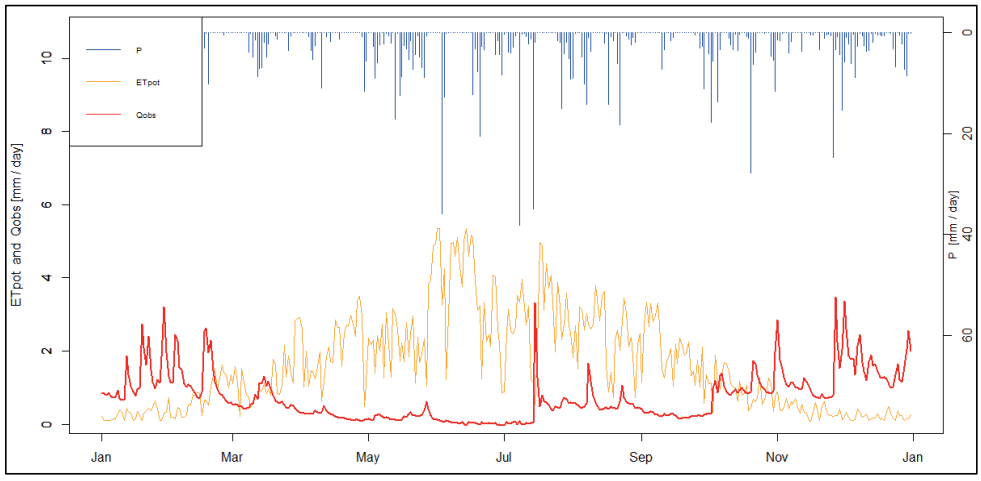


Figure 4.2: *Daily values of precipitation (P), potential evapotranspiration (ET_{pot}), and observed discharge (Q_{obs}) in the study catchment for 2021.*

4.3 Model

4.3.1 Hydrologic model

For this study, we selected the Wageningen Lowland Runoff Simulator (WALRUS) model (Brauer et al., 2014) as a parsimonious representation of flow processes in our study area. WALRUS has been developed for lowland areas and was shown to produce accurate runoff predictions (Brauer et al., 2014; de Boer-Euser et al., 2017; Pijl et al., 2018), including catchments close to our study area (Imhoff et al., 2022). The model includes three main compartments: a soil reservoir, which comprises a vadose zone and a groundwater zone, a quickflow reservoir, and a surface water reservoir (Figure 4.3). The quickflow compartment is meant to simulate water that bypasses the soil matrix due to e.g. drainage pipes, soil cracks and overland flow (Brauer et al., 2014). A wetness-dependent flow routing approach allows quickflow to dominate during the early phase of the hydrologic response. The model simulates runoff (Q) and actual transpiration (ET_{act}), plus all the 'internal' fluxes that partition and connect the

different storage compartments (i.e., P_S, P_V, P_Q, ET_V, ET_S, f_{GS}, f_{XG}, f_{XS}, and f_{QS} in Figure 4.3). For a comprehensive description of the model architecture, including its equations and parameters, we refer to (Brauer et al., 2014).

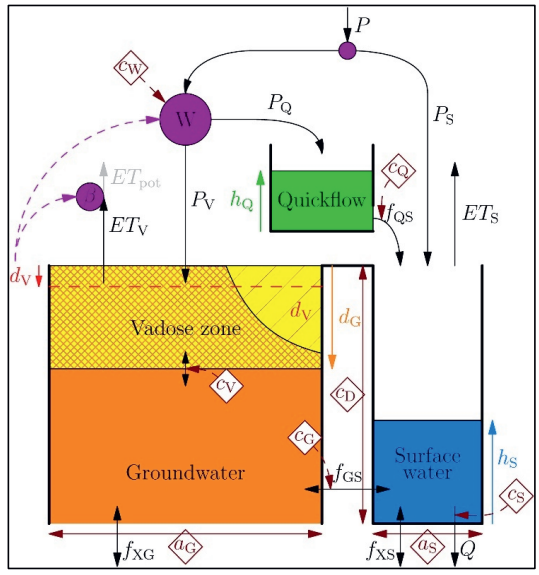


Figure 4.3: *Overview of the WALRUS model structure. Fluxes are represented with black arrows, model parameters with brown diamonds, and states are indicated in the color of the reservoir they belong to. For a complete explanation of all variables, please refer to Brauer et al. (2014), from which this figure is also sourced.*

We use WALRUS with two purposes here: 1) to estimate discharge during periods where discharge observations (Q_{obs}) are unavailable; 2) as the starting point for our transport model (Section 4.3.2). The need to reconstruct discharge observations is due to a temporal discrepancy between the available Q_{obs} and VPs measurements in surface water. The former is only available from the end of 2020, while the latter was conducted between March 25th and June 17th, 2020. We validate the model using the period with available Q_{obs}, and then estimate discharge data for the preceding period when VPs measurements are available. Our approach is analogous to the implementation by Imhoff et al. (2022), who studied a catchment close to our study area. We assume that the model parameters used in the previously mentioned study, namely the wetness index parameter (C_W), vadose zone relaxation time (C_V), groundwater reservoir constant (C_G), and quickflow reservoir constant (C_Q), are applicable to our catchment. However, we also utilize the Monte Carlo approach (Beven and Freer, 2001) to calibrate these parameters to our catchment, and we will discuss some of the observations later in this paper. The only difference with respect to Imhoff et al. (2022) is that we opted for a more conservative approach and assumed half of the groundwater leakage flux (f_{XG} in Figure 4.3). We evaluate the model

adequacy at reproducing runoff at our study site by computing the Nash-Sutcliffe (NS) efficiency (Nash and Sutcliffe, 1970), which is a widely used metric for goodness of fit in hydrologic models. The fluxes computed by WALRUS are ultimately passed over to the VPs transport model, as described in the following section.

4.3.2 VP transport model

VPs loads: The local manure application patterns have a significant impact on the distribution of VP loadings, and these patterns depend on various factors such as manure production, available agricultural land, transport, processing, and export. To calculate the distribution of VP loadings in the catchment, the INITIATOR model (Kros et al., 2019) is used based on information from Rakonjac et al. (2023). The method takes into account the applied mass distribution of VPs at the individual farm level, assuming that the applied mass is evenly distributed across fields associated with each farm. The catchment-scale VP input is computed by aggregating the contribution of each individual field. After aggregation, the annual applied mass of Sulfadiazine and Flubendazole is estimated to be 26.5 kg and 6.4 kg, respectively. The application of VPs to each field in the catchment is assumed to occur between March 12th and April 30th, inclusive, and spans 50 days. Hence, the estimated annual applied VPs masses are concentrated in the 50 days of manure application and distributed into five 10-day intervals, with application rates of 10%, 35%, 35%, 15%, and 5% for each interval, respectively, as suggested by Kros et al. (2019). The same pattern is repeated during each of the modeled years.

Coupling with WALRUS: The utilized approach is based on mass balance computations derived from the internal fluxes of the WALRUS model and the VPs loads. The key assumption here is that each compartment is 'randomly sampled' by its outflows, which is mathematically equivalent to a 'well-mixed' system. With this assumption, the transport problem is conveniently simplified and the solute outflow concentration can be computed out of simple mass balances without the need to formulate a full water-age balance (see Benettin et al. (2022)). Thus, we define water and VPs mass balance equations for each modeled storage compartment (as shown in Figure 4.3):

$$\frac{dS}{dt} = Q_{in} - Q_{out} \tag{4.1}$$

$$\frac{dM}{dt} = M_{in} - M_{out} + M_{react} \tag{4.2}$$

where S is the water in storage and Q_{in} and Q_{out} are input and output water fluxes, respectively. These fluxes are based on the internal fluxes from WALRUS. M represents the mass of VPs within the

compartment, while M_{in} and M_{out} denote the input and output mass fluxes, respectively. The term M_{react} incorporates all the reaction and sorption processes undergone by the VPs during their transport to the river network and it is modeled here as a simple first-order reaction of the type:

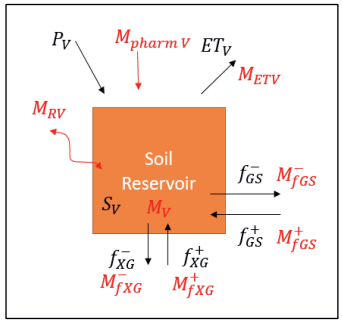
$$M_{react} = -k\,M \tag{4.3}$$

where k is the decay constant of each VP, defined through the VP half-life (DT50) as k=ln(2)/DT50.

To account for the volumes of water that are not involved in the hydrologic response but can be mixed with the system storage, a residual storage parameter (S_R) is introduced for each compartment. The residual storage parameter is fundamental to conciliate the quick runoff response with the long water transit times (Birkel et al., 2015) and it was typically shown to be larger than 1000 mm (see Benettin et al. (2015)). Here, we test different values of residual storage parameters lower than 10000 mm.

The general water and mass balance equations (eq. 4.1-4.2) are applied to each compartment in equations 4.4 to 4.14, where the hydrologic input/output fluxes to/from each compartment come from the WALRUS model. The terms indicated in light gray are considered negligible and they are not accounted for in the model. The Supplementary Material (SM) provides the notation for all used parameters and their units, as well as the initial conditions not mentioned below.

<u>For soil reservoir (vadose zone + groundwater), the balance equations are</u>:



$$\frac{dS_V}{dt} = P_V - ET_V - f_{GS}^- + f_{GS}^+ - f_{XG}^- + f_{XG}^+ \tag{4.4}$$

with the initial condition:

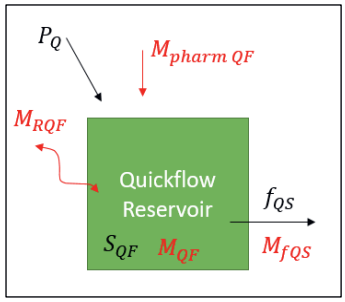
$$S_V(0) = S_{V0} + S_{VR} \tag{4.5}$$

$$\frac{dM_V}{dt} = M_{pharm\,V} - M_{ETV} - M_{fGS}^- + M_{fGS}^+ - M_{fXG}^- + M_{fXG}^+ + M_{RV}$$

$$= M_{pharm\,V} - M_{ETV} - \overline{C_V} f_{GS}^- + C_Q f_{GS}^+ - \overline{C_V} f_{XG}^- + C_{XG} f_{XG}^+ - kM_V \tag{4.6}$$

and $\overline{C_V} = M_V / S_V$ (4.7)

For quickflow reservoir, the balance equations are:



$$\frac{dS_{QF}}{dt} = P_Q - f_{QS} \tag{4.8}$$

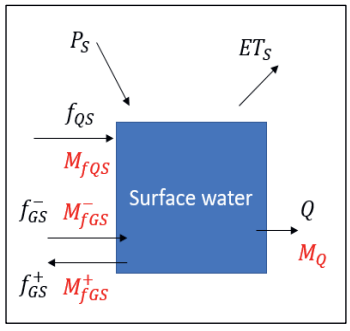
with the initial condition:

$$S_{QF}(0) = S_{QF0} + S_{QFR} \tag{4.9}$$

$$\frac{dM_{QF}}{dt} = M_{pharm\,QF} + M_{fQS} + M_{RQF}$$
$$= M_{pharm\,QF} - C_{QF} f_{QS} - k\, M_{QF} \tag{4.10}$$

and $$\overline{C_{QF}} = M_{QF}/S_{QF} \tag{4.11}$$

For surface water, the balance equations are (with the assumption that there are no storage effects present in the surface water and no mass removal):



$$Q = P_S - ET_S + f_{QS} + f_{GS}^- - f_{GS}^+ \tag{4.12}$$

$$M_Q = M_{fQS} + M_{fGS}^- - M_{fGS}^+$$
$$= C_{QS} f_{QS} + C_V f_{GS}^- - C_Q f_{GS}^+ \tag{4.13}$$

and $$\overline{C_Q} = M_Q/Q \tag{4.14}$$

To obtain an approximate solution of equations 4.4 to 4.14 at discrete time steps, we use a simple Euler Forward numerical scheme, which can be expressed as follows:

From hydrologic balance

$$S_V(i+1) = S_V(i) + \left(P_V(i) - ET_V(i) - f_{XG}^-(i) - f_{GS}^-(i)\right) * dt \tag{4.15}$$

$$S_{QF}(i+1) = S_{QF}(i) + \left(P_Q(i) - f_{QS}(i)\right) * dt \tag{4.16}$$

For each VP

$$M_V(i+1) = M_V(i) + \left(M_{pharm\,V}(i) - \overline{C_V}(i) f_{GS}^-(i) - \overline{C_V}(i) f_{XG}^-(i) - k\, M_V(i)\right) * dt \tag{4.17}$$

$$M_{QF}(i+1) = M_{QF}(i) + \left(M_{pharm\,QF}(i) - \overline{C_{QF}}(i) f_{QS}(i) - k\, M_{QF}(i)\right) * dt \tag{4.18}$$

$$\overline{C_V}(i+1) = M_V(i+1)/S_V(i+1) \tag{4.19}$$

$$\overline{C_{QF}}(i+1) = M_{QF}(i+1)/S_{QF}(i+1) \tag{4.20}$$

$$\overline{C_Q}(i+1) = \left(\overline{C_{QS}}(i+1)f_{QS}(i) + \overline{C_V}(i+1)\bar{f}_{GS}(i)\right)/(f_{QS}(i) + \bar{f}_{GS}(i)) \tag{4.21}$$

The predicted stream concentration $\overline{C_Q}$ (equation 4.21) represents a daily value, while the surface water measurements presented in Chapter 5 are based on 12-week and 4-week sampling periods. Therefore, we further take a temporal mean over windows of 12 and 4 weeks to compare our results with the measurements.

4.4 Results

4.4.1 Hydrologic model

Using the procedure described in Section 4.3.1 and model parameters from Imhoff et al. (2022), we ran the WALRUS model for the timeframe between mid-December 2020 and June 2022, corresponding to the period with available Q_{obs} data for validation. The predicted discharge Q exhibited generally good agreement with Q_{obs} (see Figure 4.4), as evidenced by a NS coefficient of 0.80. The parameters obtained through the Monte Carlo calibration are only slightly different and result in a similar NS coefficient (0.83, based on 10,000 simulations). The calibrated parameters improved the model's ability to reproduce low flow periods, but resulted in slightly worse fitting for high flow periods. The initial and calibrated parameter values are similar, but the parameterization by Imhoff et al. (2022) is based on a longer dataset and so it was deemed to be more robust. Therefore, we proceeded with the non-calibrated parameter set.

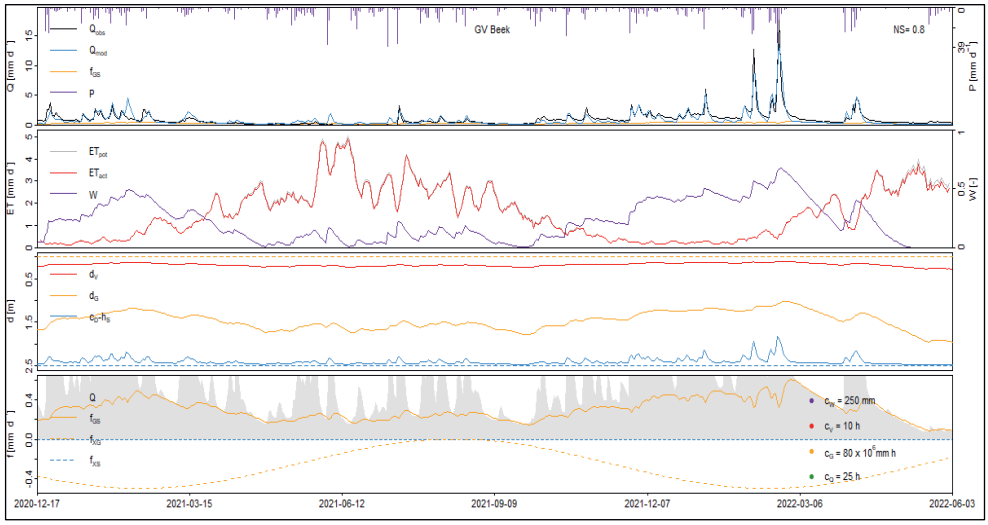


Figure 4.4: *Modeled states and fluxes with the WALRUS model for the catchment of interest. The top sub panel shows the simulated (blue) and observed (black) discharge. The NS coefficient is indicated in the top right. The purple bars indicate the rainfall input, the yellow line the groundwater drainage. Sub panel two shows the actual (red) and potential (grey) evapotranspiration, and the wetness index in purple. Sub panel three shows the storage deficit (red), the groundwater level below the surface (yellow) and the surface water level below the surface (blue). Sub panel four shows the managed groundwater outflow (downward seepage; dotted yellow line). All fluxes are in mm d⁻¹. For a complete explanation of all variables, please refer to Brauer et al. (2014).*

The good level of agreement between the model predictions and the observed flow data provides confidence in the model's ability to accurately reproduce the flow dynamics in our catchment. As an additional visual validation, we compared our model results for the year 2019 (when no measurements were available) with the discharge patterns reported in Imhoff et al. (2022) for the neighboring catchment. We found that the low flow periods were temporally aligned, indicating an overall dry period in the region, which further reinforces our confidence in the model predictions.

Understanding the relative contributions of different fluxes to stream flow is essential to assess their impact on VPs transport and water quality. Variability in water quality is largely determined by the temporal changes in the relative contributions of different sources. Thus, we investigated the relative contributions of groundwater (f_{GS}) and quickflow (f_{QS}) to total streamflow (Q) for the whole period of interest (2018-2022), as illustrated in Figure 4.5. Our analysis showed that during high flow periods, f_{QS} had a greater contribution to stream flow than f_{GS}, which is reasonable. The contribution of f_{GS} instead, is dominant in-between events and during prolonged low flow periods.

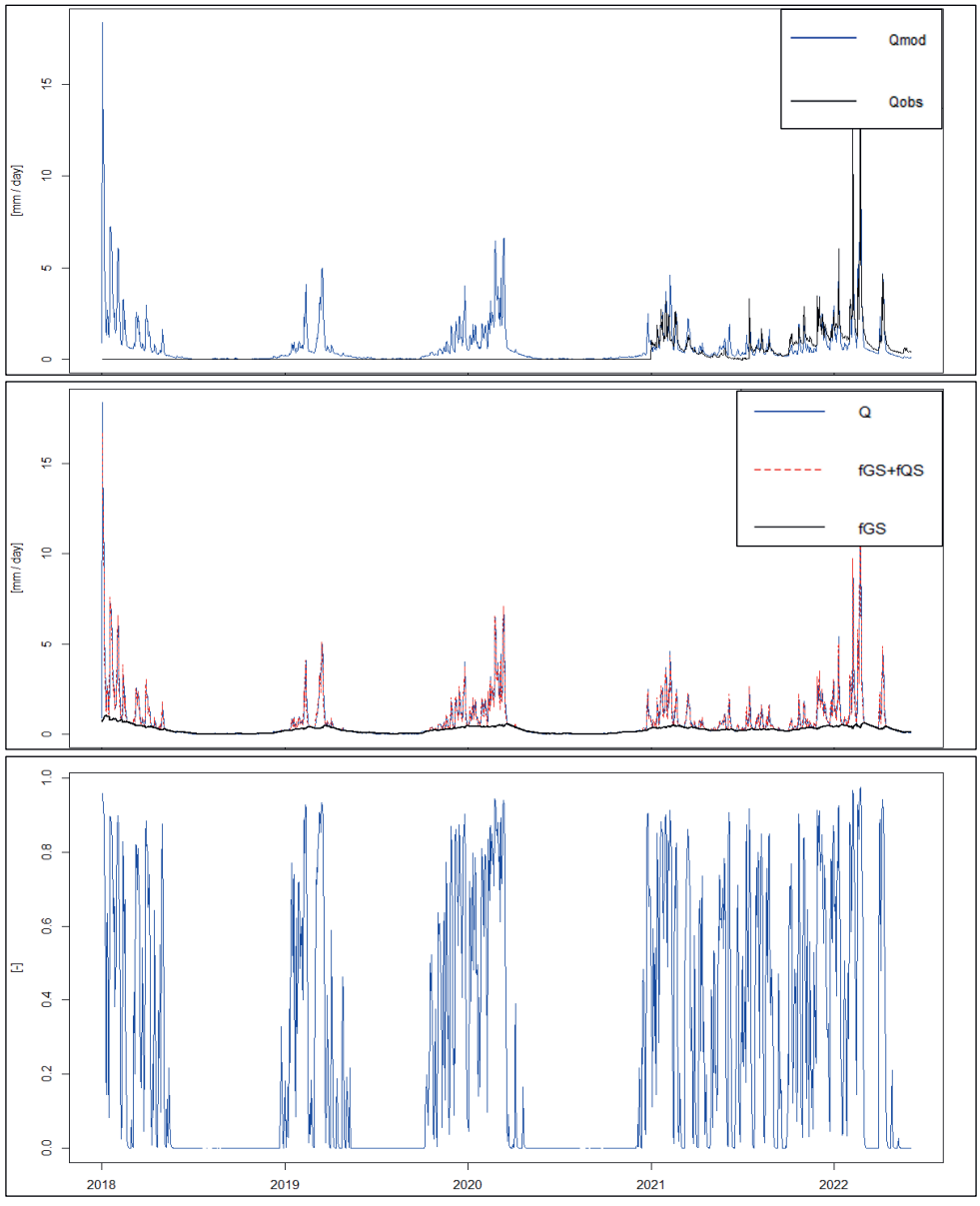


Figure 4.5: *Top figure shows the comparison between modeled (Q) and observed discharge (Q_{obs}). Middle figure shows the contribution of quickflow (f_{QS}) and groundwater flux (f_{GS}) to Q. Bottom figure shows the relative contribution of f_{QS} to Q, expressed via $f_{QS}/(f_{QS}+f_{GS})$.*

4.4.2 Water transit times and VP transport

We performed VP transport calculations as described in Section 4.3.2. We ran a first series of simulations with a fixed residual storage of 2000 mm for the soil compartment (S_{VR}) and 200 mm for the quickflow compartment (S_{QFR}), and DT50 values of 1, 10, 100 days. With these parameters, we can compute the mean, long term transit time as $\tau = \bar{S}/\bar{F}$, where $\bar{S}$ is the long term storage (computed as 2200 mm) and $\bar{F}$ is the long-term precipitation (estimated as 770 mm/y). With these values, the resulting τ is 2.8 years.

Assuming that VPs were applied mainly into the soil, but far enough from preferential flow areas, as per Dutch agricultural practices and the tendency for lower agricultural emissions, especially those related to nitrogen and phosphorus (RVO, 2022), then it is possible to neglect the mass applied directly to the quickflow compartment ($M_{pharm\ QF}$). For that case, VP simulations are shown in Figure 4.6, where the black and blue lines indicate the soil and quickflow simulated concentrations, and the red dots indicate the modeled streamflow concentration. The three simulations have similar patterns, with the concentration increasing in March-April due to the new VP input to the soil and decreasing after the application period (black vertical lines in Figure 4.6) due to dilution and degradation. The concentration generally resembles the soil compartment concentration, with occasional dilutions during periods with important quickflow contributions. The simulations with long DT50 (100 days) suggest a possible carry-over of VPs from one season to the next, because streamflow concentration does not reach a zero value during the winter. Simulations with a shorter DT50 suggest a much faster decrease in streamflow concentration after the input period, with generally lower values.

The measurement period (12 weeks, between March 25th and June 17th) occurs during and right after the input application period, when the simulated concentrations is at its highest level and then decreases. When our results are averaged over the 12-week measurement period, we see that all simulations overestimated the measured VPs by several orders of magnitude (simulated values on the order of ~1×10^7 ug against measurements of ~1×10^0 ug), regardless of the choice of residual storage and half-life parameters.

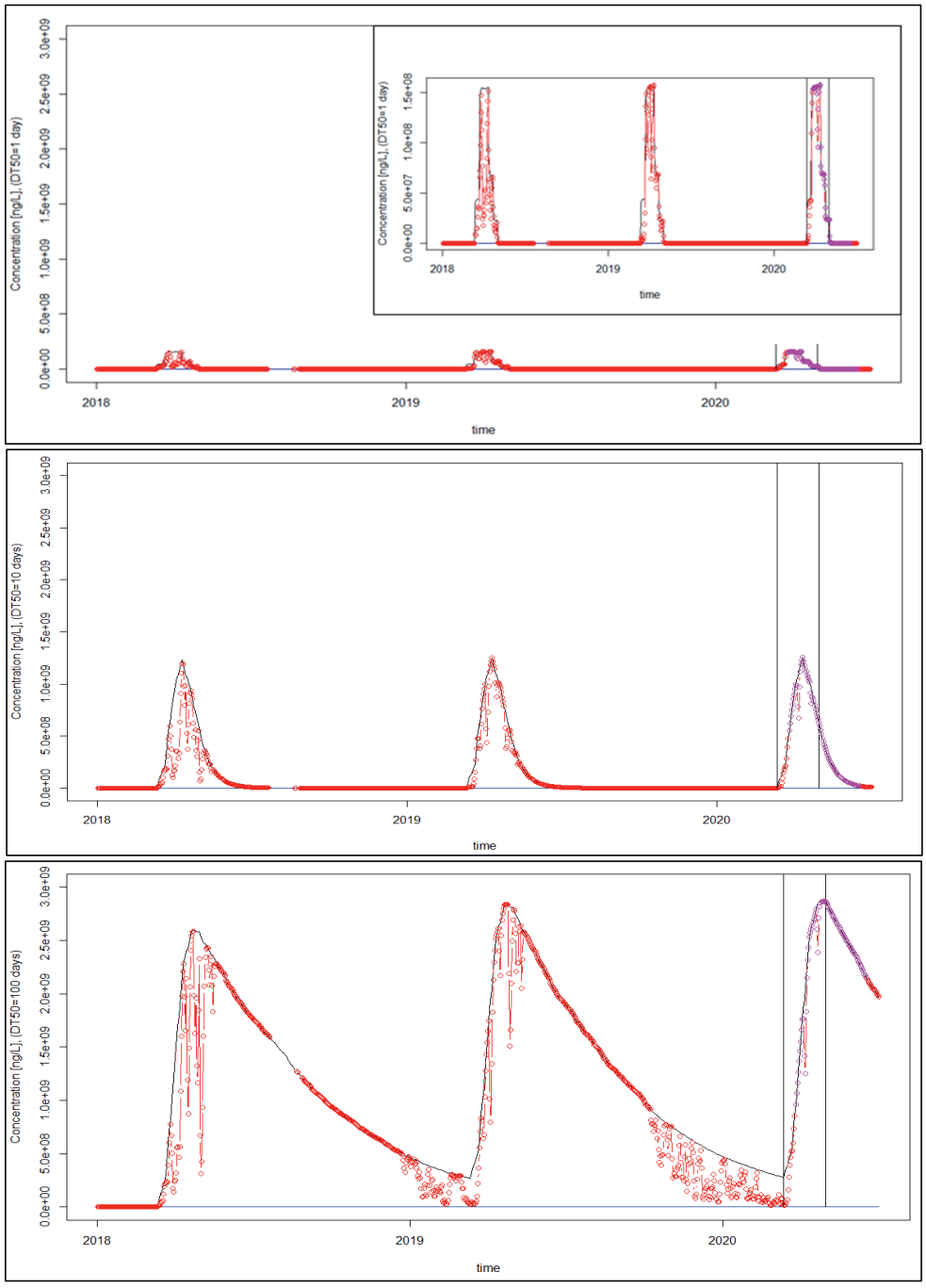


Figure 4.6: *Simulated VP concentrations (in ng/L) with varying DT50 values and their corresponding soil (black lines) and quickflow (blue lines) concentrations, along with modeled streamflow concentration (red dots). For the interest year (2020), black vertical lines indicate the VP application period, while modeled streamflow concentrations corresponding to the period of surface water measurements are indicate with magenta dots. The inset in the top figure offers a magnified view on concentrations when DT50 equals 1 day.*

4.5 Discussion

4.5.1 Model-data comparison

The current transport model implementation is based on a simplified yet widely used approximation of catchment transport processes. All the catchment compartments are assumed to be randomly sampled by the outflows and they are connected through hydrologic fluxes based on an independent hydrologic model (WALRUS in this case). Streamflow water quality is chiefly governed by the variability of the contributions from different compartments, and these in turn are controlled by rainfall variability. Previous studies (Benettin et al., 2013; Benettin et al., 2015; Hrachowitz et al., 2015) demonstrated that these models are able to simulate dynamic transport conditions, in both forested and agricultural catchments. Transit time distributions computed from such models are highly variable but they all show important contributions of water sources that are just a few days old and also contributions from older water that may have resided within the catchment for years before ending up in the stream. Even though we did not have tracer data to compare our model against, the general transport dynamics simulated by our model are fully consistent with previous catchment-scale models and our mean transit time (2.8 years) is just slightly higher than the mean transit times of 1.8 years estimated at another Dutch lowland catchment (van der Velde et al., 2010). Since the modeled transit time dynamics appear realistic but the modeled VP concentration is much higher than the measurements, we must deduce that either the first-order decay is structurally inadequate at reproducing the complexity of reactive and sorption processes, or the direct comparison of our simulated VP concentrations with the measurement is not appropriate.

In Figure 4.7 we evaluated the concentration reduction that can be achieved using a first-order decay applied to an exponential transit time distribution with different mean transit times of 0.5, 2 and 10 years (blue, red and yellow curves, respectively). All curves start from a concentration of 10^0 corresponding to a very long DT50 (1000 days) and then they decrease quickly with shorter DT50 values (note the log-log scale of the plot). However, even for the case with the largest mean transit time (yellow curve) and a DT50 as low as 10^0 days, the maximum concentration reduction does not fall below 10^{-4} (similar to our model reduction), while measurements displayed concentration reductions of 10^{-9}. One may wonder how it is possible that, with a mean transit time of 2 years and a DT50 of just 1-2 days, the concentration does not get any lower than ~10^{-3}. This issue is clarified when considering that distribution with long mean transit times still includes significant amounts of young water. This is shown in Figure 4.8, where the behaviors of a conservative (blue curve) and reactive solute with DT50 = 2 days (red curve) are shown. Due to the effect of the reaction, the reactive solute contribution

decreases exponentially with respect to the conservative solute, but the early part of the distribution (up to a few days) is almost unaffected by the reaction and thus it brings its reactive solute signature.

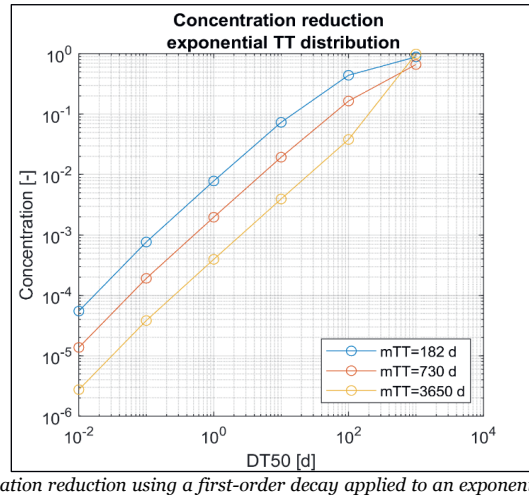


Figure 4.7: *The concentration reduction using a first-order decay applied to an exponential transit time distribution with the mean transit times of 0.5 (blue curve), 2 (red curve) and 10 years (yellow curve).*

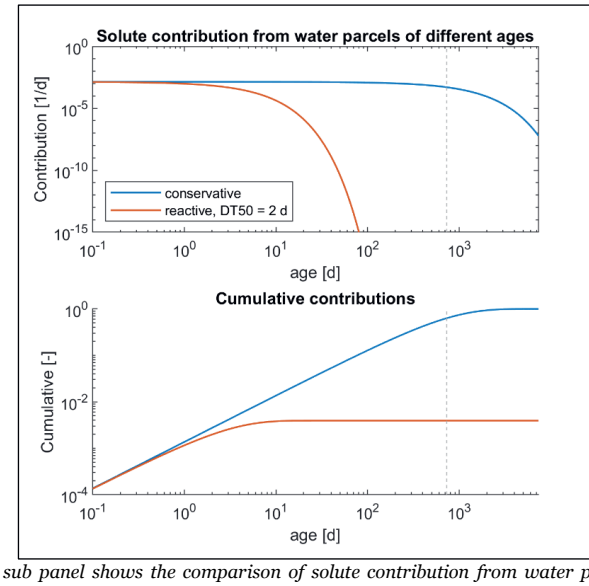


Figure 4.8: *The top sub panel shows the comparison of solute contribution from water parcels of different ages: reactive (red curve) vs conservative solute (blue curve) behavior. The bottom sub panel emphasizes the cumulative contributions of the two solutes.*

In other words, in systems where the concentration decreases quickly over time, small quantities of young water may control the overall water quality because they are almost not impacted by the reaction. Figure 4.8 (bottom), in particular shows that the cumulative contribution of water younger than 10 days is enough to explain a concentration of 10^{-3} of the input. A similar behavior cannot be

reproduced by models where only the mean water velocity is considered rather than the entire velocity distribution. The first-order decay is thus structurally unable to explain massive concentration reductions in the presence of young water transport and other processes need to be accounted for to reduce the concentration further. It is however worth to wonder whether our comparison between modeled and measured VP concentrations is appropriate. We assumed that measurements carried out with a passive sampler are representative of some average concentration over the duration of the sampling (12 weeks in this case). However, VPs which are accumulated on passive samplers are potentially affected by degradation and this degradation should perhaps be accounted for explicitly in the model in order to explain the measured data.

4.5.2 Implications for catchment scale VP circulation and future research

- The model is currently not able to explain the data, yet it suggests dynamics that are potentially interesting. The timing of streamflow sampling appears to be crucial because the concentration likely decrease quickly after the application period (Figure 4.6).
- While we looked at concentrations here, the mass export is also relevant. High VP concentrations during very low summer flows make small mass fluxes, but higher water fluxes in the spring period potentially represent hot-moments of streamflow pollution that would need to be quantified properly.
- Modeling can help design sampling campaigns. Building scenarios with a parsimonious model based on just a few parameters (the residual storage and a DT50) allows one to target a specific period or hydrologic condition and make sampling efforts more efficient.
- The model's ability to accommodate various VPs is highly valuable here, particularly when considering the high number of active substances used in countries like the Netherlands.
- In future work, it would be beneficial to model degradation and reaction using different approaches. Additionally, further investigation is needed to interpret passive sampler measurements.

4.6 Conclusions

This paper examines the temporal dynamics of VPs in lowland surface waters in a Dutch agricultural catchment by utilizing data on VP concentrations in manure and surface water. Our investigation of the catchment hydrology is based on the WALRUS rainfall-runoff model. Subsequently, we use the outputs from the WALRUS model to construct a catchment-scale transport model for VPs that differentiates between quickflow routes and slower routes through soil reservoir. The used hydrological model and associated parameters displayed a noteworthy level of accuracy in forecasting

the catchment discharge patterns over an extended period, as demonstrated by the comparison with observed flow data. However, the VP transport model developed did not provide a satisfactory fit to the surface water measurements. Given that the modeled transit time dynamics seem realistic but the modeled VP concentrations are significantly higher than the measurements, it can be inferred that either the first-order decay approach does not adequately capture the complexity of reactive and sorption processes, or direct comparison of the simulated and measured VP quantities may not be appropriate. Despite the transport model's inability to explain the field data at present, it still provided some valuable insights, including the significant impact of manure application timing on the temporal distribution of VP occurrence in a stream. The integration of complex formulations of VP transport and biogeochemical processes at the catchment scale into a single model is a challenging task. Nonetheless, it possesses the capacity to act as a fundamental foundation for constructing more robust models for water quality.

Supplementary Material

An overview of the parameters used:

Parameter	Description	Unit
P_V, P_Q, P_S, ET_V, ET_S, f_{QS}, f_{GS}^-, f_{GS}^+ f_{XG}^-, f_{XG}^+	WALRUS internal fluxes	[L T^{-1}]
M_{ETV}, M_{fQS}, M_{fGS}^-, M_{fGS}^+, M_{fXG}^-, M_{fXG}^+	Mass fluxes corresponding to the WALRUS internal fluxes	[M T^{-1}]
S	Water in storage	[L]
Q_{in}	Input water flux	[L T^{-1}]
Q_{out}	Output water flux	[L T^{-1}]
M	VP mass within the compartment	[M]
M_{in}	Input mass flux	[M T^{-1}]
M_{out}	Output mass flux	[M T^{-1}]
M_{react}	Reaction term	[M T^{-1}]
k	Decay constant	[T^{-1}]
DT50	VP half-life	[T]
S_V	Soil reservoir water storage	[L]
M_V	VP mass within soil reservoir	[M]
M_{pharmV}	VP mass flux applied to soil reservoir	[M T^{-1}]
M_{RV}	Reaction term for soil reservoir	[M T^{-1}]
$\overline{C_V}$	Mean VP concentration in soil reservoir	[M L^{-1}]
S_{QF}	Quickflow reservoir water storage	[L]
M_{QF}	VP mass within quickflow reservoir	[M]
$M_{pharmQF}$	VP mass flux applied to quickflow reservoir	[M T^{-1}]
M_{RQF}	Reaction term for quickflow reservoir	[M T^{-1}]
$\overline{C_{QF}}$	Mean VP concentration in quickflow reservoir	[M L^{-1}]
M_Q	VP flux in surface water reservoir	[M T^{-1}]
$\overline{C_Q}$	Predicted mean VP stream concentration	[M L^{-1}]

Chapter 5

Surface Water Monitoring of Chemicals Associated with Animal Husbandry in an Agricultural Region in the Netherlands using Passive Sampling

Based on:

Rakonjac, N., Roex, E., Beeltje, H. Surface Water Monitoring of Chemicals Associated with Animal Husbandry in an Agricultural Region in the Netherlands using Passive Sampling. *Environmental Pollution – under review.*

Abstract

Compounds originating from animal husbandry can reach surface water through the application of manure to soil. Typically, grab sampling is employed to detect these residues, which only provides information on the concentration at the time of sampling. To better understand the emission patterns of these compounds, we utilized passive samplers in surface water to collect data at eight locations in a Dutch agricultural region, during different time intervals. As a passive sampler, we chose the adsorption-based Speedisk® H2O-Philic DVB. In total, we targeted 46 compounds, among which 25 antibiotics, three hormones, nine antiparasitics, and nine disinfectants. From these 46 compounds, 22 compounds accumulated in passive samplers in amounts above the limit of quantification in at least one sampling location. During the 12-week deployment period, a time integrative uptake was observed in 59% of the investigated cases, while 41% did not exhibit this behavior. Our findings suggest that the proposed method of using passive samplers is more efficient than traditional grab sampling, leading to the detection of more compounds. In fact, a number of compounds originating from animal husbandry activities were quantified for the first time in Dutch surface waters. The set-up of the sampling campaign also allowed to distinguish between different pollution levels during sampling intervals on the same location. This improvement could be significant considering the frequent introduction of new compounds used in livestock and could enhance current monitoring and mitigation efforts.

5.1 Introduction

Veterinary pharmaceuticals (VPs) are compounds used to treat or prevent diseases of animals. Depending on the compound, a certain fraction is excreted unchanged via urine and feces and ends up in the animal manure after administration (Berendsen et al., 2018). In the Netherlands, the use of VPs (especially antibiotics) in livestock farming has been drastically reduced over the last decade but used quantities are still significant (de Greeff et al., 2021). In addition, the Dutch livestock farming sector produces considerable amounts of manure and consequently VPs residues frequently end up on the soil via the application of manure as fertilizer on arable land (Lahr et al., 2018; Rakonjac et al., 2022; Rakonjac et al., 2023). Depending on the physical-chemical characteristics of the compound and the soil properties, a portion of these residues may further reach the surface water via flow paths (e.g. surface runoff), potentially resulting in the pollution of surface water systems over long distances downstream of the application areas (Bailey et al., 2015).

Next to frequently used VPs, surface water in the Netherlands may also be exposed to other classes of compounds associated with animal husbandry, from which naturally occurring hormones and disinfectants are the most prominent. An extensive Dutch study (Vethaak et al., 2005) has shown that in streams in agricultural areas in the Netherlands, relatively high levels of naturally occurring hormones appear. The same study also showed that these compounds can have detrimental effects on organisms. The class of Quaternary Ammonium Compounds (QACs) belongs to the disinfectants that have the potential to come into contact with Dutch surface water. These compounds are known to have a broad range of applications (Mulder et al., 2018), and have recently been identified in manure, forage and agricultural soil samples in the Netherlands (Buijs et al., 2019). To the best of our knowledge, the occurrence of these compounds in surface water within agricultural regions of the Netherlands has not been made public thus far, regardless of their potential to pose a risk to human health upon exposure (Hrubec et al., 2021).

Despite the substantial amount of data confirming the global prevalence of the aforementioned compounds, particularly VPs, in aquatic systems (Umwelt Bundesamt), and some studies suggesting potential risks to the aquatic environment (Kemper, 2008; Kools et al., 2008), the evidence on their occurrence in surface waters remains limited and scarce due to insufficient monitoring efforts. This is especially the case for smaller streams that are not directly used as a source for drinking water but rather for agricultural activities (e.g. irrigation). Besides, these small streams act as breeding grounds for numerous aquatic species and disturbance of their habitat by a.o. VPs may have eventual severe effect on aquatic ecosystems. For this reason, the monitoring of VPs in surface water has been

highlighted by the European Commission Strategic Approach to Pharmaceuticals in the Environment (2019) as one of the research questions which remains to be tackled.

In aquatic systems, monitoring of compounds is typically based on grab sampling of the aqueous phase, followed by chemical analysis of the targeted compounds. Although grab sampling is a widely used and accepted method (e.g. in European frameworks), it only provides a snapshot of the concentration at the moment of sampling and may not account for temporal fluctuations due to irregular emission patterns or variations in water flow. This is especially relevant for chemicals with diffuse environmental pathways, such as compounds associated with manure application. To improve sampling sensitivity and integrate temporal changes, time integrative passive sampling has been proposed (Górecki and Namieśnik (2002); Vrana et al. (2005)). This method involves deploying an adsorption or absorption material in the aquatic environment for a certain time period, allowing for accumulation of compounds and monitoring of low aqueous concentrations.

Passive samplers can be broadly categorized into two types: partitioning-based samplers and adsorption-based samplers. Partitioning-based samplers mainly absorb hydrophobic substances from the aqueous phase because of their higher affinity for the sampler material (mostly polymeric) compared to the aqueous phase. Depending on the sampler-water partition coefficient (Kpw), which is unique for every compound, the concentration in the sampler will eventually reach equilibrium with the concentration in the water. If the Kpw of a certain compound is known, the sampled water volume per compound can be determined, thereby making use of the release of Performance Reference Compound (PRCs) dosed to the sampler prior to deployment of the samplers (Rusina et al., 2010). Adsorption-based samplers bind the substances at the surface of the adsorption material, even if only a part of the molecule has the appropriate affinity. Assuming that sufficient binding material is present in the sampler, adsorption-based samplers are supposed not to reach equilibrium and display compound uptake that is linear over time. Especially for substances with polar functional groups that avoid absorption by partitioning-based samplers, like VPs, adsorption-based sampler has proven to be very suitable, particularly for the purpose of qualitative screening (Moschet et al., 2015; Škodová et al., 2016; Gong et al., 2018). Opposite to partitioning based samplers, the use of PRCs to determine the sampling rate is not feasible for adsorption based samplers currently. In order to establish aqueous calculations, sampling rates for the different compounds have to derived in controlled laboratory or controlled field experiments. However, these laboratory-derived sampling rates may not be applicable to the field conditions, as flow rates may variate over time. Although attempts have been made (Hamers et al., 2018), no methods are currently available to directly determine the sampling rate of

adsorption-based samplers in the field. Therefore we decided to base our findings on the amounts per sampler.

The most comprehensive study to date in the Netherlands shows that only a limited number of VPs could be detected in surface water located in an agricultural area, which according to the authors could be partly attributed to the low monitoring frequency (Lahr et al., 2018). This was confirmed by a recent overview (Lahr et al., 2019), in which passive sampling was mentioned as an alternative method to overcome this problem. Therefore, to gain more insight into the presence of VPs in the surface waters in the Netherlands, we deployed passive samplers on a number of locations in an agricultural area with intensive livestock activities. As especially the category of VPs was our main interest, the adsorption-based Speedisk was our passive sampler of choice. We further discussed the performance of passive samplers, spatial and temporal trends, and the amount of compounds accumulated on the samplers, if detected.

5.2 Materials and methods

5.2.1 Sampling locations and deployment scheme

The study area is an agricultural region in the middle of the Netherlands. A number of locations were visited together with a representative of the regional water authority in February 2020 and assessed in terms of their suitability as potential monitoring sites. The criteria considered during those visits were: extent of livestock breeding, livestock sector, potential inputs of chemicals, manure application rates, soil and hydrological characteristics, previous VPs measurements in surface water (if available), and feasibility of passive samplers deployment. Preference was given to areas where many cow and/or pig farms were located on sandy soil, where fields (i.e. manure application areas) were located nearby water streams, and where contribution of human pharmaceuticals was minimized (e.g. without upstream discharges from WWTP). Eight sampling locations were selected using these criteria, as illustrated in Figure 5.1.

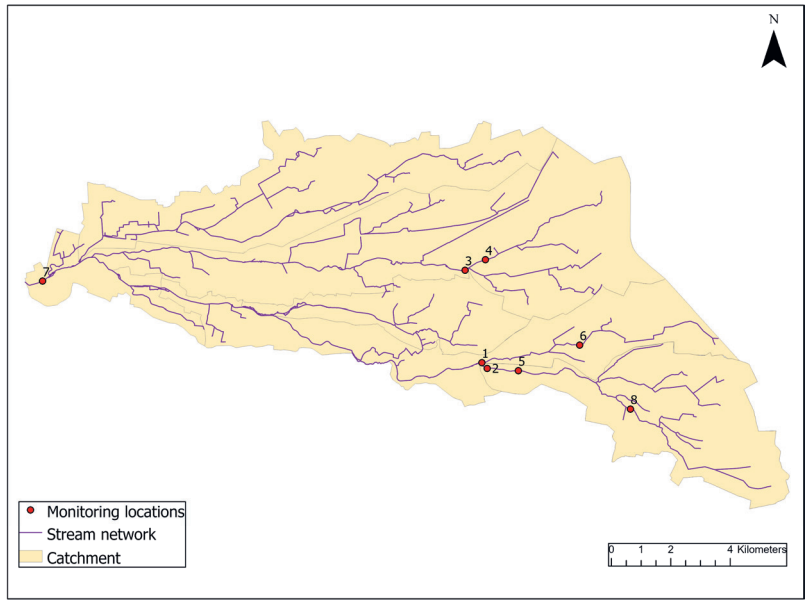


Figure 5.1: *Selected agricultural region in the middle of the Netherlands with eight monitoring locations. Water flow is in the direction from east to west, where location 7 represents the catchment outlet.*

The total catchment area, and therefore the total area draining into the sampling location number 7, is around 158 km2. This area is a valley formed in the Saale period (150 ka BC) and its shallow subsurface mostly consists of sandy deposits from peri-glacial and coastal origin. In fact, around 75 % of all fields on which manure (i.e. VPs) is applied in this area are on sandy soils, while the rest are typically on peat (11 %) and clay soils (8 %). The most dominant crop in this area is maize covering almost 80 % of the fields, and followed by grasslands with around 15 % (Kros et al., 2019).

In order to capture the most prominent annual VPs concentration peaks in surface water, the local manure application patterns were assessed. Since the majority of agricultural fields in the area have a combination of sandy soil and maize, that situation was assumed as a representative for the whole area. In that case, more than 95% of yearly (slurry) manure loads are applied on the soil between March and June (Kros et al., 2019; de Vries et al., 2023). Note that this practice might slightly deviate between farms, but is in accordance with the Dutch national regulations for manure application (RVO, 2022). Consequently, as the most suitable timeframe for sampling, period between 25th March and 17th June was chosen. In total, we targeted 46 compounds, among which 25 antibiotics, three hormones, nine antiparasitics, and nine disinfectants. The list of all targeted compounds is given in Supplementary Material (SM) of this chapter.

Sampling strategy was based on deploying two pairs of passive samplers at each location. A first set of samplers was deployed for 12 consecutive weeks. Further, three different sets of samplers were deployed, each for four consecutive weeks during the time period of the first set, for two different reasons. First, with this set-up we were able to evaluate the time integrative sampling of the passive samplers, similar to the study of de Weert et al. (2020). Second, the analysis of the samplers which were deployed for four weeks enabled us to investigate whether variability in accumulated amounts within the 12 weeks occurred. The samplers were deployed in time series following the timeline outlined in Figure 5.2.

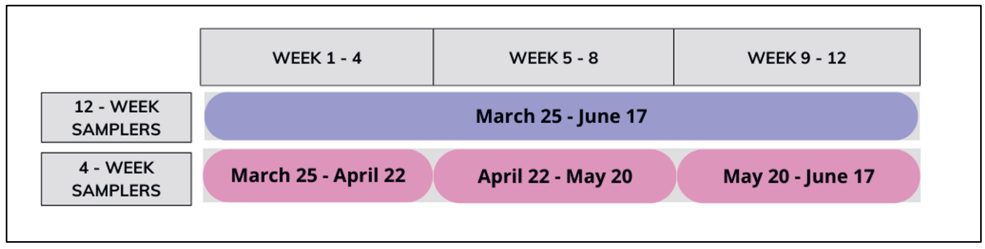


Figure 5.2: *Sampling timeline for eight investigated locations.*

5.2.2 Passive sampler preparation

In this study, the adsorption-based Speedisk (BAKERBOND® Speedisk®) was chosen as a passive sampler. Although a number of different adsorption-based samplers are being used regularly, the Speedisk has proven to be a robust sampler for different kinds of mostly polar chemical constituents in different environmental matrices, for instance for detecting VPs in groundwater (Smedes and de Weert, 2016), for pharmaceutical residues in wastewater (Zillien et al., 2019), and for pesticides in wastewater and surface water (de Weert et al., 2020). The Speedisk consists of a polypropylene container filled with ~600 mg of H2O-Philic DiVinylBenzene sorbent placed on the bottom as a uniform layer covered with a fine nylon mesh and a 0.5 mm glass fiber filter held in place by a 2 mm nylon mesh. The 0.5 mm glass fiber filter may act as a fixed water boundary layer largely regulating the diffusive uptake of compounds. The preparation of passive samplers was done by following a similar procedure as described in Hamers et al. (2018) and de Weert et al. (2020), hence we refer to those studies for more details. Per sampling site and sampling occasion, two Speedisks were deployed and considered as one sampler, of which the combined extracts were used for chemical analysis. At the end of dedicated sampling periods, samplers were pulled out and cleaned with local surface water, and then stored in jars at -20˚ C prior to extraction. During deployment, visual inspections of the samplers were performed, and when needed, plant material and debris was removed.

5.2.3 Extraction and processing of the samples

Before extraction, the Speedisks were spiked with an internal standard consisting of certified mass-labeled compounds representing the selected compounds to be analyzed, see SM6 for detailed information on the compounds selected. Extraction of the Speedisks was performed according to the Dutch concept method NEN 6597:2022 Ontw.nl (NEN). To extract as much as possible compounds from the samplers, the extraction procedure relied on different steps. First, the samplers were extracted with 2 mL methanol (Biosolve, ULC/MS), followed by an extraction with five times 5 mL dichloromethane (Merck, Supelco EMSURE). These eluates were combined, and after drying with sodium sulphate (Merck ACS reagent) over a glass frit, concentrated to 2-3 mL. Subsequently, an extraction with four times 5 ml methanol was performed, which was added to the eluate from the first step. This combined extract was further concentrated under a stream of nitrogen to a volume of approximately 0.9 ml and methanol was added to reach a volume of 1mL. In a third step, the Speedisks were eluted with four times 5 ml 1% formic acid (FA ≥ 99% (Biosolve, ULC/MS)) methanol. This extract was completely dried under a stream of nitrogen, and again methanol was added to reach a volume of 1mL. Before chemical analysis, 100 µl of the methanol and 100 µl of the FA extract were mixed, thereby reaching a 2 times dilution of the original extracts. Moreover, a 5 times and 10 times dilution were prepared by adding respectively 300 and 800 µl to the mixed extract. All three dilutions were analyzed. All extracts were stored in a dark environment at -20 °C until further analysis.

5.2.4 Chemical analysis

The final extracts were analyzed by an Agilent 1260 series high-performance liquid chromatographer coupled with an Agilent 6460 triple quadrupole LC/MS with Jetstream Electron Spray Ionization (ESI) and multiple reaction monitoring (MRM). A sample volume of 5 µL was injected with a flow rate of 0,5 mL min−1. Depending on the target compounds, three different columns and methods were used. VPs were eluted over a Gemini 3µm NX-C18 110Å (100 x 2 mm) column with a gradient of 1 mM ammonium fluoride in 100% Milli-Q water (eluent A) and 100% methanol (eluent B). The hormones were eluted over a Gemini 3µm NX-C18 110Å (100 x 2 mm) column using a gradient of 100% Milli-Q water (eluent A) and 100% methanol (eluent B), and the disinfectants were eluted on a Kinetex 2.6µm Biphenyl 100Å (100 x 2.1 mm) column, using a gradient of 100 % MQ with 0,002% Formic Acid (eluent A) and 100 % MeOH with 0,002% Formic Acid (eluate B). The target compounds were determined with one precursor ion and two product ions. For detailed information about mass-to-charge ratios, retention times and ratios see SM of this chapter. Calibration was done before measuring the samples with known amounts of the analytes in 9 steps with concentrations ranging between 0 and 50 ng mL−1.

The limit of detection (LOD) and limit of quantification (LOQ) of the analytes were determined with signal-to-noise ratios of 1:3 and 1:10 respectively. The method resulted in values for LOQ ranging between 3 and 60 ng mL−1. In a parallel experiment the recovery of all the substances on the passive sampler was tested in which a standard mixture of all the tested compounds was run over a Speedisk (n=10). Afterwards, the Speedisk were eluated, and the concentration in the eluate was compared with the concentration in the original mixture. Average analytical recoveries are given in SM of this chapter. Results were expressed as ng/sampler, where a sampler consists of 2 Speediks which were deployed, extracted and analyzed at the same time.

5.3 Results and discussion

5.3.1 Total amount per sampler

From the 46 targeted compounds, 22 accumulated in passive samplers in amounts above the LOQ in at least one sampling period on one of the eight locations. These compounds are shown in the Table 5.1, and are further addressed in this paper. The remaining compounds (24) may have been present in streams but undetected due to low recovery rates and the method's low sensitivity. This is particularly evident for certain antibiotics, where recoveries were even below 40% (see SM). The measured amounts of all the detected compounds in the Speedisk extracts, together with LOD and LOQ values, are given in the SM of this chapter.

Table 5.1: *Detected compounds, compound type, main emission source, number of detected locations, and range of detected quantities (12-week samplers).*

Compound	Type	Cas no.	Emission source	No. of locations	Detected quantities [ng/sampler] [a]
Florfenicol	Antibiotic	73231-34-2	Manure application	8/8	15 – 70.3
Flumequine	Antibiotic	42835-25-6	Manure application	8/8	6.3 – 13.3
Lincomycin	Antibiotic	154-21-2	Manure application	6/8	1.3[b] – 3.3
Oxytetracycline	Antibiotic	79-57-2	Manure application	3/8	64.5 – 142
Sulfadiazine	Antibiotic	68-35-9	Manure application	8/8	29 – 547
Sulfamethazine	Antibiotic	57-68-1	Manure application	8/8	35.2 – 139
Sulfamethoxazole	Antibiotic	723-46-6	Manure application	8/8	1.5[b] – 46.3
Sulfamethoxypyridazine	Antibiotic	80-35-3	Manure application	8/8	1.9[b] – 6
Sulfapyridine	Antibiotic	144-83-2	Manure application	5/8	1.3[b] – 13

Tilmicosine	Antibiotic	108050-54-0	Manure application	8/8	4.4 – 74
Trimethoprim	Antibiotic	738-70-5	Manure application	7/8	1.1[b] – 18
Tylosin	Antibiotic	1401-69-0	Manure application	4/8	3 – 6.2
Flubendazole	Antiparasitic	31430-15-6	Manure application	8/8	3.4 – 18.5
Mebendazole	Antiparasitic	31431-39-7	Manure application	1/8	3.3[c]
Permethrin	Antiparasitic	52645-53-1	Manure application	1/8	3.0[c]
Fipronil sulfone	Metabolite	120068-36-2	Metabolite	7/8	1.2[b] – 5.4
Estrone	Hormone	53-16-7	Naturally occurring	8/8	3 – 14.2
Benzyldimethyl-dodecylammonium chloride (BAC-C12)	Biocide	139-07-1	Disinfection	8/8	11 – 33
Benzyldimethyl-tetradecylammonium chloride (BAC-C14)	Biocide	139-08-2	Disinfection	8/8	10 – 24
Benzyldimethyl-hexadecylammonium chloride (BAC-C16)	Biocide	122-18-9	Disinfection	8/8	2.1[b] – 6.4
Benzyldimethyl-octadecylammonium chloride (BAC-C18)	Biocide	122-19-0	Disinfection	2/8	2.2[b]
Didecyldimethyl-ammonium chloride (DDAC-C10)	Biocide	7173-51-5	Disinfection	8/8	6.6 – 76

[a] *The range of detected amounts accumulated in passive samplers expressed in ng per sampler is defined by the lower border, which represents the lowest quantity (above LOD) found at one of the locations on a 12-week sampler, and the higher border, which represents the highest quantity found at one of the locations on a 12-week sampler. For the amounts on 4-week samplers, see SM of this chapter.*

[b] *Value above the LOD but below the LOQ.*

[c] *Incidental occurrence – compound detected only at one location during one of the sampling intervals. Note that Mebendazole was found only on a 4-week sampler.*

Among the 22 compounds accumulated above the LOQ, two of them were found to be insufficiently well taken up by the Speedisk which was expressed by the low recovery rate (< 70 %) in the parallel lab experiment. Mentioned compounds are Oxytetracycline and Tylosin, and those were therefore discarded from further consideration. A detailed analyses of the uptake by the passive samplers of 160 location-compound combinations (8 locations * 20 compounds) was performed. In 51 of these combinations the compound was not detected, or only detected < LOQ, hence 109 location-compound combinations remained for further investigation. Based on the ratio between the sum uptake of 1-4, 5-8, and 9-12 weeks deployment and the uptake during the 1-12 weeks deployment, we distinguished three categories: time integrative uptake, possibly time integrative uptake, and non-integrative uptake

in time. Time integrative uptake was defined as 0.5 < (sum of 4-week amounts)/(12-week amounts) < 1.5, where a value of 1 would mean the perfect time integrative uptake. In some combinations the compound was not detected during all four sampling periods, but based on the accumulated amounts (> LOQ) the uptake of samplers was possibly still time integrative thus these combinations were classified as the 2nd defined category.

The third category covered both situations when data from all four sampling periods were available but also when this was not the case. In the latter, based on the accumulated amounts (> LOQ), uptake could not be identified as time integrative on a location. Discussed observations are summarized in Figure 5.3.

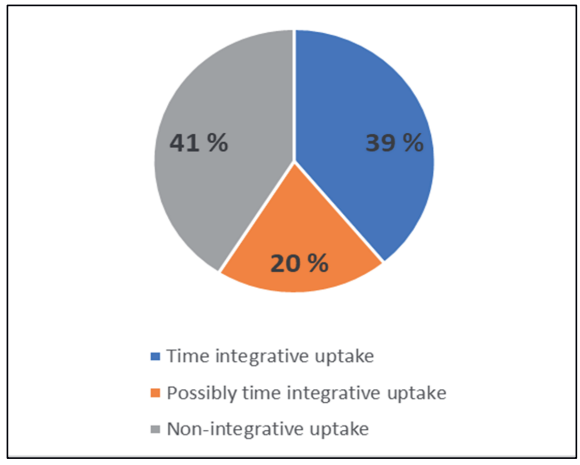


Figure 5.3: *Time integrative uptake of passive samplers. The total number of considered location-compound combinations was 109.*

In total, 59% of the combinations implied time integrative uptake (category 1 and 2), while the rest (41%) showed non-integrative uptake in time. As we were dealing with 20 different compounds and 8 different locations, we analyzed if the results of this latter category could be attributed to either specific locations or specific compounds. To do so, we calculated the average Time integrative Uptake Coefficient (TUC) as (sum of 4-week amounts)/(12-week amounts) per location and per compound. This analysis revealed that two locations showed a deviation from the normal pattern, such as location 6 and 8. The compounds on location 6 displayed a TUC which was substantially higher than 1, namely an average value of 4.6 (n=14). For location 8 the compounds showed an TUC which was substantially lower than 1, specifically 0.56 (n=11). It has been shown that the uptake rate of compounds by several types of passive samplers can be substantially influenced by hydrological conditions (i.e. water flow). For instance, both Guibal et al. (2020) and Shi et al. (2014) showed that uptake rates positively correlate with flow rates. This could be a logical explanation for the deviating uptake rates for the two

mentioned locations, as these are the most upstream locations, with the lowest flow rate, which also might fluctuate heavily in spring because of rainfall events. Besides, during some of the visual inspections, it was observed that especially at these locations, the samplers were overgrown with different macrophytes and algae, possibly diminishing flow rates at certain time intervals (Harman et al., 2012). These processes, combined with possible fluctuating aqueous concentrations on those locations may result in highly fluctuating amounts taken up by the samplers in the consecutive time intervals. Next to these two locations, also one group of compounds showed uptake rates which substantially differed from time integrative uptake, namely the QACs (see Figure 5.4. and SM). This could be caused by the lower affinity of cationic compounds, like QACs, to passive samplers, which has been reported before by Moschet et al. (2015). Other reasons for showing deviations from time integrative uptake could be triggered by measurement error of amounts close to the limit of detection. Another possible explanation could be that the Speedisk may exhibit a faster uptake for certain compounds at the beginning of the deployment. This eventually results in a higher sum uptake for the 1-4, 5-8, and 9-12 weeks deployment than for the Speedisk constantly deployed in parallel (de Weert et al., 2020). Finally, it could also be argued that for some compound-locations combination the amount of the binding material within the Speedisk might not have been sufficient enough, thereby hampering a time integrative uptake. However, based on indicative uptake rates, the DVB mass per Speedisk and the partition coefficient between DVB and water, de Weert et al. (2020) calculated a linear uptake time window for the Speedisk sampler from 128 days under very turbulent water-flow conditions to 1935 days under almost static conditions. As these calculated linear uptake windows exceed the maximum sampling period of 84 days in the present study, the Speedisk remained in the linear uptake phase during deployment.

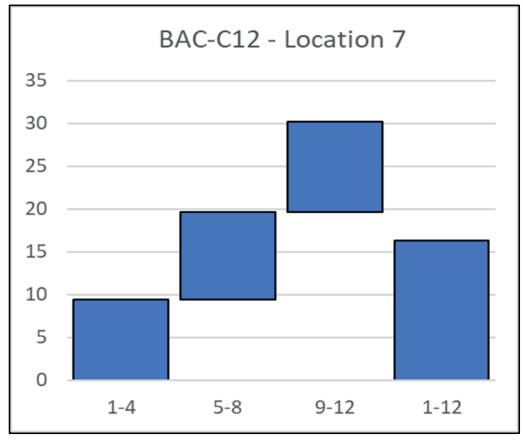


Figure 5.4: *Uptake of BAC-C12 in consecutively and in parallelly exposed Speedisk samplers at location 7, shown as the amounts on the samplers (ng/sampler) sampled during the weeks indicated at the x-axis. This example is considered as a representative for the non-integrative uptake in time.*

Out of the 13 compounds detected at all sampling locations (as listed in Table 5.1), eight of them were consistently detected across all four sampling periods. These compounds include Estrone, Florfenicol, Flubendazole, Flumequine, Sulfadiazine, Sulfamethazine, BAC-C12, and BAC-C14. While the latter two showed non-integrative uptake, Florfenicol, Flubendazole, and Flumequine demonstrated good time integrative sampling properties in most of the locations, as illustrated in Figure 5.5.

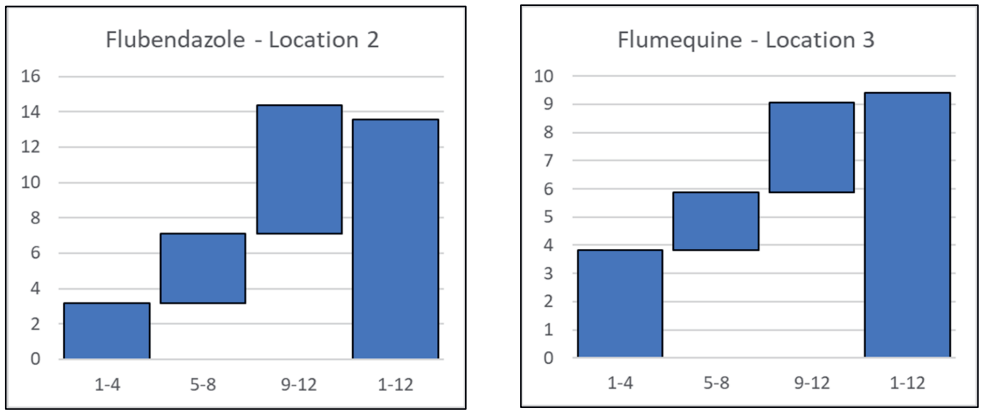


Figure 5.5: *Uptake of Flubendazole and Flumequine in consecutively and in parallelly exposed Speedisk samplers at locations 2 and 3, shown as the amounts on the samplers (ng/sampler) sampled during the weeks indicated at the x-axis. These examples are considered as a representative for the time integrative sampler uptake.*

Among the compounds detected at all locations but not during all sampling periods, Sulfamethoxazole could be seen as a representative of possibly time integrative uptake category as defined earlier. At location 5, this compound was not detected with 5-8 week samplers but found during all other time intervals, as illustrated with Figure 5.6. This effect can be caused by fluctuating concentrations of a compound in the water resulting in fluctuating uptake during the different intervals. This immediately shows the added value of the different sampling intervals; the deployment of 12 weeks produces more often a result >LOQ than the deployment periods of 4 weeks, as the deployment time of 4 weeks is sometimes not long enough to reach an amount in the sampler > LOQ. On the other hand, the results of the samplers which are deployed for 4 weeks give detailed information concerning the different time periods within these 12 weeks.

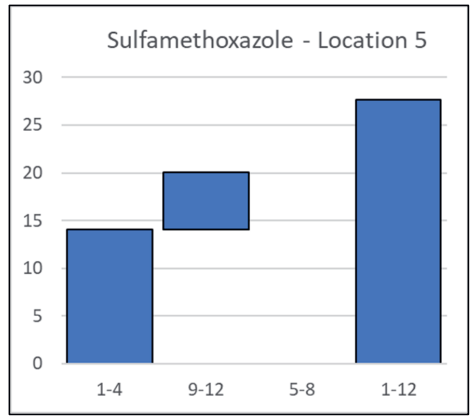


Figure 5.6: *Uptake of Sulfamethoxazole in consecutively and in parallelly exposed Speedisk samplers at locations 5, shown as the amounts on the samplers (ng/sampler) sampled during the weeks indicated at the x-axis. This example illustrates the category of possibly time integrative uptake.*

5.3.2 Spatial trends

From the 22 compounds found above LOQ in the investigated area, the highest number of compounds detected on the samplers during one sampling period was 19 at locations 3 and 5. At location 3, those were detected on the 12-week samplers, while at location 5 this was done both on 12-week and 1-4 week samplers. On average, the lowest number of detected compounds (11) was at location 4. Even though locations 3 and 4 are situated relatively close to each other (location 3 is around 700 m downstream from location 4), the number of detected compounds was quite different. All the compounds which were found on location 4 were also found on location 3, meaning that location 4 had an substantial influence on location 3. But next to that, another 8 compounds were detected on location 3, which indicates most probably the influence of an additional stream which fed location 3 from south-east side of the catchment (see Figure 5.1).

In addition, we compared the 12-week samplers at locations 2 and 5 (upstream of 2), which were fed by the approximately same catchment area. Out of the 19 compounds detected at location 5, 17 were also detected at location 2, with the remaining two compounds being close to the LOQ at location 5. No additional compounds were found at location 2. Furthermore, the compounds detected both at location 2 and 5 were also found at location 8, which is located far upstream, indicating that the stream network in that part of the catchment is consistently exposed to the same set of compounds. The same conclusion could be drawn for locations 6 and 1, as the same set of compounds was detected at these locations using both 12 and 4-week samplers. All the compounds found at location 7 were also detected somewhere upstream, typically in both investigated catchment parts. This finding indicates

that a monitoring location like location 7 may serve as a surveillance monitoring location, providing an assessment of the overall status of the upstream water bodies. As already pointed out, the other locations located further upstream are able to provide more detailed information about possible sources of compounds detected.

It is important to note that the precipitation which feeds into to a stream network can have opposite effects on fluxes of compounds in this network, substantially influenced by the physical-chemical properties of the compound. This may eventually also affect the accumulated amounts on samplers in different ways. On one hand, dilution of the compound might occur, decreasing the aqueous concentration, leading to lower amounts on the sampler. On the other hand, rainfall events are one of the main driving factors for compound transport from soil to surface water (Rozemeijer et al., 2010). However, the compound availability for transport is strongly influenced by its sorption to soil, while the duration between compound application to soil (via manure) and rainfall event directly determines the fraction available for transport (i.e. degradation occurs with time). In order to enhance understanding of the impact of precipitation on our findings, we have focused our analysis on the uppermost section of the catchment (outlet at location 2). This approach was taken due to the consistency of the detected compounds in this area, and to prevent influence of water originating from other parts of the catchment. Additionally, this area also has the highest number of monitoring sites (3) which provided us with a more comprehensive dataset. The daily precipitation amounts for the area during sampling periods were retrieved from the Royal Netherlands Meteorological Institute (KNMI) and are given in SM of this chapter. The total amount of precipitation in week 9-12 was approximately three times greater than in week 5-8 and almost fifteen times greater than in week 1-4. This suggests that the dominant water and possibly compound inputs into the stream network occurred during the last sampling period. Flubendazole demonstrated a clear trend, with higher accumulated amounts quantified during the final sampling period at all three measuring locations (2, 5, 8). In contrast, Sulfadiazine exhibited the opposite trend, with the lowest quantified amounts during the last sampling period. This inverse relationship could potentially be explained by the fact that Sulfadiazine has low soil sorption to soil, making it highly mobile and susceptible to transport to the stream network immediately after application via manure, but it rapidly degrades and does not persist long enough to be transported by subsequent rainfall events. Flubendazole, on the other hand, is more persistent in the environment and has higher soil sorption, which allows it to reside in the soil for a longer period of time (i.e. accumulate), partially allowing for degradation while also making it available for transport to the streams through succeeding rainfall events after manure application. Similar conclusions could be drawn regarding the leaching of these compounds into groundwater (Rakonjac et al., 2023). To further explore this issue, an insight into the transported water quantities (both rainfall

triggered and in the streams) is necessary, but also a deeper investigation on compound behavior in soil and water, which was out of the scope in this paper.

5.3.3 Discussion

In our earlier modelling study (Rakonjac et al., 2022), we have prioritized the most commonly soil-applied VPs in the Netherlands, namely Oxytetracycline, Doxycycline, Sulfadiazine, Flubendazole, Ivermectin, and Dexamethasone. Among them, Oxytetracycline, Doxycycline and Ivermectin are characterized with relatively high sorption to soil, hence they are less mobile and their transport towards surface water is unlikely to occur. This has been partly confirmed with our measurements, where only the incidental occurrence of Oxytetracycline was observed. Further, Flubendazole also has a lower affinity for transport due to sorption, but its persistence in the environment makes this compound more likely to reach the water bodies. Our measurements indicated its wide occurrence since it was detected at all 8 locations. According to their low sorption affinity, Sulfadiazine and Dexamethasone could be considered as a relatively mobile compounds, hence their presence in surface water might be expected. Our measurements have confirmed this for Sulfadiazine, but Dexamethasone was not detected at all. As also discussed in our earlier papers (Rakonjac et al., 2022; Rakonjac et al., 2023), Dexamethasone is applied to soil in much lower concentrations compared to other VPs, therefore it might escape environmental measurements in view of detection limits.

In a nearby region with comparable animal husbandry practices, Lahr et al. (2018) conducted a grab sampling campaign of surface water. Their study focused on over 35 compounds, including antibiotics, antiparasitics, and hormones. Of these, 18 compounds overlapped with those targeted in our study. In total, 5 compounds were detected, where Oxytetracycline, Sulfamethazine, and Progesterone were occasionally found, while for Ivermectin and Toltrazuril an incidental occurrence was observed. Despite the fact that sampling locations and periods were not identical as in our study, the difference in a number of identified compounds was profound (5 vs 22). Moreover, our measurements detected several compounds in Dutch surface water that were not previously identified (as reported in the Lahr et al. (2019) overview). Among these compounds, Flubendazole, Florfenicol, and Tilmicosine were consistently detected at all sampling locations during all sampling intervals (with the exception of Tilmicosine at one location), indicating their widespread and consistent presence.

As briefly indicated earlier, to our knowledge, this is the first study to quantify the presence of QACs (disinfectants) in surface water in agricultural regions in the Netherlands. However, their occurrence in surface water is not surprising as some of those compounds have been found on a number of farms,

in manure, food, and soil in the Netherlands (Buijs et al., 2019). According to the Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) and Registration Dossier from the European Chemicals Agency (ECHA), these compounds have a broad range of applications, such as disinfection of the materials and surfaces associated with the housing or transportation of animals, but also for more general applications like the removal of algae and other green deposits. Therefore, they have a higher chance of being present in liquid manure and eventually reach the surface water via manure application to soil (UBA, 2017). The detected amounts of QACs were moderately steady over the catchment, except at location 1 where BAC-C12, BAC-C14, and BAC-C16 were found at relatively high rates, particularly during the last sampling period. However, since the affinity of QACs to passive samplers is questionable, mentioned observations should be taken with a reserve and further investigation may be required.

Furthermore, it is noteworthy that the absence of the 24 targeted compounds above the LOQ does not necessarily indicate their absence in the streams. It is possible that these compounds were present but not detected due to low recovery rates (see SM of this chapter) and the subsequent low sensitivity of the method used. This suggests that the proposed method may not be suitable for some compounds, as they may have a low affinity for the passive sampler.

5.4 Conclusion

The similarity between the cumulative response in consecutively deployed samplers and the sampler constantly deployed in parallel, which was observed in 59% of the cases, implies that Speedisks are applicable for time integrative sampling for a number of compounds. However, also deviations from time integrative sampling were observed, probably caused by the physical-chemical properties of the compound, the environmental conditions, or a combination of those. When compared with conventional grab sampling studies done in the same region, the presented monitoring approach and results indicated a clear advantage of passive sampling over the grab sampling, particularly highlighting the potential for detecting much more compounds with passive samplers. Consequently, a number of compounds originating from animal husbandry activities were quantified for the first time in Dutch surface waters. Moreover, the used set-up and sampling strategy enabled identification of a specific time periods during which compound emissions have occurred, while the observed spatial patterns allowed for addressing the compound sources more precisely. This type of information might be very useful for spatial and temporal prioritization (e.g. location/time specific monitoring) because it indicates that different streams show a different level of pollution during the 12 week period, which can only be seen when analyzing the samplers deployed for 4 weeks. Also, our study may be used as a

first step in developing a targeted monitoring program for this type of compounds. Further, this knowledge might help in defining mitigation measures to reduce the emissions of chemicals from animal husbandry. For VPs and naturally occurring hormones, an option could be reducing manure applications in source areas, while for disinfectants a closer identification of sources is required.

<u>Supplementary Material</u>

SM1. Targeted compounds, quantified amounts, with LOD and LOQ values

Locations 1 and 6, LOD and LOQ

Description	Detection technique	CAS nummer	LOD	LOQ	location: 1-1 SD	location: 1-2 SD	location: 1-3 SD	location: 1-4 SD	location: 6-1 SD	location: 6-2 SD	location: 6-3 SD	location: 6-4 SD
Period (week)					12 week	4 week	4 week	4 week	12 week	4 week	4 week	4 week
Unit			ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
amoxicillin	LCMSMS ESI-POS (MRM)	26787-78-0	5	15	<	<	<	<	<	<	<	<
chlortetracycline	LCMSMS ESI-POS (MRM)	57-62-5	5	15	<	<	<	<	<	<	<	<
ciprofloxacin	LCMSMS ESI-POS (MRM)	85721-33-1	2	6	<	<	<	<	<	<	<	<
dexamethasone	LCMSMS ESI-POS (MRM)	50-02-2	1	3	<	<	<	<	<	<	<	<
doxycyline	LCMSMS ESI-POS (MRM)	564-25-0	5	15	<	<	<	<	<	<	<	<
estrone	LCMSMS ESI-NEG (MRM)	53-16-7	1	3	8.1	2.6	6.7	14	14	5.2	5.1	16
fenbendazole	LCMSMS ESI-POS (MRM)	43210-67-9	2	6	<	<	<	<	<	<	<	<
fipronil	LCMSMS ESI-NEG (MRM)	120068-37-3	1	3	<	<	<	<	<	<	<	<
fipronil sulfone	LCMSMS ESI-NEG (MRM)	120068-36-2	1	3	2.8	<	<	1.2	2.4	1.6	<	1.4
florfenicol	LCMSMS ESI-NEG (MRM)	73231-34-2	1	3	27	1.4	13	1.9	15	1.5	5.1	12
flubendazole	LCMSMS ESI-POS (MRM)	31430-15-6	1	3	7.2	1.0	2.5	6.5	3.4	1.2	1.1	3.2
flumequine	LCMSMS ESI-POS (MRM)	42835-25-6	1	3	8.0	2.8	3.4	4.0	6.3	5.3	3.2	5.1
Ivermectine B1b	LCMSMS ESI-POS (MRM)	70288-86-7	20	60	<	<	<	<	<	<	<	<
lincomycin	LCMSMS ESI-POS (MRM)	154-21-2	1	3	1.3	1.1	<	<	3.1	1.7	<	2.0
mebendazole	LCMSMS ESI-POS (MRM)	31431-39-7	1	3	<	<	<	<	<	<	<	<
norfloxacine	LCMSMS ESI-POS (MRM)	70458-96-7	1	3	<	<	<	<	<	<	<	<
oxytetracycline	LCMSMS ESI-POS (MRM)	79-57-2	10	30	<	<	<	<	<	<	<	<
penicilline G	LCMSMS ESI-POS (MRM)	113-98-4	1	3	<	<	<	<	<	<	<	<
permethrin 1	LCMSMS ESI-POS (MRM)	52645-53-1	1	3	<	<	<	<	<	<	<	<
permethrin 2	LCMSMS ESI-POS (MRM)	52645-53-1	1	3	<	<	<	<	<	<	<	<
progesterone	LCMSMS ESI-POS (MRM)	57-83-0	1	3	2.8	1.8	1.9	1.9	1.2	1.0	<	<
sulfachloropyridazine	LCMSMS ESI-POS (MRM)	80-32-0	2	6	<	<	<	<	<	<	<	<
sulfadiazine	LCMSMS ESI-POS (MRM)	68-35-9	1	3	63	26	13	10	37	41	9.5	15
sulfadimethoxine	LCMSMS ESI-POS (MRM)	122-11-2	1	3	<	<	<	<	<	<	<	<
sulfadoxine	LCMSMS ESI-POS (MRM)	2447-57-6	1	3	<	<	<	<	<	<	<	<
sulfamerazine	LCMSMS ESI-POS (MRM)	127-79-7	1	3	<	<	<	<	<	<	<	<
sulfamethazine	LCMSMS ESI-POS (MRM)	57-68-1	1	3	71	25	20	21	78	50	21	54
sulfamethoxazole	LCMSMS ESI-NEG (MRM)	723-46-6	1	3	13	4.6	<	<	19	<	3.5	23
sulfamethoxypyridazine	LCMSMS ESI-POS (MRM)	80-35-3	1	3	3.5	1.2	<	1.1	3.4	2.0	1.0	2.4
sulfapyridine	LCMSMS ESI-POS (MRM)	144-83-2	1	3	<	<	<	<	<	<	<	<
sulfathiazole	LCMSMS ESI-POS (MRM)	72-14-0	1	3	<	<	<	<	<	1.1	<	<
tetracycline	LCMSMS ESI-POS (MRM)	60-54-8	10	30	<	<	<	<	<	<	<	<
tiamulin	LCMSMS ESI-POS (MRM)	55297-95-5	1	3	<	<	<	<	<	<	<	<
tilmicosin	LCMSMS ESI-POS (MRM)	108050-54-0	1	3	4.4	<	<	<	74	15	21	44
toltrazuril	LCMSMS ESI-NEG (MRM)	69004-03-1	5	15	<	<	<	<	<	<	<	<
trimethoprim	LCMSMS ESI-POS (MRM)	738-70-5	1	3	2.5	<	<	<	9.3	<	1.8	5.7
tylosin	LCMSMS ESI-POS (MRM)	1401-69-0	1	3	<	<	<	<	<	<	<	<
Benzyldimethyloctylammonium chloride (BAC-C8)	LCMSMS ESI-POS (MRM)	959-55-7	2	6	<	<	<	<	<	<	<	<
Benzyldimethyldecylammonium chloride (BAC-C10)	LCMSMS ESI-POS (MRM)	965-32-2	2	6	<	<	<	3.3	<	<	<	<
Benzyldimethyldodecylammonium chloride (BAC-C12)	LCMSMS ESI-POS (MRM)	139-07-1	2	6	21	17	559	1415	17	17	41	12
Benzyldimethyltetradecylammonium chloride (BAC-C14)	LCMSMS ESI-POS (MRM)	139-08-2	2	6	22	20	841	1851	15	15	49	11
Benzyldimethylhexadecylammonium chloride (BAC-C16)	LCMSMS ESI-POS (MRM)	122-18-9	2	6	6.4	3.0	223	457	3.6	2.4	9.2	<
Benzyldimethyloctadecylammonium chloride (BAC-C18)	LCMSMS ESI-POS (MRM)	122-19-0	2	6	<	<	2.8	6	<	<	<	<
Dimethyldioctylammonium chloride (DDAC-C8)	LCMSMS ESI-POS (MRM)	5538-94-3	2	6	<	<	<	<	<	<	<	<
Didecyldimethylammonium chloride (DDAC-C10)	LCMSMS ESI-POS (MRM)	7173-51-5	2	6	12	4.4	5.7	7.0	24	11	40	16
Didodecyldimethylammonium chloride (DDAC-C12)	LCMSMS ESI-POS (MRM)	3401-74-9	2	6	<	<	<	<	<	<	<	<

Locations 2 and 5

Description	Detection technique	CAS nummer	location 2-1 SD	location 2-2 SD	location 2-3 SD	location 2-4 SD	location 5-1 SD	location 5-2 SD	location 5-3 SD	location 5-4 SD
Period (week)			12 week	4 week	4 week	4 week	12 week	4 week	4 week	4 week
Unit			ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
amoxicillin	LCMSMS ES+POS (MRM)	26787-78-0	<	<	<	<	<	<	<	<
chlortetracycline	LCMSMS ES+POS (MRM)	57-62-5	<	<	<	<	<	<	<	<
ciprofloxacin	LCMSMS ES+POS (MRM)	85721-33-1	<	<	<	<	<	<	<	<
dexamethasone	LCMSMS ES+POS (MRM)	50-02-2	<	<	<	<	<	<	<	<
doxycycline	LCMSMS ES+POS (MRM)	564-25-0	<	<	<	<	<	<	<	<
estrone	LCMSMS ES-NEG (MRM)	53-16-7	4.2	2.3	3.9	2.5	7.6	4.5	4.3	7.5
fenbendazole	LCMSMS ES+POS (MRM)	43210-67-9	<	<	<	<	<	<	<	<
ivermectin	LCMSMS ES+POS (MRM)	70288-86-7	<	<	<	<	<	<	<	<
lignocaine	LCMSMS ES+POS (MRM)	137-58-6	1.2	<	<	<	2	2	<	<
lignocaine sulfone	LCMSMS ES-NEG (MRM)	120068-36-2	<	<	<	<	<	<	<	<
florfenicol	LCMSMS ES-NEG (MRM)	73231-34-2	18	5.5	4.5	4.1	65	53	7.0	7.6
flubendazole	LCMSMS ES+POS (MRM)	31430-15-6	13.6	3.2	3.9	7.3	18.5	9.4	5.7	11.5
flumequine	LCMSMS ES+POS (MRM)	42835-25-6	7.7	2.2	1.8	3.2	13	7.7	2.9	4.8
ivermectine B1b	LCMSMS ES+POS (MRM)	70288-86-7	<	<	<	<	<	<	<	<
mebendazole	LCMSMS ES+POS (MRM)	31431-39-7	<	<	<	<	3.3	2.8	<	<
metronidazole	LCMSMS ES+POS (MRM)	443-48-1	<	<	<	<	<	<	<	<
norfloxacine	LCMSMS ES+POS (MRM)	70458-96-7	<	<	<	<	<	<	<	<
oxytetracycline	LCMSMS ES+POS (MRM)	79-57-2	65	66	23	<	142	176	55	<
penicilline G	LCMSMS ES-POS (MRM)	113-98-4	<	<	<	<	<	<	<	<
permethrin 1	LCMSMS ES+POS (MRM)	52645-53-1	<	<	<	<	3	<	<	<
permethrin 2	LCMSMS ES+POS (MRM)	52645-53-1	<	<	<	<	<	<	<	<
progesterone	LCMSMS ES+POS (MRM)	57-83-0	13	<	13	<	14	<	14	<
sulfachloropyridazine	LCMSMS ES+POS (MRM)	80-32-0	<	<	<	<	<	<	<	<
sulfadiazine	LCMSMS ES+POS (MRM)	68-35-9	101	35	29	26	255	329	41	32
sulfadimethosine	LCMSMS ES+POS (MRM)	122-11-2	<	<	<	<	<	<	<	<
sulfadoxine	LCMSMS ES+POS (MRM)	2447-57-6	13	<	<	<	2.7	2.6	11	<
sulfamerazine	LCMSMS ES+POS (MRM)	127-79-7	<	<	<	<	<	<	<	<
sulfamethazine	LCMSMS ES+POS (MRM)	57-68-1	71	17	24	21	139	96	36	29
sulfamethoxazole	LCMSMS ES+NEG (MRM)	723-46-6	2.8	<	<	2.1	28	14	<	6
sulfamethoxypyridazine	LCMSMS ES+POS (MRM)	80-35-3	3.2	<	1.2	1.1	6.0	4.6	1.6	1.3
sulfapyridine	LCMSMS ES+POS (MRM)	144-83-2	2.1	<	<	1.0	8.0	1.1	1.2	2.3
sulfathiazole	LCMSMS ES+POS (MRM)	72-14-0	<	<	<	<	<	<	<	<
tiamulin	LCMSMS ES+POS (MRM)	55297-95-5	<	<	<	<	<	<	<	<
tylmicosin	LCMSMS ES+POS (MRM)	108050-54-0	7.1	7.6	1.5	4.0	31	185	2.8	3.5
tolfenpyrad	LCMSMS ES+NEG (MRM)	129558-76-5	<	<	<	<	<	<	<	<
trimethoprim	LCMSMS ES+POS (MRM)	738-70-5	3.1	<	<	<	13.8	5.7	<	4.6
tylosin	LCMSMS ES+POS (MRM)	1401-69-0	<	<	<	<	<	4.8	<	<
Benzyldimethyloctylammonium chloride (BAC-C8)	LCMSMS ES+POS (MRM)	959-55-7	<	<	<	<	<	<	<	<
Benzyldimethyldecylammonium chloride (BAC-C10)	LCMSMS ES+POS (MRM)	965-32-2	2.1	<	<	<	<	<	<	<
Benzyldimethyldodecylammonium chloride (BAC-C12)	LCMSMS ES+POS (MRM)	139-07-1	13	14	18	11	24	20	18	12
Benzyldimethyltetradecylammonium chloride (BAC-C14)	LCMSMS ES+POS (MRM)	139-08-2	13	15	20	12	24	22	19	12
Benzyldimethylhexadecylammonium chloride (BAC-C16)	LCMSMS ES+POS (MRM)	122-18-9	3.2	<	3.2	<	6.1	5.7	3.6	<
Benzyldimethyloctadecylammonium chloride (BAC-C18)	LCMSMS ES+POS (MRM)	122-19-0	<	<	<	<	<	<	<	<
Dimethyldioctylammonium chloride (DDAC-C8)	LCMSMS ES+POS (MRM)	5538-94-3	<	<	<	<	<	<	<	<
Didecyldimethylammonium chloride (DDAC-C10)	LCMSMS ES+POS (MRM)	7173-51-5	8.9	3.8	2.9	3.0	21	10	4.3	8.9
Didodecyldimethylammonium chloride (DDAC-C12)	LCMSMS ES+POS (MRM)	3401-74-9	<	<	<	<	<	<	<	<

Locations 3 and 8

Description	Detection technique	CAS nummer	location: 8-1 SD	location: 8-2 SD	location: 8-3 SD	location: 8-4 SD	location: 3-1 SD	location: 3-2 SD	location: 3-3 SD	location: 3-4 SD
Period (week)			12 week	4 week	4 week	4 week	12 week	4 week	4 week	4 week
Unit			ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
amoxicillin	LCMSMS ESI-POS (MRM)	26787-78-0	<	<	<	<	<	<	<	<
chlortetracycline	LCMSMS ESI-POS (MRM)	57-62-5	<	<	<	<	<	<	<	<
ciprofloxacin	LCMSMS ESI-POS (MRM)	85721-33-1	<	<	<	<	<	<	<	<
dexamethasone	LCMSMS ESI-POS (MRM)	50-02-2	<	<	<	<	<	<	<	<
doxycycline	LCMSMS ESI-POS (MRM)	564-25-0	7.0	<	9.9	<	<	<	<	<
estrone	LCMSMS ESI-NEG (MRM)	53-16-7	4.8	5.7	10	19	9.5	2.7	1.7	3.4
fenbendazole	LCMSMS ESI-POS (MRM)	43210-67-9	<	<	<	<	<	<	<	<
flumequine	LCMSMS ESI-NEG (MRM)	42835-25-6	<	14	<	13	23	<	<	<
fipronil sulfone	LCMSMS ESI-NEG (MRM)	120068-36-2	2.4	2.4	1.9	2.2	4.8	<	<	<
florfenicol	LCMSMS ESI-NEG (MRM)	73231-34-2	56	65	21	6.5	70	12	17	4.0
flubendazole	LCMSMS ESI-POS (MRM)	31430-15-6	4.3	2.5	3.1	5.2	5.9	1.6	1.4	2.3
flumequine	LCMSMS ESI-POS (MRM)	42835-25-6	10	7.5	4.1	3.6	9.4	3.8	2.0	3.2
ivermectine B1b	LCMSMS ESI-POS (MRM)	70288-86-7	<	<	<	<	<	<	<	<
lincomycin	LCMSMS ESI-POS (MRM)	154-21-2	<	16	<	<	1.9	<	<	<
mebendazole	LCMSMS ESI-POS (MRM)	31431-39-7	<	<	<	<	<	<	<	<
norfloxacine	LCMSMS ESI-POS (MRM)	70458-96-7	<	<	<	<	<	<	<	<
oxytetracycline	LCMSMS ESI-POS (MRM)	79-57-2	<	<	16	<	<	<	<	<
penicilline G	LCMSMS ESI-POS (MRM)	113-98-4	<	<	<	<	<	<	<	<
permethrin 1	LCMSMS ESI-POS (MRM)	52645-53-1	<	<	<	<	<	<	<	<
permethrin 2	LCMSMS ESI-POS (MRM)	52645-53-1	<	<	<	<	<	<	<	<
progesterone	LCMSMS ESI-POS (MRM)	57-83-0	1.3	14	<	<	1.3	1.1	<	<
sulfachloropyridazine	LCMSMS ESI-POS (MRM)	80-32-0	<	<	<	<	<	<	<	<
sulfadiazine	LCMSMS ESI-POS (MRM)	68-35-9	102	591	146	80	5947	16	3.5	14
sulfadimethoxine	LCMSMS ESI-POS (MRM)	122-11-2	<	<	<	<	<	<	<	<
sulfamerazine	LCMSMS ESI-POS (MRM)	2441-57-6	<	<	2	<	14	<	<	<
sulfamerazine	LCMSMS ESI-POS (MRM)	127-79-7	<	<	<	<	<	<	<	<
sulfamethazine	LCMSMS ESI-POS (MRM)	57-68-1	70	57	34	39	135	34	12	8.6
sulfamethoxazole	LCMSMS ESI-NEG (MRM)	723-46-6	18	41	<	<	46	<	5.7	<
sulfamethoxypyridazine	LCMSMS ESI-POS (MRM)	80-35-3	3.4	4.3	1.6	1.8	6.0	1.5	<	<
sulfapyridine	LCMSMS ESI-POS (MRM)	144-83-2	1.9	2.6	5.3	11	13	<	<	<
sulfathiazole	LCMSMS ESI-POS (MRM)	72-14-0	<	<	<	<	<	<	<	<
tetracycline	LCMSMS ESI-POS (MRM)	60-54-8	<	<	<	<	<	<	<	<
tiamulin	LCMSMS ESI-POS (MRM)	55297-95-5	<	<	<	<	<	<	<	<
trimethoprim	LCMSMS ESI-POS (MRM)	738-70-5	20	21	32	14	65	2.9	2.2	4.4
tylosin	LCMSMS ESI-POS (MRM)	1401-69-0	6.5	5.5	1.1	<	18	<	2.8	<
Benzyldimethyloctylammonium chloride (BAC-C8)	LCMSMS ESI-POS (MRM)	959-55-7	<	<	<	<	<	<	<	<
Benzyldimethyldecylammonium chloride (BAC-C10)	LCMSMS ESI-POS (MRM)	958-32-2	<	<	<	<	<	<	<	<
Benzyldimethyldodecylammonium chloride (BAC-C12)	LCMSMS ESI-POS (MRM)	139-07-1	13	21	40	24	33	8.2	13	8.1
Benzyldimethylhexadecylammonium chloride (BAC-C14)	LCMSMS ESI-POS (MRM)	139-08-2	14	14	25	14	23	7.4	14	6.0
Benzyldimethylhexadecylammonium chloride (BAC-C16)	LCMSMS ESI-POS (MRM)	122-18-9	3.2	2.6	3.7	<	5.9	<	<	<
Benzyldimethyloctadecylammonium chloride (BAC-C18)	LCMSMS ESI-POS (MRM)	122-19-0	<	<	<	<	2.2	<	<	<
Dimethyldidecylammonium chloride (DDAC-C8)	LCMSMS ESI-POS (MRM)	5538-94-3	<	<	<	<	<	<	<	<
Didecyldimethylammonium chloride (DDAC-C10)	LCMSMS ESI-POS (MRM)	7173-51-5	15	42	57	64	76	<	<	<
Didodecyldimethylammonium chloride (DDAC-C12)	LCMSMS ESI-POS (MRM)	3401-74-9	<	<	<	<	<	<	<	<

Locations 4 and 7

Description	Detection technique	CAS nummer	location 4-1 SD	location 4-2 SD	location 4-3 SD	location 4-4 SD	location 7-1 SD	location 7-2 SD	location 7-3 SD	location 7-4 SD
Period (week.)			12 week.	4 week.	4 week.	4 week.	12 week.	4 week.	4 week.	4 week.
Unit			ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
amoxicillin	LCMSMS ESI-POS (MRM)	26787-78-0	<	<	<	<	<	<	<	<
chlortetracycline	LCMSMS ESI-POS (MRM)	57-62-5	<	<	<	<	<	<	<	<
ciprofloxacin	LCMSMS ESI-POS (MRM)	85721-33-1	<	<	<	<	<	<	<	<
desamethasone	LCMSMS ESI-POS (MRM)	50-02-2	<	<	<	<	<	<	<	<
doxycycline	LCMSMS ESI-POS (MRM)	564-25-0	<	<	<	<	<	<	<	<
estrone	LCMSMS ESI-NEG (MRM)	53-16-7	2.9	20	2.5	6.1	3.3	2.0	6.2	6.9
fenbendazole	LCMSMS ESI-POS (MRM)	43210-67-9	<	<	<	<	<	<	<	<
fipronil	LCMSMS ESI-NEG (MRM)	120068-37-3	<	<	<	<	2.1	<	<	<
fipronil sulfone	LCMSMS ESI-NEG (MRM)	120068-36-2	<	<	<	<	5.4	1.7	<	1.3
florfenicol	LCMSMS ESI-NEG (MRM)	73231-34-2	22	37	2.1	<	44	10	6.1	6.8
flubendazole	LCMSMS ESI-POS (MRM)	31430-15-6	5.7	25	2.3	3.8	13.2	3.9	3.9	6.1
flumequine	LCMSMS ESI-POS (MRM)	42835-25-6	13	44	4.7	4.8	8.6	7.2	3.1	2.9
ivermectine B1b	LCMSMS ESI-POS (MRM)	70209-81-3	<	<	<	<	<	<	<	<
lincomycin	LCMSMS ESI-POS (MRM)	154-21-2	<	<	<	<	1.4	<	<	<
mebendazole	LCMSMS ESI-POS (MRM)	31431-39-7	<	<	3.3	<	<	<	<	<
norfloxacine	LCMSMS ESI-POS (MRM)	70458-96-7	<	<	<	<	<	<	<	<
oxytetracycline	LCMSMS ESI-POS (MRM)	79-57-2	<	<	<	<	<	<	<	<
penicillins G	LCMSMS ESI-POS (MRM)	113-98-4	<	<	<	<	<	<	<	<
permetrin 1	LCMSMS ESI-POS (MRM)	52645-53-1	<	<	<	<	<	<	<	<
permetrin 2	LCMSMS ESI-POS (MRM)	52645-53-1	<	<	<	<	<	<	<	<
progesterone	LCMSMS ESI-POS (MRM)	57-83-0	2.3	<	<	13	<	<	<	<
sulfachloropyridazine	LCMSMS ESI-POS (MRM)	80-32-0	<	<	<	<	<	<	<	<
sulfadiazine	LCMSMS ESI-POS (MRM)	68-35-9	29	56	5.8	2.1	29	10	4.0	3.3
sulfadimethoxine	LCMSMS ESI-POS (MRM)	122-11-2	<	<	<	<	<	<	<	<
sulfadoxine	LCMSMS ESI-POS (MRM)	2447-57-6	<	<	<	<	<	<	<	<
sulfamerazine	LCMSMS ESI-POS (MRM)	127-79-7	<	<	<	<	<	<	<	<
sulfamethazine	LCMSMS ESI-POS (MRM)	57-68-1	84	66	19	6	35	8.0	8.3	7.5
sulfamethoxazole	LCMSMS ESI-NEG (MRM)	723-46-6	15	<	11	<	8.2	<	<	<
sulfamethoxypyridazine	LCMSMS ESI-POS (MRM)	80-35-3	4.2	29	<	<	1.9	<	<	<
sulfapyridine	LCMSMS ESI-POS (MRM)	144-83-2	<	<	<	<	1.3	<	<	<
sulfathiazole	LCMSMS ESI-POS (MRM)	72-14-0	<	<	<	<	<	<	<	<
tetracycline	LCMSMS ESI-POS (MRM)	60-54-8	<	<	<	<	<	<	<	<
tiamulin	LCMSMS ESI-POS (MRM)	55297-95-5	<	<	<	<	<	<	<	<
tilmicosin	LCMSMS ESI-POS (MRM)	108050-54-0	8.2	81	4.7	5.1	9.7	4.2	1.8	4.9
toltrazuril	LCMSMS ESI-NEG (MRM)	69004-03-1	<	<	<	<	<	<	<	<
trimethoprim	LCMSMS ESI-POS (MRM)	738-70-5	<	<	<	<	1	<	<	<
tylosin	LCMSMS ESI-POS (MRM)	1401-69-0	<	<	<	<	2.9	<	<	<
Benzyldimethyloctylammonium chloride (BAC-C8)	LCMSMS ESI-POS (MRM)	959-55-7	<	<	<	<	<	<	<	<
Benzyldimethyldecylammonium chloride (BAC-C10)	LCMSMS ESI-POS (MRM)	965-32-2	<	<	<	<	<	<	<	<
Benzyldimethyldodecylammonium chloride (BAC-C12)	LCMSMS ESI-POS (MRM)	139-07-1	11	10	8.0	7.6	16	9.5	10	11
Benzyldimethyltetradecylammonium chloride (BAC-C14)	LCMSMS ESI-POS (MRM)	139-08-2	10	94	6.7	5.3	18	9.1	15	9.8
Benzyldimethylhexadecylammonium chloride (BAC-C16)	LCMSMS ESI-POS (MRM)	122-18-9	2.1	<	<	<	5.5	<	2.0	<
Benzyldimethyloctadecylammonium chloride (BAC-C18)	LCMSMS ESI-POS (MRM)	122-19-0	<	<	<	<	<	<	<	<
Didecyldimethylammonium chloride (DDAC-C10)	LCMSMS ESI-POS (MRM)	7173-51-5	<	<	<	<	<	<	<	<
Didecyldimethylammonium chloride (DDAC-C10)	LCMSMS ESI-POS (MRM)	7173-51-5	6.6	26	<	3.3	13	4.5	3.6	<
Didodecyldimethylammonium chloride (DDAC-C12)	LCMSMS ESI-POS (MRM)	3401-74-9	<	<	<	<	<	<	<	<

SM2. Recovery rates

Unit	added concentration	52020068-SA1 QW+SA SD	52020068-SA2 QW+SA SD	52020068-SA3 QW+SA SD	52020068-SA4 QW+SA SD	52020068-SA5 QW+SA SD	52020068-SA6 QW+SA SD	52020068-SA7 QW+SA SD	52020068-SA8 QW+SA SD	52020068-MBL1 method blank QW SD	52020068-MBL2 method blank QW SD	average recovery (Tv_r) (n=8)	Intra-laboratory repeatable standard deviation (s_r) (n=8)	Intra-laboratory repeatable coefficient of variation (vc_r) (n=8)	Uj	Precision (U_r) (n=8)	Extended measurement Uncertainty Ue (Ue_r) (n=8)
	ng/ml	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
amoxicillin	11	7.8	7.4	8.9	9.4	11	6.9	10	8.1	0	0	8.8	1.5	17	10	-91	187
chlortetracycline	10	49	30	40	48	35	30	38	35	1	0	38	7.3	19	10	-62	131
ciprofloxacin	10	40	40	44	38	38	36	37	41	6	1	39	2.7	6.9	10	-61	124
doxycyline	12	18	18	19	31	17	21	22	19	3	2	21	4.5	22	10	-79	166
fenbendazole	10	81	80	85	82	80	85	79	83	2	1	82	2.2	2.7	10	-18	42
fipronil	10	89	86	80	89	86	88	83	82	1	0	85	3.5	4.1	10	-15	36
fipronil sulfone	11	98	97	98	95	97	99	96	95	1	1	97	1.5	1.6	10	-3	21
florfenicol	10	97	104	98	96	104	96	97	92	0	0	98	4.2	4.3	10	-2	22
flubendazole	11	86	86	92	85	86	88	89	86	5	9	87	2.3	2.6	10	-13	33
flumequine	10	64	63	67	50	76	66	76	75	0	6	75	12	16	10	-25	63
Ivermectine	162	80	87	92	99	93	88	97	105	0	6	93	8.0	8.6	10	-7	30
lincomycin	11	82	81	80	76	78	81	79	81	0	0	80	2.0	2.5	10	-20	45
mebendazole	11	82	82	89	79	82	78	83	85	1	0	82	3.6	4.4	10	-18	41
norfloxacine	12	80	55	67	72	61	76	83	66	0	9	70	9.5	14	10	-30	69
oxytetracycline	13	33	32	34	36	29	36	35	30	4	2	33	2.8	8.4	10	-67	137
penicilline G	13	10	10	9.0	9.0	10	10	9.1	9.0	0	0	10	0.5	5.7	10	-90	182
permethrin 1	3	86	78	82	85	85	78	76	83	0	0	82	3.8	4.7	10	-18	43
permethrin 2	8	87	80	85	80	85	81	73	84	0	0	82	4.4	5.4	10	-18	43
sulfachloropyridazine	11	39	38	49	46	42	34	55	39	5	2	43	6.8	16	10	-57	120
sulfadiazine	11	69	74	79	67	69	66	69	74	0	5	71	4.5	6.3	10	-29	63
sulfadimethoxine	13	96	95	96	98	95	91	96	97	2	1	96	2.2	2.3	10	-4	22
sulfadoxine	10	103	105	103	105	104	94	101	106	0	0	103	3.7	3.6	10	3	22
sulfamerazine	11	100	103	103	101	101	95	100	104	1	1	101	2.6	2.6	10	1	21
sulfamethazine	11	112	111	111	110	113	112	109	112	9	6	111	1.3	1.2	10	11	30
sulfamethoxazole	10	86	72	69	74	79	73	79	81	2	1	76	5.4	7.1	10	-24	53
sulfamethoxypyridazine	12	102	100	102	100	101	100	99	103	1	0	101	1.6	1.6	10	1	20
sulfapyridine	11	101	100	100	101	99	102	99	101	0	0	100	1.2	1.2	10	0	20
sulfathiazole	10	59	80	81	50	71	64	75	75	0	0	69	11	16	10	-31	72
tetracycline	24	13	12	12	11	15	13	13	15	1	3	13	1.5	11	10	-87	176
tiamulin	12	87	88	89	91	89	90	88	88	0	1	89	1.4	1.6	10	-11	30
tilmicosin	12	96	86	102	95	93	82	103	81	1	0	92	8.3	9.0	10	-8	31
toltrazuril	11	106	88	100	105	97	98	102	100	2	2	99	5.6	5.6	10	-1	23
trimethoprim	12	101	99	104	99	100	104	97	104	0	0	101	2.8	2.7	10	1	21
tylosin	11	58	61	76	64	64	59	59	49	0	0	61	7.5	12	10	-39	83
Benzyldimethyloctylammonium chloride (BAC-C8)	10	107	100	97	105	106	104	113	107	0	0	105	4.9	4.6	10	5	24
Benzyldimethyldecylammonium chloride (BAC-C10)	12	99	99	85	95	92	85	102	100	0	0	95	6.7	7.1	10	-5	27
Benzyldimethyldodecylammonium chloride (BAC-C12)	12	102	107	81	82	87	95	99	100	0	0	94	9.6	10	10	-6	31
Benzyldimethyltetradecylammonium chloride (BAC-C14)	11	92	81	99	88	83	103	90	89	0	0	91	7.5	8.3	10	-9	32
Benzyldimethylhexadecylammonium chloride (BAC-C16)	13	100	82	89	88	87	104	101	83	0	0	92	8.8	9.6	10	-8	32
Benzyldimethyloctadecylammonium chloride (BAC-C18)	10	79	93	81	96	85	99	83	82	0	0	87	7.6	8.8	10	-13	37
Dimethyldioctylammonium chloride (DDAC-C8)	12	92	88	108	88	106	90	107	103	0	0	98	9.0	9.2	10	-2	28
Didecyldimethylammonium chloride (DDAC-C10)	10	107	83	91	82	81	93	95	91	0	0	91	8.7	9.6	10	-9	34
Didodecyldimethylammonium chloride (DDAC-C12)	12	111	93	114	100	101	99	101	107	0	0	103	7.0	6.8	10	3	25
17-alpha-Estradiol	12	136	82	170	111	108	102	100	113	0	0	115	27	23	10	15	59
17-beta-Estradiol	11	125	100	93	114	105	106	105	103	3	7	106	9.5	8.9	10	6	30
Altrenogest	11	103	93	100	89	106	81	108	101	0	0	98	9.0	9.2	10	-2	28
Androstendion	12	114	101	112	92	111	87	110	118	1	0	105	11	11	10	5	31
Estrone	12	96	100	107	88	95	100	101	102	1	0	99	5.8	5.8	10	-1	23
Progesterone	12	119	110	119	100	118	94	127	123	3	0	114	12	10	10	14	40
Testosterone	11	125	117	126	100	115	91	126	124	1	0	116	13	11	10	16	43

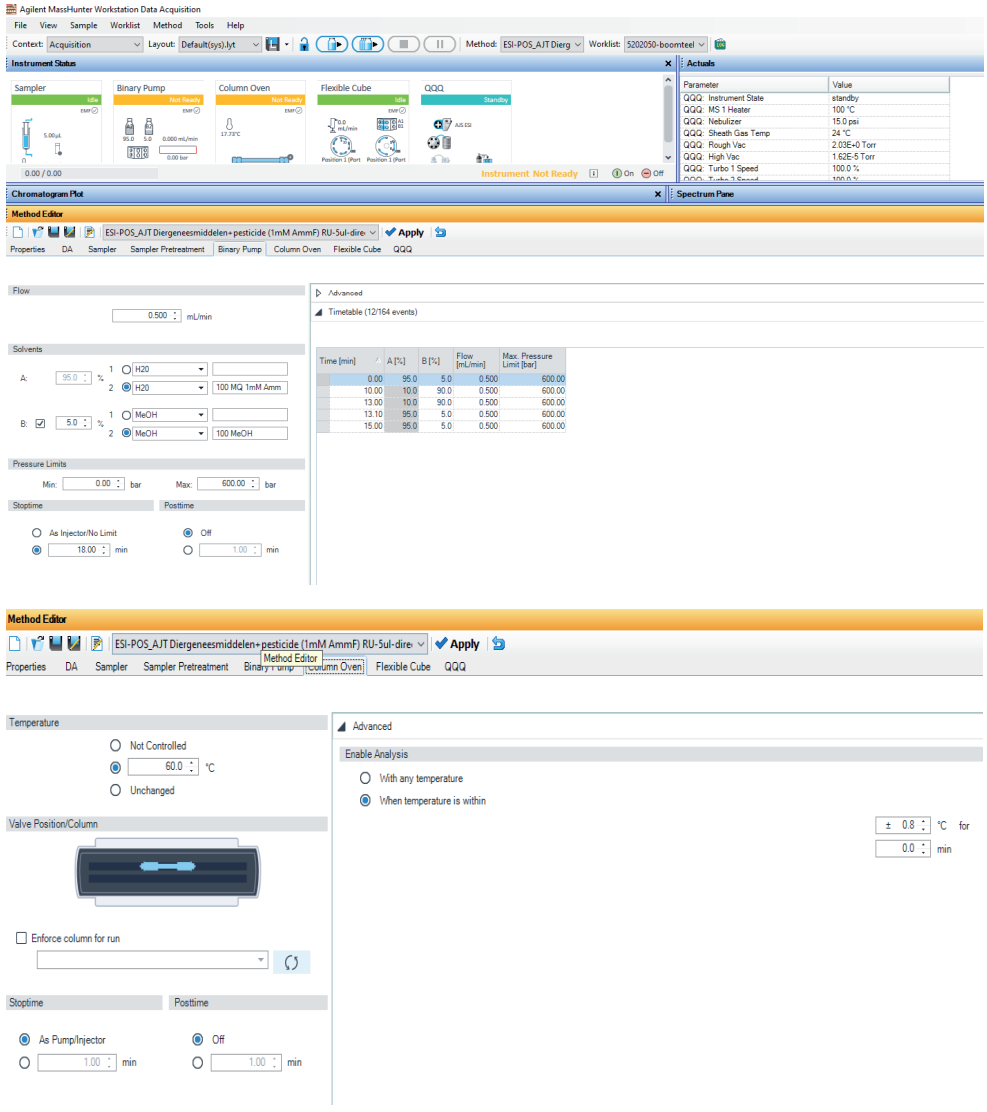
SM3. Time integrative Uptake Coefficient (TUC)

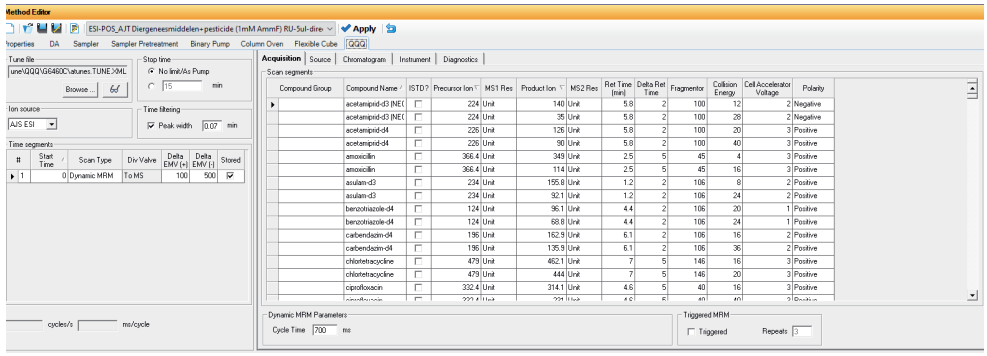
Location	CAS number	location: 1	location: 6	location: 2	location: 5	location: 8	location: 3	location: 4	location: 7
		TUC	TUC	TUC	TUC	TUC	TUC	TUC	TUC
estrone	53-16-7	2.86	1.89		2.15	7.42	0.83		4.50
fipronil sulfone	120068-36-2								
florfenicol	73231-34-2	0.61	1.23	0.78	1.04	1.32	0.47	0.52	0.53
flubendazole	31430-15-6	1.39	1.63	1.06	1.44	2.53	0.91	1.53	1.06
flumequine	42835-25-6	1.27	2.18	0.92	1.20	1.52	0.96	1.05	1.54
lincomycin	154-21-2								
metendazole	31431-39-7								
permethrin 1	52645-53-1								
sulfadiazine	68-35-9	0.78	1.77	0.90	1.58	8.00	0.06	2.18	0.62
sulfamethazine	127-79-7	0.93	1.60	0.87	1.16	2.43	0.41	1.07	0.67
sulfamethoxazole	723-46-6		1.40		0.73	2.28	0.12		
sulfamethoxypyridazine	80-35-3			0.70	1.25	2.26		0.69	
sulfapyridine	144-83-2				0.57	10.25			
tilmicosin	108050-54-0		1.09	1.85	3.92	3.46	0.14	2.18	1.13
trimethoprim	738-70-5				0.75	2.10	0.16		
Benzyldimethyldodecylammonium chloride (BAC-C12)	139-07-1	96.70	4.23	3.34	2.14	6.36	0.89	2.42	1.86
Benzyldimethyltetradecylammonium chloride (BAC-C14)	139-08-2	123.03	4.96	3.52	2.20	3.69	1.17	2.15	1.90
Benzyldimethylhexadecylammonium chloride (BAC-C16)	122-18-9	106.64	3.23						
Benzyldimethyloctadecylammonium chloride (BAC-C18)	122-19-0								
Didecyldimethylammonium chloride (DDAC-C10)	7173-51-5	1.48	2.79	1.08	1.13	11.02		0.90	

SM4. Additional details on the methods and instrument settings

VPs

Analysis column: GEMINI NX C18

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Sampler
Sampler Pretreatment
Binary Pump
Column Oven
Flexible Cube
QQQ
Tune file
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Stop time
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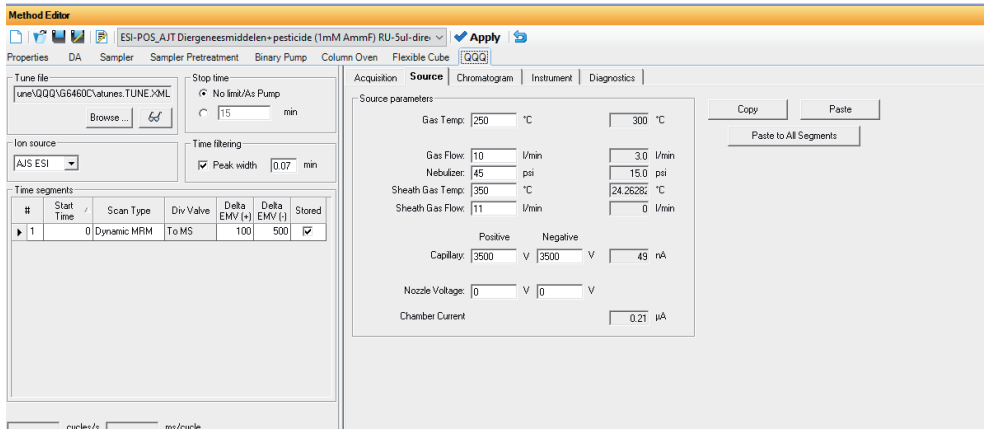
min
Ion source
AJS ESI
Time filtering
Peak width
0.07
min
Time segments
| Start Time | Scan Type | Div Valve | Delta EMV (+) | Delta EMV (-) | Stored
1 | 0 | Dynamic MRM | To MS | 100 | 500
cycles/s
ms/cycle
Acquisition
Source
Chromatogram
Instrument
Diagnostics
Scan segments
Compound Group | Compound Name | ISTD? | Precursor Ion | MS1 Res | Product Ion | MS2 Res | Ret Time [min] | Delta Ret Time | Fragmentor | Collision Energy | Cell Accelerator Voltage | Polarity
acetamiprid-d3 (NEC | 224 | Unit | 140 | Unit | 5.8 | 2 | 100 | 12 | 2 | Negative
acetamiprid-d3 (NEC | 224 | Unit | 35 | Unit | 5.8 | 2 | 100 | 28 | 2 | Negative
acetamiprid-d4 | 228 | Unit | 126 | Unit | 5.8 | 2 | 100 | 20 | 3 | Positive
acetamiprid-d4 | 228 | Unit | 90 | Unit | 5.8 | 2 | 100 | 40 | 3 | Positive
amoxicillin | 368.4 | Unit | 349 | Unit | 2.5 | 5 | 45 | 4 | 3 | Positive
amoxicillin | 368.4 | Unit | 114 | Unit | 2.5 | 5 | 45 | 16 | 3 | Positive
asulam-d3 | 234 | Unit | 155.8 | Unit | 1.2 | 2 | 105 | 8 | 2 | Positive
asulam-d3 | 234 | Unit | 92.1 | Unit | 1.2 | 2 | 105 | 24 | 2 | Positive
benzotriazole-d4 | 124 | Unit | 96.1 | Unit | 4.4 | 2 | 105 | 20 | 1 | Positive
benzotriazole-d4 | 124 | Unit | 68.8 | Unit | 4.4 | 2 | 105 | 24 | 1 | Positive
carbendazim-d4 | 196 | Unit | 162.9 | Unit | 6.1 | 2 | 105 | 16 | 2 | Positive
carbendazim-d4 | 196 | Unit | 135.9 | Unit | 6.1 | 2 | 105 | 36 | 2 | Positive
chlortetracycline | 479 | Unit | 462.1 | Unit | 7 | 5 | 145 | 16 | 3 | Positive
chlortetracycline | 479 | Unit | 444 | Unit | 7 | 5 | 145 | 20 | 3 | Positive
ciprofloxacin | 332.4 | Unit | 314.1 | Unit | 4.6 | 5 | 40 | 16 | 3 | Positive
Dynamic MRM Parameters
Cycle Time
700
ms
Triggered MRM
Triggered
Repeats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ESI-POS_AJT Diergeneesmiddelen+pesticide (1mM AmmF) RU-5ul-dire
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Properties
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Sampler
Sampler Pretreatment
Binary Pump
Column Oven
Flexible Cube
QQQ
Tune file
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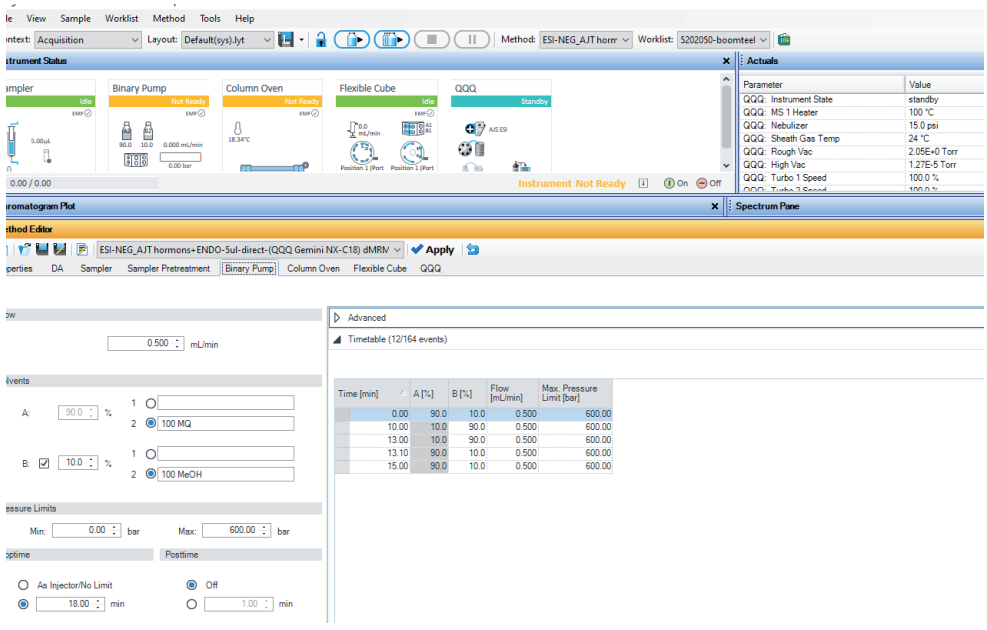
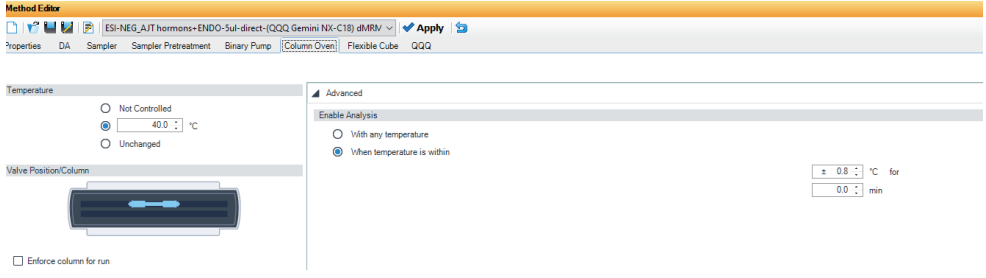
Stop time
No limit/As Pump
15
min
Ion source
AJS ESI
Time filtering
Peak width
0.07
min
Time segments
| Start Time | Scan Type | Div Valve | Delta EMV (+) | Delta EMV (-) | Stored
1 | 0 | Dynamic MRM | To MS | 100 | 500
cycles/s
ms/cycle
Acquisition
Source
Chromatogram
Instrument
Diagnostics
Source parameters
Gas Temp: 250 °C 300 °C
Gas Flow: 10 l/min 3.0 l/min
Nebulizer: 45 psi 15.0 psi
Sheath Gas Temp: 350 °C 24.2628 °C
Sheath Gas Flow: 11 l/min 0 l/min
Positive
Negative
Capillary: 3500 V 3500 V 49 nA
Nozzle Voltage: 0 V 0 V
Chamber Current 0.21 µA
Copy
Paste
Paste to All Segments

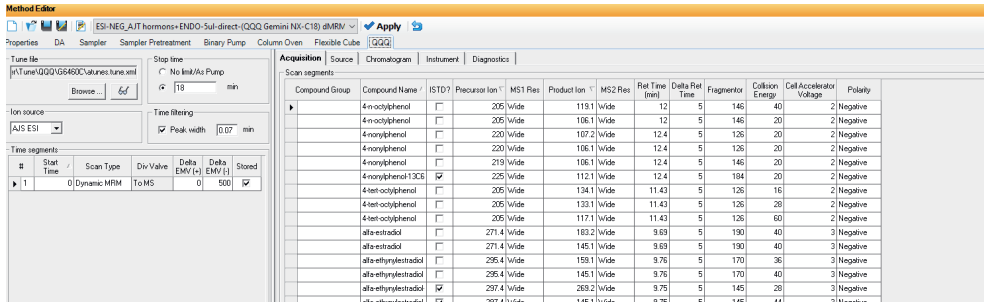
Dynamic MRM																							
Compoun	Compound Name	ISTD?	Precursor	MS1 Res	Product Io	MS2 Res	Primary	Trigger	Threshold	Ret Time (	Delta Ret	Fragment	Collision E	Cell Accel	Polarity	Trigger En	Trigger De	Trigger W	IsLogicEna	Trigger Lo	Trigger Ra	Trigger Ra	Ignore MR
	acetamiprid-d3 (NEG)	FALSE	224	Unit	35	Unit	TRUE	FALSE	0	5.8	2	100	28	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	acetamiprid-d3 (NEG)	FALSE	224	Unit	140	Unit	TRUE	FALSE	0	5.8	2	100	12	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	acetamiprid-d4	FALSE	226	Unit	126	Unit	TRUE	FALSE	0	5.8	2	100	20	3	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	acetamiprid-d4	FALSE	226	Unit	90	Unit	TRUE	FALSE	0	5.8	2	100	40	3	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	amoxicillin	FALSE	366.4	Unit	349	Unit	TRUE	FALSE	0	2.5	5	45	4	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	amoxicillin	FALSE	366.4	Unit	114	Unit	TRUE	FALSE	0	2.5	5	45	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	asulam-d3	FALSE	234	Unit	155.8	Unit	TRUE	FALSE	0	1.2	2	106	8	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	asulam-d3	FALSE	234	Unit	92.1	Unit	TRUE	FALSE	0	1.2	2	106	24	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	benzotriazole-d4	FALSE	124	Unit	96.1	Unit	TRUE	FALSE	0	4.4	2	106	20	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	benzotriazole-d4	FALSE	124	Unit	68.8	Unit	TRUE	FALSE	0	4.4	2	106	24	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	carbendazim-d4	FALSE	196	Unit	162.9	Unit	TRUE	FALSE	0	6.1	2	106	16	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	carbendazim-d4	FALSE	196	Unit	135.9	Unit	TRUE	FALSE	0	6.1	2	106	36	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	chlortetracycline	FALSE	479	Unit	462.1	Unit	TRUE	FALSE	0	7	5	146	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	chlortetracycline	FALSE	479	Unit	444	Unit	TRUE	FALSE	0	7	5	146	20	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	ciprofloxacin	FALSE	332.4	Unit	314.1	Unit	TRUE	FALSE	0	4.6	5	40	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	ciprofloxacin	FALSE	332.4	Unit	231	Unit	TRUE	FALSE	0	4.6	5	40	40	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	desisopropylatrazine-d5	FALSE	179	Unit	69.1	Unit	TRUE	FALSE	0	4.7	2	126	36	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	desisopropylatrazine-d5	FALSE	179	Unit	44.1	Unit	TRUE	FALSE	0	4.7	2	126	40	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	dichloorvos-d6	FALSE	227	Unit	115	Unit	TRUE	FALSE	0	7.6	2	106	16	4	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	dichloorvos-d6	FALSE	227	Unit	83	Unit	TRUE	FALSE	0	7.6	2	106	32	4	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	diuron-d6	FALSE	239	Unit	78.1	Unit	TRUE	FALSE	0	8.7	2	106	24	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	diuron-d6	FALSE	239	Unit	52.1	Unit	TRUE	FALSE	0	8.7	2	106	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	diuron-d6 (NEG)	FALSE	237	Unit	185.9	Unit	TRUE	FALSE	0	8.7	2	106	16	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	diuron-d6 (NEG)	FALSE	237	Unit	149.9	Unit	TRUE	FALSE	0	8.7	2	106	24	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	doxycyline	FALSE	445	Unit	428.1	Unit	TRUE	FALSE	0	6.9	5	126	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	doxycyline	FALSE	445	Unit	201.1	Unit	TRUE	FALSE	0	6.9	5	126	48	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	erythromycin-13C3	FALSE	736.9	Unit	578.4	Unit	TRUE	FALSE	0	8.5	2	135	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	erythromycin-13C3	FALSE	736.9	Unit	160.2	Unit	TRUE	FALSE	0	8.5	2	135	28	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	fenbendazole	FALSE	300	Unit	268	Unit	TRUE	FALSE	0	9.9	2	116	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	fenbendazole	FALSE	300	Unit	159	Unit	TRUE	FALSE	0	9.9	2	116	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	fipronil	FALSE	436	Unit	330.9	Unit	TRUE	FALSE	0	10.5	2	106	12	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	fipronil	FALSE	436	Unit	319	Unit	TRUE	FALSE	0	10.5	2	106	20	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	Fipronil sulfone 1	FALSE	450.8	Unit	414.9	Unit	TRUE	FALSE	0	10.7	2	136	16	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	Fipronil sulfone 1	FALSE	450.8	Unit	281.9	Unit	TRUE	FALSE	0	10.7	2	136	36	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	Fipronil sulfone 2	FALSE	452.8	Unit	416.9	Unit	TRUE	FALSE	0	10.7	2	136	16	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	Fipronil sulfone 2	FALSE	452.8	Unit	282	Unit	TRUE	FALSE	0	10.7	2	136	36	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	Florfenicol	FALSE	358	Unit	338	Unit	TRUE	FALSE	0	5.3	2	126	8	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	Florfenicol	FALSE	358	Unit	184.9	Unit	TRUE	FALSE	0	5.3	2	126	20	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	flubendazole	FALSE	314.1	Unit	282	Unit	TRUE	FALSE	0	8.9	2	146	24	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	flubendazole	FALSE	314.1	Unit	123	Unit	TRUE	FALSE	0	8.9	2	146	44	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	flubendazole-d3	TRUE	317.1	Unit	282	Unit	TRUE	FALSE	0	8.9	2	126	24	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	flubendazole-d3	TRUE	317.1	Unit	123	Unit	TRUE	FALSE	0	8.9	2	126	40	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	flumequine	FALSE	262.3	Unit	244	Unit	TRUE	FALSE	0	7.8	2	115	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	flumequine	FALSE	262.3	Unit	201.9	Unit	TRUE	FALSE	0	7.8	2	115	36	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	ibuprofen-13C2	FALSE	208.3	Unit	163.2	Unit	TRUE	FALSE	0	6.3	2	70	0	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	imidacloprid-d4	FALSE	260	Unit	213	Unit	TRUE	FALSE	0	5.3	2	100	12	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	imidacloprid-d4	FALSE	260	Unit	179.1	Unit	TRUE	FALSE	0	5.3	2	100	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	isoproturon-d6	FALSE	213	Unit	78.1	Unit	TRUE	FALSE	0	8.7	2	106	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	isoproturon-d6	FALSE	213	Unit	52.1	Unit	TRUE	FALSE	0	8.7	2	106	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Ivermectine B1a	FALSE	893.5	Unit	570.3	Unit	TRUE	FALSE	0	13.1	5	136	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Ivermectine B1a	FALSE	893.5	Unit	145.1	Unit	TRUE	FALSE	0	13.1	5	136	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Ivermectine B1b	FALSE	893.5	Unit	570.3	Unit	TRUE	FALSE	0	14.1	5	136	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Ivermectine B1b	FALSE	893.5	Unit	145.1	Unit	TRUE	FALSE	0	14.1	5	136	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	lincomycin	FALSE	407	Unit	359.2	Unit	TRUE	FALSE	0	7.1	2	146	16	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	lincomycin	FALSE	407	Unit	126.1	Unit	TRUE	FALSE	0	7.1	2	146	32	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	Mebendazole	FALSE	296.1	Unit	264	Unit	TRUE	FALSE	0	8.6	2	136	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Mebendazole	FALSE	296.1	Unit	77.1	Unit	TRUE	FALSE	0	8.6	2	136	60	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	naproxen-13C -d3	FALSE	236.3	Unit	190.2	Unit	TRUE	FALSE	0	9.1	2	85	8	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	naproxen-13C -d3	FALSE	236.3	Unit	171.2	Unit	TRUE	FALSE	0	9.1	2	85	28	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	Norfloxacine	FALSE	320.1	Unit	302.1	Unit	TRUE	FALSE	0	4.7	5	106	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Norfloxacine	FALSE	320.1	Unit	231	Unit	TRUE	FALSE	0	4.7	5	106	56	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	oxytetracycline	FALSE	461.4	Unit	443.1	Unit	TRUE	FALSE	0	5.5	2	45	8	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	oxytetracycline	FALSE	461.4	Unit	426.1	Unit	TRUE	FALSE	0	5.5	2	45	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	paracetamol-13C2	FALSE	154.2	Unit	111	Unit	TRUE	FALSE	0	2	2	95	12	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	paracetamol-13C2	FALSE	154.2	Unit	93	Unit	TRUE	FALSE	0	2	2	95	20	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	pendimethalin-d5	FALSE	287	Unit	213.1	Unit	TRUE	FALSE	0	11.7	2	80	8	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	pendimethalin-d5	FALSE	287	Unit	46.1	Unit	TRUE	FALSE	0	11.7	2	80	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Penicilline G	FALSE	335	Unit	176.1	Unit	TRUE	FALSE	0	9.1	5	88	12	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Penicilline G	FALSE	335	Unit	160	Unit	TRUE	FALSE	0	9.1	5	88	12	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	permethrin 1	FALSE	408	Unit	355.1	Unit	TRUE	FALSE	0	12.5	2	88	8	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	permethrin 1	FALSE	408	Unit	183.1	Unit	TRUE	FALSE	0	12.5	2	88	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	permethrin 2	FALSE	408	Unit	355.1	Unit	TRUE	FALSE	0	12.7	2	88	8	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	permethrin 2	FALSE	408	Unit	183.1	Unit	TRUE	FALSE	0	12.7	2	88	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	pirimicarb-d6	FALSE	245	Unit	185.1	Unit	TRUE	FALSE	0	8.6	2	100	12	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	pirimicarb-d6	FALSE	245	Unit	78.1	Unit	TRUE	FALSE	0	8.6	2	100	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	propamocarb-d7	FALSE	196	Unit	103.1	Unit	TRUE	FALSE	0	1.5	2	100	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	propamocarb-d7	FALSE	196	Unit	75.1	Unit	TRUE	FALSE	0	1.5	2	100	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	propyzamid-d3	FALSE	260	Unit	148	Unit	TRUE	FALSE	0	5.3	2	80	36	3	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	propyzamid-d3	FALSE	260	Unit	44	Unit	TRUE	FALSE	0	5.3	2	80	28	3	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	simazine-d10	FALSE	212	Unit	134.1	Unit	TRUE	FALSE	0	7.6	2	126	20	3	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	simazine-d10	FALSE	212	Unit	105	Unit	TRUE	FALSE	0	7.6	2	126	28	3	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	sulfachloropyridazine	FALSE	285.7	Unit	157	Unit	TRUE	FALSE	0	4.7	2	40	8	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfachloropyridazine	FALSE	285.7	Unit	65	Unit	TRUE	FALSE	0	4.7	2	40	56	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfadiazine	FALSE	251	Unit	156	Unit	TRUE	FALSE	0	2.8	2	110	15	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfadiazine	FALSE	251	Unit	92	Unit	TRUE	FALSE	0	2.8	2	110	25	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfadiazine-13C6	TRUE	257.1	Unit	162	Unit	TRUE	FALSE	0	2.8	2	100	12	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfadiazine-13C6	TRUE	257.1	Unit	98.1	Unit	TRUE	FALSE	0	2.8	2	100	32	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	Sulfadimethoxine	FALSE	311.1	Unit	156	Unit	TRUE	FALSE	0	6.2	2	116	24	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfadimethoxine	FALSE	311.1	Unit	92.1	Unit	TRUE	FALSE	0	6.2	2	116	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfadoxine	FALSE	311.1	Unit	156	Unit	TRUE	FALSE	0	5.2	2	96	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfadoxine	FALSE	311.1	Unit	92.1	Unit	TRUE	FALSE	0	5.2	2	96	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfamerazine	FALSE	265.1	Unit	156	Unit	TRUE	FALSE	0	4.2	2	96	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfamerazine	FALSE	265.1	Unit	92.1	Unit	TRUE	FALSE	0	4.2	2	96	40	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	sulfamethazine	FALSE	279.3	Unit	186	Unit	TRUE	FALSE	0	4.9	2	45	12	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethazine	FALSE	279.3	Unit	124.1	Unit	TRUE	FALSE	0	4.9	2	45	24	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole	FALSE	252.3	Unit	156	Unit	TRUE	FALSE	0	4.7	2	95	8	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole	FALSE	252.3	Unit	64	Unit	TRUE	FALSE	0	4.7	2	95	32	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole-13C6	TRUE	258.3	Unit	162.1	Unit	TRUE	FALSE	0	4.7	2	90	8	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole-13C6	TRUE	258.3	Unit	98.1	Unit	TRUE	FALSE	0	4.7	2	90	20	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	Sulfamethoxypyridazine	FALSE	281	Unit	156	Unit	TRUE	FALSE	0	4.8	2	106	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfamethoxypyridazine	FALSE	281	Unit	92.1	Unit	TRUE	FALSE	0	4.8	2	106	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfapyridine	FALSE	250.1	Unit	156	Unit	TRUE	FALSE	0	3.9	2	96	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfapyridine	FALSE	250.1	Unit	92.1	Unit	TRUE	FALSE	0	3.9	2	96	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfathiazole	FALSE	256	Unit	156	Unit	TRUE	FALSE	0	3.8	2	106	16	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Sulfathiazole	FALSE	256	Unit	92.1	Unit	TRUE	FALSE	0	3.8	2	106	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	tebuconazole-d9	FALSE	317	Unit	125.3	Unit	TRUE	FALSE	0	10.5	2	126	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	tebuconazole-d9	FALSE	317	Unit	70	Unit	TRUE	FALSE	0	10.5	2	126	24	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	terbutylazine-d5	FALSE	235	Unit	179.1	Unit	TRUE	FALSE	0	9.6	2	106	16	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	terbutylazine-d5	FALSE	235	Unit	69.1	Unit	TRUE	FALSE	0	9.6	2	106	44	2	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	tetracycline	FALSE	445	Unit	427.2	Unit	TRUE	FALSE	0	5.7	2	126	8	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	tetracycline	FALSE	445	Unit	410.1	Unit	TRUE	FALSE	0	5.7	2	126	16	3	Positive	0	0	0	FALSE	AND	1	1	FALSE
	Tiamulin	FALSE	494.3	Unit	192.1	Unit	TRUE	FALSE	0	9.4	2	106	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Tiamulin	FALSE	494.3	Unit	119	Unit	TRUE	FALSE	0	9.4	2	106	56	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Tilmicosin	FALSE	869.6	Unit	696.5	Unit	TRUE	FALSE	0	8.8	5	290	60	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Tilmicosin	FALSE	869.6	Unit	174.1	Unit	TRUE	FALSE	0	8.8	5	290	60	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	Toltrazuril	FALSE	424	Unit	99	Unit	TRUE	FALSE	0	10.8	2	136	20	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	Toltrazuril	FALSE	424	Unit	42.1	Unit	TRUE	FALSE	0	10.8	2	136	24	1	Negative	0	0	0.5	FALSE	AND	1	1	FALSE
	trimethoprim	FALSE	291.3	Unit	261.1	Unit	TRUE	FALSE	0	5.2	2	110	24	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	trimethoprim	FALSE	291.3	Unit	230.1	Unit	TRUE	FALSE	0	5.2	2	110	24	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	tylosin	FALSE	917.1	Unit	174.1	Unit	TRUE	FALSE	0	9.9	2	255	36	5	Positive	0	0	0	FALSE	AND	1	1	FALSE
	tylosin	FALSE	917.1	Unit	101	Unit	TRUE	FALSE	0	9.9	2	255	44	5	Positive	0	0	0	FALSE	AND	1	1	FALSE

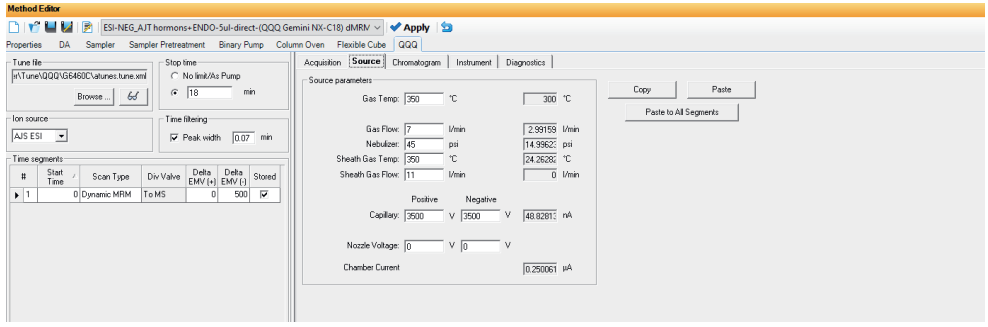
Hormones

Analysis column: Gemini NX C18







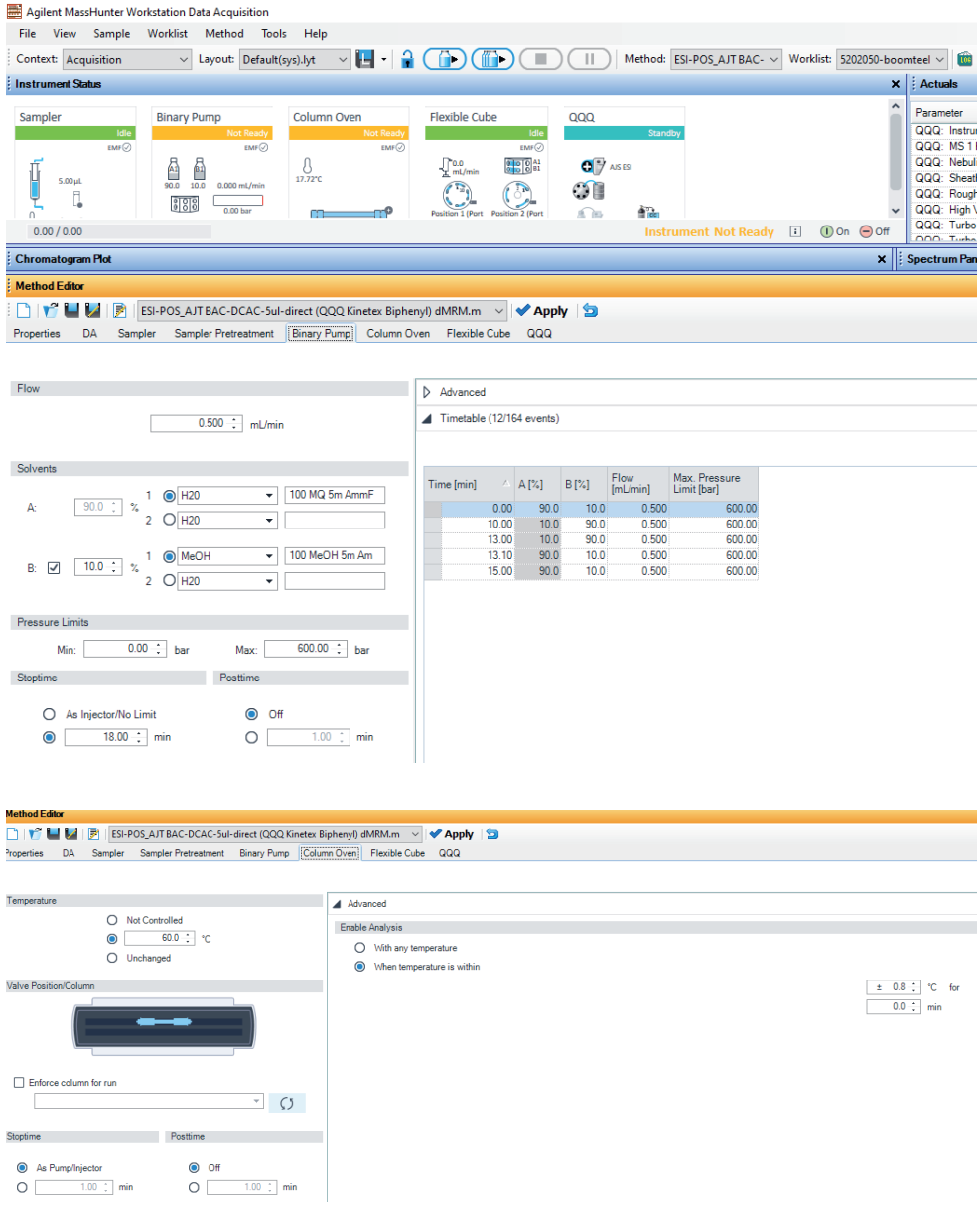


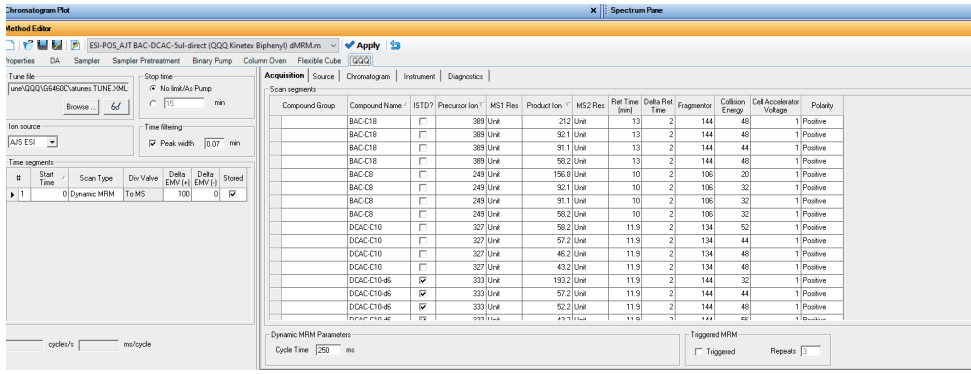
Dynamic MRM

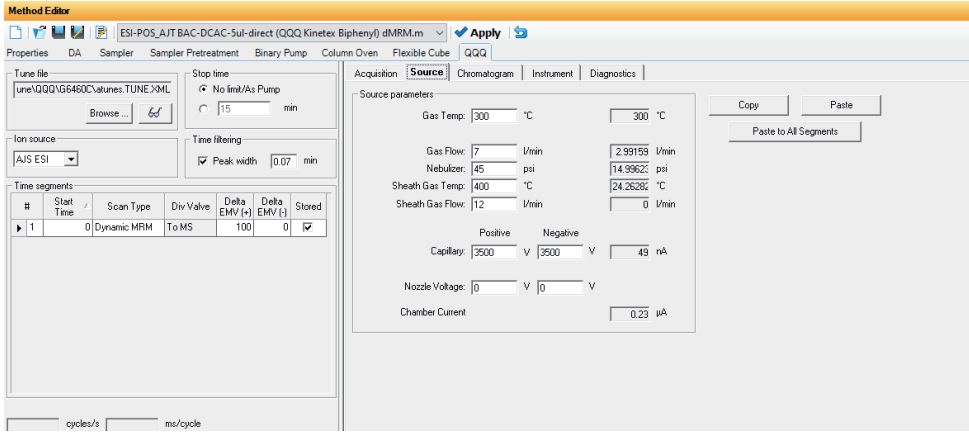
Compoun	Compound Name	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Primary	Trigger	Threshold	Ret Time (	Delta Ret	Fragmentc	Collision (	Cell Accel	Polarity	Trigger En	Trigger De	Trigger Wi	IsLogicEnc	Trigger Lo	Trigger Ra	Trigger Ra	Ignore MR
	4-n-octylphenol	FALSE	205	Wide	106.1	Wide	TRUE	FALSE	0	12	5	146	20	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-n-octylphenol	FALSE	205	Wide	119.1	Wide	TRUE	FALSE	0	12	5	146	40	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-nonylphenol	FALSE	220	Wide	107.2	Wide	TRUE	FALSE	0	12.4	5	126	20	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-nonylphenol	FALSE	220	Wide	106.1	Wide	TRUE	FALSE	0	12.4	5	126	20	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-nonylphenol	FALSE	219	Wide	106.1	Wide	TRUE	FALSE	0	12.4	5	146	20	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-nonylphenol-13C6	TRUE	225	Wide	112.1	Wide	TRUE	FALSE	0	12.4	5	184	20	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-tert-octylphenol	FALSE	205	Wide	134.1	Wide	TRUE	FALSE	0	11.43	5	126	16	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-tert-octylphenol	FALSE	205	Wide	133.1	Wide	TRUE	FALSE	0	11.43	5	126	28	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	4-tert-octylphenol	FALSE	205	Wide	117.1	Wide	TRUE	FALSE	0	11.43	5	126	60	2	Negative	0	0	0	FALSE	AND	1	1	FALSE
	alfa-estradiol	FALSE	271.4	Wide	183.2	Wide	TRUE	FALSE	0	9.69	5	190	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	alfa-estradiol	FALSE	271.4	Wide	145.1	Wide	TRUE	FALSE	0	9.69	5	190	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	alfa-ethynylestradiol	FALSE	295.4	Wide	159.1	Wide	TRUE	FALSE	0	9.76	5	170	36	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	alfa-ethynylestradiol	FALSE	295.4	Wide	145.1	Wide	TRUE	FALSE	0	9.76	5	170	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	alfa-ethynylestradiol-13C2	TRUE	297.4	Wide	269.2	Wide	TRUE	FALSE	0	9.75	5	145	28	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	alfa-ethynylestradiol-13C2	TRUE	297.4	Wide	145.1	Wide	TRUE	FALSE	0	9.75	5	145	44	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	beta-estradiol	FALSE	271.4	Wide	183.2	Wide	TRUE	FALSE	0	9.81	5	190	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	beta-estradiol	FALSE	271.4	Wide	145.1	Wide	TRUE	FALSE	0	9.81	5	190	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A	FALSE	227	Unit	212.1	Unit	TRUE	FALSE	0	9.02	5	126	20	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A	FALSE	227	Unit	133.1	Unit	TRUE	FALSE	0	9.02	5	126	28	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A	FALSE	227	Unit	93.1	Unit	TRUE	FALSE	0	9.02	5	126	56	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A-13C12	TRUE	239	Unit	224.1	Unit	TRUE	FALSE	0	9.02	5	126	20	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A-13C12	TRUE	239	Unit	223	Unit	TRUE	FALSE	0	9.02	5	126	28	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A-13C12	TRUE	239	Unit	139.2	Unit	TRUE	FALSE	0	9.02	5	126	28	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A-d16	FALSE	241	Wide	223.2	Wide	TRUE	FALSE	0	9.06	5	120	12	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	bisphenol-A-d16	FALSE	241	Wide	142.1	Wide	TRUE	FALSE	0	9.06	5	120	24	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	chloramphenicol	FALSE	322.1	Wide	258	Wide	TRUE	FALSE	0	6.48	5	100	4	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	chloramphenicol	FALSE	322.1	Wide	153	Wide	TRUE	FALSE	0	6.48	5	100	12	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	diethylstilbestrol	FALSE	267.4	Wide	251.1	Wide	TRUE	FALSE	0	9.91	5	145	20	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	diethylstilbestrol	FALSE	267.4	Wide	237.1	Wide	TRUE	FALSE	0	9.91	5	145	24	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	diethylstilbestrol-d8	TRUE	275.4	Wide	259.2	Wide	TRUE	FALSE	0	9.88	5	150	24	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	diethylstilbestrol-d8	TRUE	275.4	Wide	245.2	Wide	TRUE	FALSE	0	9.88	5	150	28	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	estradiol-13C2	TRUE	273.4	Wide	185.2	Wide	TRUE	FALSE	0	9.69	5	125	44	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	estradiol-13C2	TRUE	273.4	Wide	147.1	Wide	TRUE	FALSE	0	9.69	5	125	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	estrone	FALSE	269.4	Wide	145.1	Wide	TRUE	FALSE	0	9.64	5	145	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	estrone	FALSE	269.4	Wide	143.1	Wide	TRUE	FALSE	0	9.64	5	145	60	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	estrone-13C2	TRUE	271.4	Wide	185.1	Wide	TRUE	FALSE	0	9.64	5	160	36	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	estrone-13C2	TRUE	271.4	Wide	147.1	Wide	TRUE	FALSE	0	9.64	5	160	40	3	Negative	0	0	0	FALSE	AND	1	1	FALSE
	hydrohlorothiazide	FALSE	296	Unit	268.9	Unit	TRUE	FALSE	0	1.5	5	162	17	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	hydrohlorothiazide	FALSE	296	Unit	205	Unit	TRUE	FALSE	0	1.5	5	162	25	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	progesterone	FALSE	315.5	Unit	109	Unit	TRUE	FALSE	0	10.59	5	120	28	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	progesterone	FALSE	315.5	Unit	97	Unit	TRUE	FALSE	0	10.59	5	120	24	1	Positive	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole	FALSE	252.3	Wide	156	Wide	TRUE	FALSE	0	5.3	5	95	8	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole	FALSE	252.3	Wide	64	Wide	TRUE	FALSE	0	5.3	5	95	32	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole-13C6	TRUE	258.3	Wide	162.1	Wide	TRUE	FALSE	0	5.3	5	90	8	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	sulfamethoxazole-13C6	TRUE	258.3	Wide	98.1	Wide	TRUE	FALSE	0	5.3	5	90	20	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA	FALSE	543	Unit	419.8	Unit	TRUE	FALSE	0	12.55	5	164	44	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA	FALSE	543	Unit	417.8	Unit	TRUE	FALSE	0	12.55	5	164	44	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA	FALSE	543	Unit	80.9	Unit	TRUE	FALSE	0	12.55	5	164	60	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA	FALSE	543	Unit	78.9	Unit	TRUE	FALSE	0	12.55	5	164	56	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA-13C12	TRUE	555	Unit	430.9	Unit	TRUE	FALSE	0	12.55	5	224	48	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA-13C12	TRUE	555	Unit	428.8	Unit	TRUE	FALSE	0	12.55	5	224	48	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA-13C12	TRUE	555	Unit	80.9	Unit	TRUE	FALSE	0	12.55	5	224	60	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	TBBPA-13C12	TRUE	555	Unit	78.9	Unit	TRUE	FALSE	0	12.55	5	224	60	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	triclosan	FALSE	288	Unit	35	Unit	TRUE	FALSE	0	11.38	5	80	8	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	triclosan	FALSE	287	Unit	35	Unit	TRUE	FALSE	0	11.38	5	60	12	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	triclosan-13C12	TRUE	300	Unit	35.1	Unit	TRUE	FALSE	0	11.38	5	80	12	1	Negative	0	0	0	FALSE	AND	1	1	FALSE
	triclosan-13C12	TRUE	299	Unit	35	Unit	TRUE	FALSE	0	11.38	5	80	8	1	Negative	0	0	0	FALSE	AND	1	1	FALSE

Disinfectants

Analysis: Kinetex Biphenyl





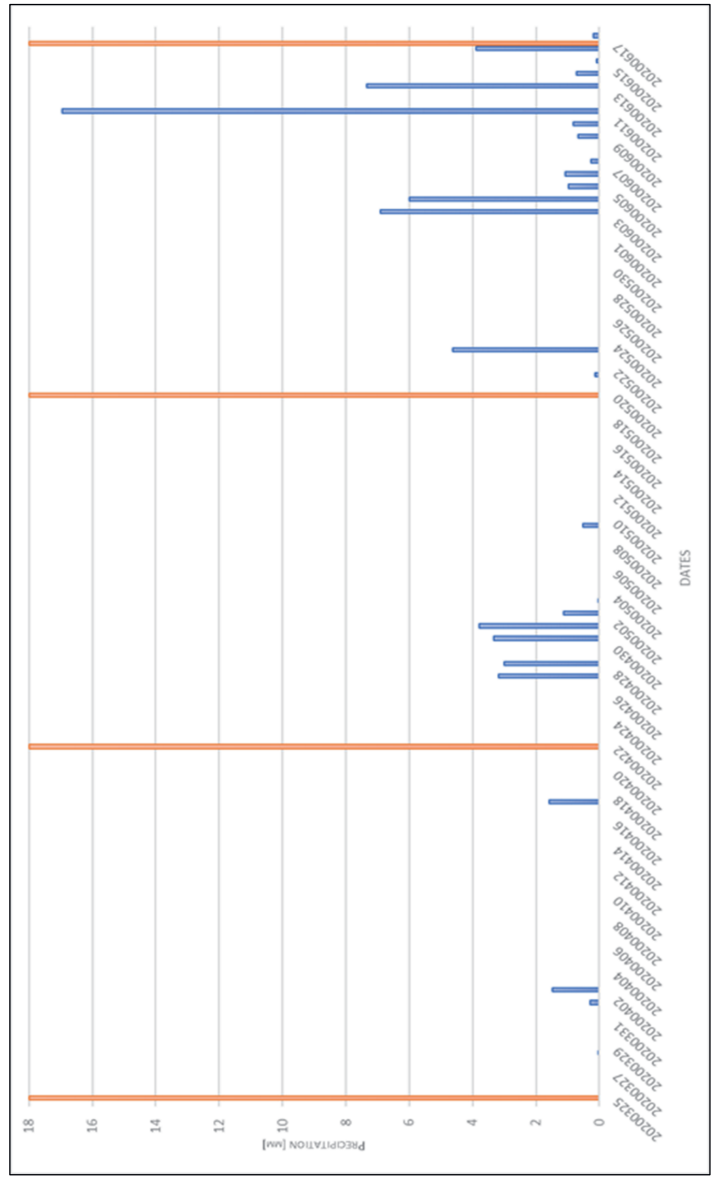


Dynamic MRM

Compoun	Compound Name	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Primary	Trigger	Threshold	Ret Time	Delta Ret	Fragment	Collision	Cell Accel	Polarity	Trigger En	Trigger De	Trigger We	IsLogicEna	Trigger Lo	Trigger Ra	Trigger Ra	Ignore MR
	BAC-C10	FALSE	277	Unit	92.2	Unit	TRUE	FALSE	0	11	2	96	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C10	FALSE	277	Unit	91.1	Unit	TRUE	FALSE	0	11	2	96	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C10	FALSE	277	Unit	58.2	Unit	TRUE	FALSE	0	11	2	96	32	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C12	FALSE	305	Unit	213.2	Unit	TRUE	FALSE	0	11.4	2	106	24	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C12	FALSE	305	Unit	92.2	Unit	TRUE	FALSE	0	11.4	2	106	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C12	FALSE	305	Unit	91.1	Unit	TRUE	FALSE	0	11.4	2	106	40	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C12	FALSE	305	Unit	58.2	Unit	TRUE	FALSE	0	11.4	2	106	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14	FALSE	333	Unit	241.2	Unit	TRUE	FALSE	0	11.9	2	116	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14	FALSE	333	Unit	92.2	Unit	TRUE	FALSE	0	11.9	2	116	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14	FALSE	333	Unit	91.1	Unit	TRUE	FALSE	0	11.9	2	116	40	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14	FALSE	333	Unit	58.2	Unit	TRUE	FALSE	0	11.9	2	116	40	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14-d7	TRUE	340	Unit	241.2	Unit	TRUE	FALSE	0	11.9	2	106	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14-d7	TRUE	340	Unit	99.2	Unit	TRUE	FALSE	0	11.9	2	106	40	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14-d7	TRUE	340	Unit	98.2	Unit	TRUE	FALSE	0	11.9	2	106	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C14-d7	TRUE	340	Unit	58.2	Unit	TRUE	FALSE	0	11.9	2	106	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C16	FALSE	361	Unit	269.3	Unit	TRUE	FALSE	0	12.4	2	126	28	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C16	FALSE	361	Unit	92.2	Unit	TRUE	FALSE	0	12.4	2	126	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C16	FALSE	361	Unit	91.1	Unit	TRUE	FALSE	0	12.4	2	126	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C16	FALSE	361	Unit	58.2	Unit	TRUE	FALSE	0	12.4	2	126	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C18	FALSE	389	Unit	212	Unit	TRUE	FALSE	0	13	2	144	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C18	FALSE	389	Unit	92.1	Unit	TRUE	FALSE	0	13	2	144	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C18	FALSE	389	Unit	91.1	Unit	TRUE	FALSE	0	13	2	144	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C18	FALSE	389	Unit	58.2	Unit	TRUE	FALSE	0	13	2	144	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C8	FALSE	249	Unit	156.8	Unit	TRUE	FALSE	0	10	2	106	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C8	FALSE	249	Unit	92.1	Unit	TRUE	FALSE	0	10	2	106	32	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C8	FALSE	249	Unit	91.1	Unit	TRUE	FALSE	0	10	2	106	32	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C8	FALSE	249	Unit	58.2	Unit	TRUE	FALSE	0	10	2	106	32	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10	FALSE	327	Unit	58.2	Unit	TRUE	FALSE	0	11.9	2	134	52	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10	FALSE	327	Unit	57.2	Unit	TRUE	FALSE	0	11.9	2	134	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10	FALSE	327	Unit	46.2	Unit	TRUE	FALSE	0	11.9	2	134	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10	FALSE	327	Unit	43.2	Unit	TRUE	FALSE	0	11.9	2	134	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10-d6	TRUE	333	Unit	193.2	Unit	TRUE	FALSE	0	11.9	2	144	32	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10-d6	TRUE	333	Unit	57.2	Unit	TRUE	FALSE	0	11.9	2	144	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10-d6	TRUE	333	Unit	52.2	Unit	TRUE	FALSE	0	11.9	2	144	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C10-d6	TRUE	333	Unit	43.2	Unit	TRUE	FALSE	0	11.9	2	144	56	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C12	FALSE	383	Unit	58.2	Unit	TRUE	FALSE	0	12.6	2	154	56	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C12	FALSE	383	Unit	57.2	Unit	TRUE	FALSE	0	12.6	2	154	52	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C12	FALSE	383	Unit	46.2	Unit	TRUE	FALSE	0	12.6	2	154	56	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C12	FALSE	383	Unit	43.2	Unit	TRUE	FALSE	0	12.6	2	154	60	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C8	FALSE	271	Unit	58.2	Unit	TRUE	FALSE	0	10.9	2	146	40	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C8	FALSE	271	Unit	57.2	Unit	TRUE	FALSE	0	10.9	2	146	36	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C8	FALSE	271	Unit	46.2	Unit	TRUE	FALSE	0	10.9	2	146	44	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	DCAC-C8	FALSE	271	Unit	43.2	Unit	TRUE	FALSE	0	10.9	2	146	48	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE
	BAC-C10	FALSE	277	Unit	184.8	Unit	TRUE	FALSE	0	11	2	96	20	1	Positive	0	0	0.5	FALSE	AND	1	1	FALSE

SM5. Daily precipitation

This graph depicts the precipitation trends on a daily basis within the specified region during the sampling duration. Blue bars – rainfall intensity, orange bars – days when the passive samplers were installed, changed, or collected.



SM6. Internal standards used for extraction procedure

- d6-didecyldimethylammonium iodide (HPC Standards; 674541)
- d7-benzyldimethyltetradecylammonium chloride (HPC Standards; 674611)
- benzotriazole-d4 (Sigma-Aldrich; 32566)
- erythromycin-13C2 (CIL; CLM-3672-MT-S)
- flubendazole-d3 (Sigma-Aldrich; 32839)
- naproxen-13C -d3 (CIL; CLM-7665-S)
- paracetamol-13C2 (CIL; CLM-3726-S)
- sulfadiazine-13C6 (Sigma-Aldrich; 32518)
- sulfamethoxazole-13C6 (CIL; CLM-6944-S)
- alfa-ethynylestradiol-13C2 (CIL; CLM-3375-S)
- estradiol-13C2 (CIL; CLM-803-S)
- estrone-13C2 (CIL; CLM-673-S)

Chapter 6

Synthesis

6.1 General discussion

Veterinary pharmaceuticals (VPs) are used worldwide to treat diseases and to protect the health of animals. The primary pathway for their entry into the environment is via the application of (slurry) manure to agricultural land. Once VPs enter the terrestrial environment, their residues may accumulate in the soil, or be further transported towards water bodies. The fate of these compounds in soil and whether they will leach to groundwater depends on the compound persistence and mobility, as well as on the soil structure, soil properties (e.g. fraction of organic matter), groundwater levels, and hydraulic conditions. Similarly, VPs transport towards surface water depends on the compound behavior, but also on the physical processes occurring during the transport, hydrological conditions, the way manure is applied to soil, and the distance to surface water. In general, most of the aforementioned processes and conditions are interdependent, and their simultaneous interactions are incompletely comprehended, while they might significantly impact the presence of VPs residues in the environment.

Numerous studies have confirmed the presence of VPs in various environmental compartments worldwide, including several studies conducted in the Netherlands. There are multiple factors that contribute to the Netherlands being exposed to the risks of appearance of VPs in the environment. Some of the key factors are:

1. Intensive livestock farming: The Netherlands is one of the largest producers of meat and dairy products in the European Union. The high concentration of livestock in intensive farming systems means that large quantities of VPs are used to treat and prevent diseases, resulting in potentially high VPs concentrations in manure.

2. Agricultural activities: Due to the high concentration of livestock, the Netherlands is a significant producer of manure. Additionally, the country is a major agricultural producer, including crops. As a result, the application of produced manure to land as a fertilizer is a common and widespread practice in the Netherlands. In fact, even though being assigned as a vulnerable zone with respect to admissible nitrogen and phosphorus application rates (EU Nitrates Directive 91/676/EEC), the EC has granted a derogation for the Netherlands implying a maximum application rates. Accordingly, significant quantities of VPs residues may enter the environment via manure application.

3. Dutch water system: Due to the vast network of rivers and canals in the Netherlands, the impact of agricultural emissions on water quality can be considerable. In particular, the proximity of agricultural land to waterways means that compounds applied to land, like VPs via manure, might relatively easily enter the water bodies, which makes the Dutch water system vulnerable to these emissions. In response to this issue, the Dutch government has implemented a number of regulations and guidelines to manage agricultural activities (e.g. manure application timing), but has also put some efforts into water quality monitoring in order to ensure the safety of (drinking) water and protect aquatic ecosystems. Nonetheless, the monitoring often bypasses minor waterways that are not utilized as a primary drinking water source, despite the fact that these streams may be directly susceptible to VPs. In addition, these streams can serve as important habitats for many aquatic species, and exposure to VPs or other contaminants can disrupt the delicate balance of these ecosystems and cause significant harm.

To better understand the fate and transport of VPs residues, researchers have developed a range of modelling approaches that can help to predict their behavior in different environmental compartments. The Chapter 1 of this thesis highlights some of the commonly implemented global modelling approaches, as well as those specifically tailored to the Netherlands. Most of the latter are based on the pesticide-targeted models, hence the origin of VPs and the impact of spatially variable conditions on their environmental pathways remain highly uncertain across all approaches. Additionally, the majority of previous modelling efforts are limited to a single environmental compartment and a small number of compounds, while in the Netherlands alone, there are nearly 2700 registered VPs products, with approximately 900 active substances. As it is impractical to monitor the environmental fate of every VP, there is a need for an integrated, yet adaptable, approach for both compounds and environmental conditions. Furthermore, there is a need for tools that can aid relevant authorities in rapidly screening and prioritizing compounds with the available information. By using effective screening tools, decision-makers can identify and prioritize the most urgent pollutants for further investigation or regulatory action, thus improving the efficiency and effectiveness of soil/water quality management efforts.

This thesis takes into account the various factors that influence the occurrence of VPs in the Dutch environment, with a specific focus on developing an automated integrative approach in the form of a model (see Figure 1.2, Chapter 1). The model illustrates the sequence of processes starting from the usage of VPs, then moving through excretion, manure storage, manure application to land, emissions to groundwater and surface water, and finally, detection and quantification of VPs in surface water.

Four general objectives of this thesis were derived to appropriately link all of the aforementioned sequences. These objectives were addressed in four separate chapters, and are discussed in the introduction (Chapter 1). The principal results concerning those objectives are outlined in the ensuing section.

6.2 Major findings

6.2.1 Objective 1: Understand and quantify the chain of processes that lead to VPs concentrations in soil-applied manure – addressed in the Chapter 2.

The processes that result in the concentrations of VPs in soil-applied manure are complex and influenced by various factors. These factors include the type and dosage of VPs administered to the animal, the metabolic processes involved in excretion, and the management practices employed on the farm. In this thesis, Chapter 2 examines the aforementioned processes by focusing on Dutch conditions. Firstly, it identifies the VPs of potential concern within the four most relevant livestock sectors in the Netherlands: dairy cow, veal calf, fattening pig, and sow. Secondly, it estimates the administered quantities of the identified VPs by analyzing data from around 400 farms. In addition, it employs information on the amount of produced manure and excretion rates to estimate the concentration of VPs in manure just prior to storage. Moreover, this chapter describes the approach to model the manure storage and its impact on the VPs concentrations in manure. The outcomes of the storage model are used to develop an indicator that quantifies the residue potential of VPs in soil-applied manure at a national level.

The primary outcomes of the Chapter 2 are:

- The quantities of VPs administered to animals between 2015 and 2018 have shown a reduction for nearly all the examined VPs.

- Differences in the usage and excretion of VPs have resulted in varying emissions across livestock sectors.

- Furthermore, the residues of VPs can be affected by manure production and storage practices.

- VPs that are most commonly used may not necessarily be the ones that end up with high concentrations on the soil.

- Comparison of the predicted concentrations of VPs in manure and the residue indicator prioritization with literature data reveals good agreement.

6.2.2 Objective 2: Generate a modelling approach to identify groundwater vulnerability from VPs at different spatial scales (local to national) – addressed in the Chapter 3.

In Chapter 3 of this thesis, an analytical framework for estimating the leaching potential of VPs on a national scale is presented. The framework takes into account soil-applied VPs concentrations, soil-hydraulic and soil-chemical properties, groundwater levels, and sorption and degradation of VPs. The approach is used to analyze six commonly soil-applied VPs in the Netherlands, as identified in Chapter 2, resulting in a spatially distributed estimation of VPs leaching to groundwater. This approach is probably the first of its kind to integrate the abovementioned input data at the local scale, resulting in an efficient national-scale model for estimating VPs leaching to groundwater.

The primary outcomes of the Chapter 3 are:

- Various processes impact the spatial distribution of VPs leaching to groundwater, and their relative significance varies between different VPs.

- The importance of soil-applied VPs quantities for leaching to groundwater is determined by the environmental properties of the substance.

- Oxytetracycline, Doxycycline, and Ivermectin exhibit low leaching potential to groundwater in the Netherlands.

- Sulfadiazine and Flubendazole show a leaching potential that is limited and varies based on location. Future groundwater monitoring could focus on prioritized locations when targeting the VPs that share similar environmental properties as Sulfadiazine and Flubendazole.

- Dexamethasone is given priority for environmental risk assessment due to its high leaching potential.

6.2.3 Objective 3: Build a VPs transport model suitable for lowland catchments – addressed in the Chapter 4.

Lowland catchments are typically characterized by a network of slow-moving water bodies, as well as by extensive agricultural land use. In these catchments, the balance between precipitation, evaporation, runoff, and subsurface water storage is complex and influenced by a variety of factors, including soil properties, vegetation cover, land use practices, and climate variability. The transport of soil-applied VPs in lowland catchments can be triggered by e.g. surface runoff, which can occur when rainfall intensity exceeds the soil's infiltration capacity. This can result in a high volume of water and VPs being transported rapidly overland to nearby surface waters. Additionally, VPs have the potential to be transported from fields to surface water via macropore flow through drainpipes or soil cracks, as well as by infiltrating the vadose zone and reaching phreatic groundwater, which can then drain further into surface water.

In Chapter 4 of this thesis, the temporal dynamics of VPs in lowland surface waters of an agricultural catchment in the Netherlands are investigated. An existing rainfall-runoff model is utilized to differentiate between quick flow transport routes and slower routes through the vadose zone and groundwater zone. Predicted water fractions are then combined with data on VPs concentrations in manure (from Chapter 2) and local manure application patterns. The biogeochemical processes of VPs are simplified into a decay parameter based on an exponential degradation model.

The primary outcomes of the Chapter 4 are:

- The used hydrological model and associated parameters display a noteworthy level of accuracy in forecasting the catchment discharge patterns over an extended period, as demonstrated by the comparison with observed flow data.

- The relative contribution of quick flow routes to discharge varies over time, and this variability can significantly impact the transport patterns of VPs.

- The temporal distribution of VPs occurrence in a stream is significantly impacted by the timing of manure application.

- The application of the reactive VPs transport model in this chapter did not yield a satisfactory level of accuracy in predicting VPs concentrations in streams.

Regarding the last mentioned finding, the validation of the predicted VPs concentrations against the surface water measurements from Chapter 5 was unsuccessful, indicating that the original objective 3 was not fully achieved. The underlying reason is that either the first-order decay approach does not adequately capture the complexity of reactive and sorption processes, or direct comparison of the simulated and measured VP quantities may not be appropriate. Therefore, the objective was slightly modified to focus on explaining the dominant transport routes and the influence of manure application, as well as identifying ways to improve the model, as discussed in Chapter 4.

6.2.4 Objective 4: Quantify the presence of compounds associated with livestock husbandry in surface waters via sampling – addressed in the Chapter 5.

The experimental component of this thesis is presented in Chapter 5, which outlines a field campaign conducted in 2020. The aim of the campaign was to assess the occurrence of VPs in a specific agricultural area of the Netherlands through the utilization of passive samplers. Eight sampling locations were selected, and each location was equipped with two pairs of samplers. One pair of samplers was left in place for a period of three months, while the second pair was replaced every four weeks. The sampling campaign targeted 46 compounds, including 25 antibiotics, three hormones, nine antiparasitics, and nine disinfectants.

The primary outcomes of the Chapter 5 are:

- The passive samplers accumulated 22 distinct compounds in quantities above the limit of quantification. Out of these, 13 compounds were detected in all sampling locations, and 8 were found during all sampling intervals as well.

- The results of the sampling campaign clearly demonstrate the advantages of passive sampling over grab sampling, as evidenced by the greater number of detected compounds.

- Quantification of several compounds in Dutch surface waters is reported for the first time.

- This chapter provides a preliminary foundation for developing a targeted monitoring program for these types of compounds and may also assist in identifying potential mitigation measures to decrease chemical emissions from animal husbandry.

6.3 Implications and recommendations

The main focus of this thesis is to investigate the fate of VPs in the environment by dividing the processes affecting individual environmental compartments into dependent partitions (chapters) and analyzing them separately. However, in the course of these analyses, a number of assumptions are made that may adversely affect the results. This section discusses the most significant assumptions and provides recommendations on how to improve these uncertainties in future research.

6.3.1 Uncertainty in input data on VPs usage

The VPs usage requires additional attention as it serves as a first input for the model train developed in this thesis. As discussed in Chapter 2, there is a lack of publicly disclosed data on individual VP usage in the Netherlands. Dutch reports typically only provide information on the annual usage of veterinary antibiotic groups in different livestock sectors. This thesis makes an important contribution to current knowledge by reporting on individual VP usage for selected compounds, not limited to antibiotics. The usage is estimated based on a dataset collected directly from farms, and the author(s) of this thesis are aware of the locations of these farms, although they are not disclosed or used further in the thesis due to privacy reasons. Therefore, the calculations performed in all other chapters (except Chapter 5) rely on nationally averaged yearly administered VP quantities, even though individual farms may administer VPs in quantities that deviate from these averages. It is worth highlighting that this thesis utilizes spatially variable data, including information on manure application, soil properties, groundwater levels, and hydrology. Therefore, incorporating spatially distributed VPs usage data could significantly enhance the accuracy of the model train. Thus, it is highly recommended that future research efforts combine the existing information in this thesis with spatially distributed VPs usage data. However, the author(s) of this thesis acknowledge that privacy issues make this a challenging task, especially given the sensitivity of anything related to manure application in the current Dutch nitrogen crisis.

6.3.2 Uncertainty in model parameters

This thesis involves the development of multiple models, including those for predicting VPs concentrations in manure, fractions leached to groundwater, and transport from fields to surface water, utilizing over 25 different parameters. Many of these parameters are obtained from literature, estimated based on available information, or calibrated, leading to some level of uncertainty. Determining which parameters and underlying assumptions could significantly affect the results if

altered is challenging and depends on the range of variations considered. Rather than discussing each parameter individually, which is already covered in relevant chapters, we will only highlight those with a potential significant impact on the overall modelling results.

- Notably, little is known about VPs excretion rates, particularly for compounds other than antibiotics, and variations between animal types. Most of the existing knowledge on VPs excretion in specific animals dates back over 20 years, resulting in many (new) VPs being left unconsidered. The excretion rates of VPs have a direct impact on the fraction of VPs that end up in manure, and consequently, they strongly influence the availability of VPs residues for further transport processes. Therefore, their accurate estimation is crucial for predicting VPs concentrations in manure and their subsequent transport to groundwater and surface water. However, the author(s) of this thesis recognize that determining standard excretion rates is challenging due to the complexity of metabolic systems in animals and variations within each livestock sector (e.g., age, weight, health status).

- There is a common agreement among researchers that the behavior of VPs in soil can greatly vary. It is possible for some VPs to be retained in the upper soil layer because of their low mobility and strong sorption to soil particles, while others may move down into deeper soil layers and potentially contaminate groundwater. However, there is significant variability across the literature in the parameters characterizing the behavior of VPs, such as degradation and sorption, even for the same VPs and soil types. In developing our groundwater leaching model, we selected VPs to model primarily based on studies that experimentally determined these parameters for the Dutch tailored situation. This has two general consequences: firstly, the limited number of compounds that can be modeled due to the focus of experiments on a restricted set of compounds; and secondly, the use of highly uncertain input when no data are available for the specific environmental conditions. Similar effects of parameters related to VPs behavior may also arise when modeling the transport of VPs from fields to surface water.

- The behavior of VPs in the mixture of soil and manure could potentially impact our understanding of the transport processes in the system. However, due to the scarcity of studies in this area and the considerable variability of data in the literature concerning both manure and soil, investigating the behavior of VPs in this mixture was considered beyond the scope of this thesis. Nonetheless, this aspect could alter our approach to the problem of VPs application to soil, particularly with different manure application techniques such as spreading, injection, or incorporation. The addition of (rainfall) water as a third component to

this system could further complicate the situation. Therefore, acquiring knowledge in this area would be highly valuable.

6.3.3 Dexamethasone in the Dutch Environment

Considering that Dexamethasone was identified as potentially widespread and environmentally relevant in both manure and groundwater within the context of this thesis, it is crucial to recognize that this could be due to some rough assumptions made regarding its behavior. Dexamethasone belongs to the class of synthetic corticosteroid hormones and is utilized for treating various animal conditions (e.g. inflammation). Its administration can occur in different ways, while the appropriate dosage and treatment duration vary based on factors like the animal's size, condition, and response to the medication. According to the usage data inventory presented in Chapter 2, Dexamethasone is likely used on over 50% of farms in the Netherlands, with variation depending on the livestock sector. Despite its lower estimated administered quantities compared to other investigated VPs, its assumed behavior (i.e. persistence) in manure storage and soil justifies its prioritization for environmental risk assessment, as discussed in Chapters 2 and 3 of this thesis. However, it is worth noting that our approximations of Dexamethasone's behavior in manure storage and soil may be subject to significant uncertainties due to the scarcity of available literature data. Moreover, Dexamethasone may not have been a focus of environmental monitoring due to its low concentrations that fall below the detection limits, as observed in our surface water measurements presented in Chapter 5. Given its frequent use in the Netherlands and incomplete understanding of its environmental behavior and impacts, further research is needed to investigate Dexamethasone more closely.

6.4 Concluding remarks

The work of Dutch scientists over the past two decades has yielded valuable knowledge and data regarding the risks and occurrence of VPs in the Dutch environment. Building on this foundation, we have developed tools and models to predict the environmental fate of these compounds to some extent. While not yet fully validated for all environmental compartments, the indicators and models presented in this thesis are suitable for rapid screening and prioritization of VPs, requiring less information and accounting for calculation processing efficiency. As a result, the insights gained from this thesis can provide valuable inputs for the Dutch scientific community and policymakers. However, it is clear that addressing this issue requires a collaborative effort and action from various stakeholders, including regulatory authorities, producers, veterinarians, farmers, and scientists.

The conceptual framework and modelling approach presented in this thesis can have broader applicability beyond the Dutch context. It could be used as a reference for other countries or regions that are facing similar environmental concerns with VPs residues. Although some modifications may be needed to adapt the modelling parameters to the local conditions, the basic approach could be applied universally. The models developed in this research are flexible and can be easily updated as new knowledge becomes available, ensuring their continued relevance and usefulness. Ultimately, this work highlights the importance of continued research in this area to further our understanding of the behavior and impacts of VPs in the environment, and to develop effective strategies for their management and control.

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Summary

The Netherlands is characterized by intensive livestock farming and a high manure production. The manure is typically applied to soil in a slurry form, and in the case of calves and pigs more than 70% of it is applied onto agricultural land untreated. Accordingly, Netherlands is one of the highest nitrogen input regions in Europe. As a result of combined efforts of the Dutch authorities, the livestock sectors and the veterinarians, the overall sales and usage of veterinary pharmaceuticals (VPs) decreased over the past decade. However, these trends are not equally distributed across the farms and livestock sectors, and could differ substantially. Moreover, nearly 2700 VPs products (around 900 active substances) are registered in the Netherlands. These can be administered to animals in many different ways (e.g. injected, applied to skin), while after administration their residues may end up in the environment via manure. Upon the eventual emission to the soil surface with manure application, VPs may be further transported in the soil-water system, where the major transport pathways are the leaching towards groundwater and (overland) flow into surface water. Some earlier scientific and monitoring studies affirm the occurrence of these compounds in different environmental compartments in the Netherlands but those findings are still relatively scarce and unevenly distributed across the country. In addition, information concerning the origin of compounds and impacts of spatially variable conditions on the environmental pathways is largely unknown. It is thus imperative that methods to better understand such issues are of current interest. Therefore, this thesis investigates the chain of processes that lead to quantification of VPs residues in manure, soil-groundwater, and surface water in the Netherlands.

Chapter 2 of this thesis analyzes the use of VPs in the four most relevant Dutch livestock sectors, and based on substance properties, excretion rates, and manure storage, estimates the VPs concentrations in soil-applied manure. This investigation focuses on 17 VPs of potential concern in the Netherlands for the period 2015-2018. To estimate the administered VPs quantities, raw use data are obtained from the Dutch Farm Accountancy Data Network and processed. The results suggest that the quantities administered to the animals during the period 2015–2018 have decreased for almost all investigated VPs. Furthermore, this chapter specifically illustrates an approach to model the manure storage and its effect on the VPs concentrations in manure. Based on the storage model outcomes, an indicator that quantifies the VPs residue potential in soil-applied manure at a national level has been developed. The comparison with literature data shows a good agreement both for the predicted VPs concentrations in manure and the residue indicator prioritization.

Currently, besides pesticide-targeted models, a detailed model that characterizes the VPs leaching to groundwater in the Netherlands, and that pays heed to spatio-temporal heterogeneity is not available in the literature yet. To fill this gap, Chapter 3 combines data on soil-applied VPs, manure quantities, soil-hydraulic and soil-chemical properties, groundwater levels, and sorption and degradation of compounds to provide spatially distributed quantification of VPs leaching to groundwater at the spatial resolution of the field. This model is applied at a national scale to the Netherlands, and according to our knowledge, it is the first VP-targeted approach to combine the abovementioned input data at local scales. In total, more than 1 million fields are analyzed and results show that some of the most frequently soil-applied VPs have a very low leaching potential, hence not posing a threat for the groundwater chemical quality. On the other hand, the results also suggest VPs for which a location specific or widespread leaching is possible. These VPs require a closer examination when performing an environmental risk assessment of VPs. The results further indicate that the spatial patterns of VPs leaching to groundwater are affected by many environmental conditions and processes, and that the relative importance of those processes differs between the VPs. This implies that for assessing the factors with the highest impact on VPs leaching, a substance individual approach is recommended. In general, this chapter can be used as a guidance to identify groundwater vulnerability to VPs at different spatial scales, thereby providing an important asset in environmental risk assessment of VPs.

Chapter 4 investigates the transport of VPs from agricultural fields to and along the stream network. This chapter illustrates a modelling approach to determine the temporal dynamics of VPs in lowland surface waters. The approach uses an existing rainfall-runoff model (WALRUS) and the data on soil-applied VPs (including concentrations in manure and manure application patterns) to build a VP transport model suitable for lowland catchments. In principle, the output of a hydrologic model is utilized to input into the VP transport model. The transport model considers all the reaction processes experienced by the VPs during their transfer to the stream network through a basic first-order reaction. The results suggest that the hydrologic model performs well in simulating the catchment discharge patterns, but the VP transport model has inadequate fit to the surface water measurements. As the chapter specifically addresses VPs residence times in surface waters, it helps to understand the dependency of water quality on agricultural practices, and it allows to identify suspected risks of relevance for policy improvement.

Chapter 5 represents the experimental part of this thesis, and describes a field campaign done in 2020, when the surface water sampling was done with passive samplers at eight locations in a typical agricultural region in the Netherlands. The sampling strategy was based on the deployment of two pairs of samplers at each location, where one pair was kept for three months, and a second pair was

changed every four weeks. This allowed for assessing the influence of manure applications patterns and some hydrological aspects (e.g. rainfall). In total, 46 compounds were targeted, among which 25 antibiotics, three hormones, nine antiparasitics, and nine disinfectants. 22 compounds accumulated in the passive samplers in quantities above the limit of quantification in at least one sampling location. Particularly, 13 compounds were detected at all sampling locations, while 8 of them were found also during all sampling intervals. The conducted sampling campaign indicated a clear advantage of passive sampling over the grab sampling, expressed via the number of detected compounds. Moreover, due to lower detection limits compared to the grab sampling, a number of compounds originating from animal husbandry activities were quantified for the first time in Dutch surface waters. Therefore, this chapter may be used as a first step in developing a targeted monitoring program for this type of compounds, and might also help in defining mitigation measures to reduce the emissions of chemicals from animal husbandry.

Altogether, the results of the work presented in this thesis suggest that in agricultural regions with intensive livestock activities the emissions of VPs to the environment are almost inevitable. However, this thesis also shows that the most commonly administered VPs are frequently not the ones ending up in the environment in high concentrations. Furthermore, emissions of VPs show to vary per livestock sector and although some generalization on their environmental pathways is possible, a substance individual approach is probably the best option to assess their fate. The indicators and models provided in this thesis are suitable for fast screening and prioritization of compounds as they typically require less information and take into account the time processing efficiency of the calculations. Considering the number of used VPs in the Netherlands, the mentioned tools and results may also help policy makers to identify both relevant compounds and hotspot regions, hence providing an important knowledge for a proper risk assessment of VPs. Still, it is crucial to establish better monitoring program for VPs residues in all environmental compartments. This would instantly provide valuable information for responsible authorities, while also supplying useful data for the respective models.

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Nikola Rakonjac was born on January 14, 1993, in Serbia. After completing his BSc degree in Civil Engineering at Belgrade University in 2016, he made the decision to venture beyond Serbia and pursue his master's studies in Civil Engineering for Risk Mitigation at Politecnico di Milano in Italy. He was awarded a scholarship for outstanding international students and successfully graduated in 2018. Right after graduation, he embarked on his PhD journey at Wageningen University, where he became part of the Dutch Research Council (NWO) project, focusing on decision support tools for risk-based prioritization and control of contaminants of emerging concern. During the final year of his PhD, he also took on the role of a visiting PhD student at EPFL in Switzerland. Throughout his PhD research, he actively participated in numerous conferences, mentored students, and contributed to lectures. He holds memberships in various international associations and task forces focused on water quality. Currently, Nikola serves as a post-doctoral researcher at Soil Physics and Land Management Group at Wageningen University.

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Publications

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Netherlands Research School for the
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D I P L O M A

for specialised PhD training

The Netherlands research school for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Nikola Rakonjac

born on the 14th of January in Belgrade, Serbia

has successfully fulfilled all requirements of the
educational PhD programme of SENSE.

Wageningen, 19th of June 2023

Chair of the SENSE board

Prof. dr. Martin Wassen

The SENSE Director

Prof. Philipp Pattberg

The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)

KONINKLIJKE NEDERLANDSE
AKADEMIE VAN WETENSCHAPPEN

The SENSE Research School declares that **Nikola Rakonjac** has successfully fulfilled all requirements of the educational PhD programme of SENSE with a work load of 53.4 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2018)
- Research in context activity: (online) international symposium hosted by the O'Higgins University, Chile (2021)

Other PhD and Advanced MSc Courses

- ORCHESTRA course, Wageningen University (2019)
- Applications in Soil and Water Chemistry, Wageningen University (2019)
- Subsurface Solute Transport, Wageningen University (2019)
- Adapting the expertise acquired from developed countries to address environmental concerns in Serbia, Institute of Forestry (2023)

External training at a foreign research institute

- Attending workshops and courses related to damages and losses caused by natural disasters, Politecnico di Milano (2019-2021)
- Research visit to the École Polytechnique Fédérale de Lausanne, Switzerland (2022)

Selection of Management and Didactic Skills Training

- Member of the Pharmaceutical Interest Group at SETAC (2022 - present)
- Member of the Water Quality Board at IWRA (2022 - present)
- Moderator of two sessions at the UNESCO-IWRA Conference 17-19 January 2023.
- Supervising BSc student with thesis (2020-2021)
- Teaching in the BSc course 'Hydrogeology' (2018-2022) and MSc course 'European Workshop Environmental Sciences and Management' (2019)

(Selection of) Oral Presentations

- *The Advantages of Using Passive Sampling in Monitoring Veterinary Pharmaceuticals.* SETAC North America, 13-17 November 2022, Online
- *Emissions of veterinary pharmaceuticals from livestock breeding: experience from the Netherlands*. 5th International scientific conference 'Village and Agriculture' 30 September – 1 October 2022 Bijeljina, Bosnia and Herzegovina
- *Transport of Veterinary Pharmaceuticals in Lowland Catchments: a Lumped Modelling Approach.* UNESCO-IWRA Conference on 'Emerging Pollutants: Protecting Water Quality for the Health of People and the Environment', 17-19 January 2023, Online
-

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen

Colophon

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