Managing the Release of Emerging Agricultural Contaminants into the Environment

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The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others. This thesis is presented as an alternative thesis which was deemed suitable as two chapters (Chapters 2 and 3) have already been published and Chapters 4 and 5 are to be submitted as soon as possible. Presented below is the status of each chapter and the author contributions.

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Abstract

Recently there has been an increased interest regarding the environmental fate of veterinary medicines, however it is apparent that variability and knowledge gaps exist within the literature. The presented doctoral thesis employed novel, practical, and thorough experimental and analytical techniques to bridge these knowledge gaps but also to contribute towards a more robust and harmonised risk assessment.

Reported veterinary medicine half-lives in animal manures are highly variable, this indicates that current exposure assessments are inaccurate, consequently predicted soil concentrations maybe under or overestimated. Manure properties are highly heterogenic and the current guidance permits the use of just one manure per animal type. Therefore, an experiment was designed to investigate whether commonly reported pig slurry pH's and anaerobic redox potentials are contributing towards variable degradation rates. The results demonstrate these parameters to have a significant and compound specific effect on degradation rates. Therefore, to conduct accurate and realistic environmental fate assessments manure degradation studies should encompass numerous manures with differing properties.

A semi-field experiment which evaluated the transfer of veterinary medicines under varying application techniques was conducted where eight veterinary medicines were identified to be transported to receiving waters. Incorporating slurries into soils and injection were identified to substantially reduce the transport of veterinary medicines over that of broadcast. A year-long field monitoring study was conducted to evaluate the fate of veterinary medicines at the farm scale and validate the modelling suite FOCUS_PEARL. Antibiotics recently utilized at the farm were detected in slurries, soils, and groundwaters. Antibiotics were observed to persist within groundwaters throughout the study, however, there was little compelling evidence to link this to their presence in slurries or soils. Moreover, the modelled predictions were observed to surpass that of the concentrations detected in groundwater.

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Chapter 1

1.0 Literature review

1.1 Introduction

Veterinary medicines are a group of chemical compounds used extensively for the treatment and prevention of bacterial and parasiticidal diseases within livestock. Administration is required for protection and improvement of animal health within animal husbandry. Predominately these consist of antibiotics, coccidiostats, anthelmintic, antifungal, hormones and immunological products (Capleton et al., 2006). In addition, disinfectants are routinely used for the domestic cleaning of rearing/housing facilities; biocides are also used for this purpose but also for veterinary purposes. Due to the increasing global populations coupled with the increasing demand for rich protein diets in both developed and developing countries, livestock farming has evolved, shifting animal husbandry from extensive to intensive (Delgado, 2005; Thronton, 2010; and Wanapat et al., 2015). This has resulted in larger intensive-feeding operations with greater veterinary medicine requirements becoming more common (Keyzer et al., 2005; Spielmeyer, 2018).

1.1.1 Legacy and status of veterinary pharmaceuticals within the Environment

The usage of veterinary medicines within agriculture is not a new phenomenon, and their use results in the release of bioactive chemicals to the environment. Therefore, increasing scientific interest has arisen regarding the fate and effect of veterinary medicines within ecosystems. Improved analytical techniques and sensitivity have made this possible via providing a capable means to quantify concentrations to parts per billion (ppb) and even parts per trillion (ppt) (Ptrović et al., 2003). Contaminants arising from agriculture have been topical for some time, for example Rachel Carson's book Silent Spring which was published in 1962 highlighted the influence of pesticide usage on bird populations, the book specifically targeted DDT (Dichlorodiphenyltrichloroethane) as well as other organochlorines and organophosphates (Carson, 1962). The publication of this book is thought to of brought insight towards environmental issues and processes such as, bioaccumulation, resistances towards pesticides and persistence of these synthetic organic compounds in the environment. This publication is thought to have driven changes within agricultural policy as well as igniting the environmental risk assessments we see today.

Some pharmaceuticals arising from veterinary usage are now considered as emerging contaminants, some of these include, antibiotics, anti-inflammatories, pesticides, cosmetics, care products and analgesics (Kümmerer, 2009; Rivera-Utrilla et al., 2012). There are now >700 emerging contaminants and metabolites, in order to achieve good environmental standards, it is critical to have a comprehensive understanding of their fate and toxicity in the environment (Geiseen et al., 2015; NORMAN, 2016). Of these contaminants antibiotics and hormones are considered to have the greatest risk towards society (Doughton and Ternes, 1999), their environmental presence is well known to result in, irreversible antimicrobial resistance, endocrine disruption, bioaccumulation as well as non-target effects towards terrestrial and aquatic organisms (Aris et al., 2014; Richter et al., 2016; Singh et al., 2019; Zhang et al., 2020). Some of these chemicals are now on the Water Framework Directive watchlist (2000/60/EC), as a result of their toxicological effect, risks, or persistence within the environment. Some of these include, erythromycin, clarithromycin, azithromycin (macrolides), amoxicillin and ciprofloxacin, neonicotinoids, metaflumizone, ethinylestradiol and Methiocarb (European Commission, 2018).

Research has linked the presence of veterinary medicines within the environment to a series of negative ecological effects. For example, the administration of diclofenac to ruminants in Pakistan has resulted in <95% decline in the Oriental White-Blacked Vulture population. Decaying deceased ruminants are often eaten by scavenging birds in these regions, resulting in a bio-accumulative transfer from the animal fats and tissues to the vulture ultimately resulting in visceral grout (Oaks et al., 2014). Meanwhile, the usage of organophosphates as pour on dips for sheep was banned within the EU in 1992; these compounds were reported to have resulted in a number of surface water and pollution events such as deterioration of the salmon populations within the River Tweed and Biggar Water (1978 and 1980) (Hooda et al., 2000; Virtue and Clayton, 1997). Famphur was commonly used to treat warbles on cattle but has now been identified to decrease the *Pica Pica, Buteo Jamaicensis and Haliaeetus* populations via bioaccumulation and biomagnification (Henry et al., 1985; Franson et

al., 1985). These examples highlight the requirement for a thorough environmental risk assessment on synthetic agricultural substances.

This chapter presents a review on the occurrence, fate, and effects of veterinary medicines within the environment with specific attention towards antibiotics, the environmental risk assessment and common agricultural management practices which influence environmental fate.

1.2 Usage excretion and occurrence of veterinary antibiotics

As a consequence of intensification, animals are now being reared in poor housing conditions and veterinary medicines are now routinely administered for protective and therapeutic purposes. Animal manures containing bioactive pharmaceuticals are applied to land as a form of organic fertilizer but also as a means of waste disposal which presents a pathway for veterinary medicines to enter the environment (Thiele-Bruhn 2003; Carvalho and Santos, 2016; Xu et al., 2021).

Veterinary medicines within the environment could be considered somewhat ubiquitous with a range of concentrations being detected (ng/kg to mg/kg) within various environmental matrices such as manures, soils, sediments, surface water and groundwater (Halling-Sorensen et al., 1998; Holten Lutzhoft, 2000; and aus der Beek et al., 2016). The extent of veterinary medicine exposure differs regionally due to differences within animal husbandry, policies and veterinary practices (Ghitadini et al., 2020). The following sections within this chapter are going to discuss current veterinary antibiotic usage, excretion rates and veterinary medicine occurrence within the environment.

1.2.1 Veterinary antibiotic usage

Veterinary medicine usage within agriculture consists of therapeutic, prophylactic and for the promotion of livestock growth (subtherapeutic) (Subbiah et al., 2011). Growth promotion is considered using synthetic or essential metals and minerals to increase the overall body weight of the animal. Antibiotics are known to be used to promote animal growth, in some circumstances body weight can be increased by up to 16.4% (Sarmah et al., 2006; Dibner and Richards. 2004). Certain antibiotics can be used to alter the gastric intestinal micro-flora which reduces nutrient losses during microbial fermentation (Gaskins et al., 2002). Due to the increased risk of antimicrobial resistance the use of antibiotics for sub therapeutic purposes was banned in 2006 throughout all

EU member states under directive 70/524/EEC, and regulation 1831/2003/EC (EC, 2003).

Within the UK 262 tonnes of antibiotics were sold in 2020 for veterinary purposes, this equates to 37 mg/kg of active ingredient used per use in food-producing animal species (Veterinary Medicines Directorate, 2020). As presented by Arikan et al., (2009), antibiotic use was much greater in 1999 prior to the ban on sub therapeutic usage, there was a total of 5000 tons of antibiotics sold, of which 70% was for sub-therapeutic purposes (Fig.1). The European Medicine Agency has implemented a plan known as the 'One Health Action Plan', which was implemented to recognize and harmonise human health, animal health and the environment with regards to antibiotic usage. This is in order to reduce the risk of antimicrobial resistance which would render veterinary antibiotics ineffective (European Commission, 2017). Moreover, the RONAFA opinion was established in 2017, this specifically focussed on animal husbandry within the EU with regards to the usage of antimicrobials (EMA, 2017).

Whilst antibiotic usage within the UK and some of the EU member states is decreasing (Fig.1), the importing of meat is becoming more frequent especially within the UK (Poppy et al., 2019). This presents a concern as imports may originate from regions with fewer policies controlling the use of antimicrobials in animal husbandry. Whilst the current status in the UK is improving this is not the case for USA, Korea, China, Africa, Brazil, Russia, India, and New Zealand, where sub-therapeutic antibiotic use still occurs. For example, global usage is expected to exhibit a 67% increase in sales from 2010 to 2030 (Van Boeckel et al., 2015).

As a result of poor housing standards in swine and broiler facilities, 52% of antibiotic sales in 2018 sales were in these sectors (Kim et al., 2011; Lhermie et al., 2018). Moreover, administration and usage are driven by the compound's pharma-kinetics, target disease/infection (interspecies complexity), animal product (i.e., dairy requires antibiotics that do not bioaccumulate or transfer into milk easily), and growth stage requirements (gestation and farrowing periods in pigs) (De Briyne et al., 2014; Filippitzi et al., 2014; Carlvalho and Santas, 2016; Lekagul et al., 2019). For example,

pigs have high application rates of sulphonamide which are often used synergistically with trimethoprim (16%) in their early growth stages, whereas this ranges between 7-10% for poultry and cows (UK VARSS, 2017).

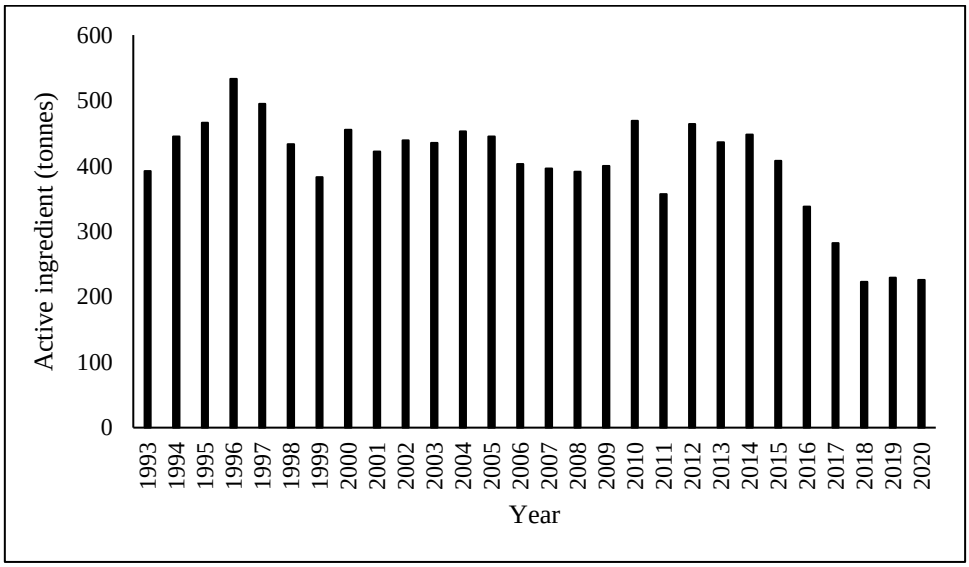

Figure 1.0: Antibiotic sales within the UK from 1993-2020, data from 1993 to 2004 was obtained from ESVAC, whilst data from 2004-2017 and 2017 to 2020 was taken from VARS (UK VARSS, 2017; and UK-VARSS 2021).

1.2.2 Veterinary medicine excretion rates

Veterinary medicines are poorly absorbed within the gastrointestinal tract of the animal which often leads to large percentages of the active being excreted. The extent of which is typically governed by the compound's pharmacokinetics and pharmacodynamic processes (Table 1.0) (Thiele-bruhn, 2003; Sukul et al., 2009). Pharmaceuticals which are low in polarity or hydrophobic are generally found to have higher excretion rates. For example, enrofloxacin and lincomycin have differing octanol-water coefficient values (Log K_{ow} measure of hydrophobicity) of 4.7 and 0.56 respectively and their excretion rates are 11.65% and 32% (Zhou et al., 2008; Kutcha and Cesna, 2009) (Table.1). Moreover, tetracyclines are highly polar and all exhibit low excretion rates whereas erythromycin, trimethoprim and metronidazole contain soluble lipids which results in higher excretion rates (Kumar et al., 2005). Other properties such as solubility

govern pharmacokinetic processes, for example most antibiotics are expected to be deposited via urine due to their high aqueous solubility (Halling-Sørensen, 2001).

Within the gastrointestinal tract both phase I and II metabolites have the potential to form due to metabolism processes. For example, the administration of difloxacin and sulfadiazine have been identified to transform into sarfloxacin, IS-$^{13}C^{15}N$-difloxacin, N-acetylsulfadiazine and 4-hydroxy-SAZ via metabolism (Lamshöft et al., 2010). The metabolic transformation of parent compounds is often excluded within laboratory studies via dosing directly into animal manures, it could be argued that this is unrealistic of environmental processes as it overlooks the ability for enzymatic and oxidative processes forming Phase I metabolites as well as conjugate formation in phase II (Sukul et al., 2009). Therefore, the environmental fate and toxicity of such metabolites is often overlooked or unknown, for example acetylated, glucuronide conjugation, and aromatic hydroxylation metabolites (N^1 and N^4) are a common but work seldom explores their toxicity and fate (García-Galán et al., 2012). Metabolism studies are generally infrequent within the risk assessment due to their efficacy and requirement, but when they are conducted should metabolite equate to 5-10% of the administered dose it can be subtracted from the total excreted (Slana and Dolenc, 2013).

Table 1.0: Collated literature regarding veterinary medicine excretion rates in differing animals.

Compound	Animal species	Percentage excreted	Reference
Chlortetracycline	Bull	75%	Elmund et al., (1971)
Monensin	Deer	75%	Herberg et al., (1978)
Enrofloxacin	Pigs	7.9% (faeces) and 3.75% (urine)	Zhou et al., (2008)
Ivermectin	Rat, sheep and cattle	1.51-62%	Chiu et al., (1990)

Oxytetracycline	Young bull	17-75%	Montforts, (1999)
	Sheep	21%	
	Pigs	75% (urine)	Xia et al., (1983)
	Pigs	72%	Winckler and Grafe, (2001)
Tetracycline	Cattle	80-90%	Kühne et al., (2000)
	Pigs	42-72%	Wincler and Grafe, (2001)
Tylosin	Pigs	20-35%	Feinman and Matheson, (1978)
	Sheep	11%	Ishikawa et al,. (2018)
Streptomycin	Weanling pigs	3%	Kutch and Cessna (2009)
Virginiamycin	Pigs	31%	Feinman and Matheson, (1978)
Sulphamethoxine		15%	Hirsch et al., (1999)
Sulfachloropyridazine	Pigs	62%	Qiu et al., (2016)
Sulfadimoxine	Pigs	38%	Qiu et al., (2016)
Sulfamerazine	Pigs	43%	Qiu et al., (2016)
Sulfaquinoxaline	Pigs	79%	Qiu et al., (2016)
Sulfadiazine	Pigs	95% (44% as parent)	Lamshöft et al., (2007)
Amoxicillin	Layer hen	55.82-67.88%	Peng et al., (2016)
Lincomycin	Weanling pigs	32%	Kutchta and Cessna (2009)
Ciprofloxacin	Layer hen	44.87-51.85%	Peng et al., (2016)
	Pigs	22.51%	Zhou et al., (2008)
Doxycline	Layer hen	82.67-75.72%	Peng et al., (2016)

1.2.3 The occurrence of veterinary medicines in animal manures

As a result of high excretion rates veterinary medicines are frequently detected in manures with concentrations ranging from ng/kg to mg/kg (Table 1.1). Martínez-

Carballo et al., (2007) reported chlortetracycline concentrations as high as 46 mg/kg in liquid slurries. Moreover, similar detections of enrofloxacin and sulfadimidine have been reported (Höper et al., 2002; Christian et al., 2005). As a result of differences in veterinary practices concentrations generally differ regionally, for example in China the average oxytetracycline and ciprofloxacin concentrations that were detected in eight provinces were 59.06 mg/kg and 33.98 mg/kg (Zhao et al., 2010). Whilst Berendsen et al., (2015) conducted a similar survey on 20 pig farms in Germany and reported a median concentration of oxytetracycline to be 21 µg/kg. Other reasons for differences in the reported concentrations of veterinary medicines within manures include, differences in sampling times, storage mechanisms (compost or slurry lagoon), and factors effecting chemical degradation (temperature, moisture, and microbial diversity) (Wohde et al., 2016).

Table 1.1: The occurrence and concentration of veterinary medicines in animal manures or slurries.

Veterinary medicine	Location	Animal-farm	Detection	Reference
Chlortetracycline			2.7 mg/kg	Kummerer 2008
	Australia	Liquid manure	46 mg/kg	Martínez-Carballo et al., (2007)
Enrofloxacin	Germany	Pig	8.3 mg/kg	Sattelberger et al., (2005)
Enrofloxacin	Germany	Poultry	0.040 mg/kg	Weiss et al., (2007)
Flubendazole (anthelmintic)	Germany	Poultry	0.010 – 0.056 mg/kg	Weiss et al., (2007)
Oxytetracycline			19 mg/kg	Kummerer 2008
	Australia	Liquid manure	29 mg/kg	Martínez-Carballo et al., (2007)
Sulfachloropyridazine	UK – Osgathope Leicestershire	Pig farm	0.0006 mg/kg	Blackwell et al., (2004)

Sulfachloropyridazine	Germany	Pig farm	20 mg/kg	Höper et al., (2002)
Sulfadimidine	Switzerland		0.012 mg/kg	Burkhardt and Stamm (2007)
Sulfagidine	Jiangsu China	Pig manure, 178 samples	7.1 mg/kg	Chen et al., (2008)
Sulfamethoxypyridazine	Germany lower Saxony		0.20 mg/kg	Höper et al., (2002)
Tetracycline			66 mg/kg	Kummerer, (2008)
Tiamulin			0.040 mg/kg	Schlüsener et al., (2003)
Toltrazuril	Denmark		0.114 mg/kg	Olsen et al., (2012)
Tylosin	Germany Bovaria		0.320 mg/kg	Weiss et al., (2007)

1.2.4 The occurrence of veterinary medicines in soils

Manure applications to land acts as a source of veterinary medicines into soils. As a result numerous veterinary medicines have been detected within arable soils (Table 1.2) (Du and Liu, 2012). The concentrations of veterinary medicines are comparatively lower than that of animal manures with concentrations generally ranging from ng/kg to µg/kg, but in some circumstances concentrations of mg/kg have been identified (Table 1.2). The lower concentrations in soils are to be expected due to dissipation during storage (see section 5.1), adsorption to soil particulates (see section 5.3), and incorporation (De Liguoro et al., 2003; Grote et al., 2007). The occurrence of veterinary medicines within soils is well known and therefore specific interest regarding their fate upon exposure is of a commonplace within research (Thiele-Bruhn, 2003; Grenni et al., 2018; Conde-Cid et al., 2020). Their fate in soils is dependent on their physical-chemical properties (Boxall et al., 2010) and properties of the soil such as organic matter content, pH, and moisture (Chatterjee et al., 2013; Thiele-Bruhn, 2013).

Following application to land, some veterinary medicines can be transported to receiving waters via surface water runoff and leachate whilst others remain persistent in the soils and can be taken up by plants (Kay et al., 2005; Tasho et al., 2016; Joy et al, 2013; Blackwell et al., 2009, Popova et al., 2014, Aga et al., 2016, and Pan and Chu, 2017a).

Table 1.2: Occurrence and concentrations of veterinary medicines found in soils.

Chemical	Location	Animal/farm	Detection	Reference
Chlortetracycline	Germany		3-39 µg/kg	Hamscher et al., (2005)
	Denmark		20-30 µg/kg	Halling-Sørensen et al., (2005)
Enrofloxacin	Brazil		22.93 µg/kg	Leal et al., (2012)
Ivermectin	Canada		0.0960 µg/kg	Metcalfe et al., (2008)
Oxolinic acid	France- Paris		5.9 µg/kg	Tamtam et al., (2011)
Sulfachoropyridazine	Turkey		0.12 mg/kg	Karcı & Balcıoğlu (2009)
Sulfadimethoxine	USA	Dairy farms	457 µg/kg	Watanbe et al., (2010)
Sulfadimethoxine	Germany		1 µg/kg	Hamscher et al., (2004)
Sulfadimidine	Switzerland		130 µg/kg	Burkardt, (2007)
Sulfadimidine	Germany		16800 µg/kg	Christian, (2005)
Sulfamethazine	Germany		11 µg/kg	Hamscher et al., (2004)
Sulfamethazine	Korea	Swine facility	1.1 µg/kg	Awad et al., (2014)

Toltrazuril (antiparasitics)	Denmark	0.000335 µg/kg	Olsen et al., (2012)
Tetracycline	Germany	295 µg/kg	Hamscher et al., (2005)
Tiamulin	Germany	0.7 µg/kg	Hamscher et al., (2004)
Tylosin	Denmark	57.4 µg/kg	Jacobsen et al., (2004)
Tylosin	Malaysia	678.9 µg/kg	Ho et al., (2012)

1.2.5 The occurrence of veterinary medicines in waters

The transport of veterinary medicines from manured soils following a rainfall event is well known (Kay et al., 2005; Burkhardt et al., 2005; Blackwell et al., 2007; Knäbel et al., 2018; Spielmeyer et al., 2017; Kreuzig et al., 2005; Dolliver and Gupta, 2007; Popova et al., 2013; Blackwell et al., 2009). Generally, this phenomenon is exacerbated when a rainfall event occurs close to the timing of application. The transport of veterinary medicines is enhanced via the presence of manures through elevated, pH (ionized state) concentration and moisture but as well as through colloidal facilitated transport (Pan and Chu, 2017b; Blackwell et al., 2009; Kim et al., 2010). Veterinary medicine transport is compound specific and related to their dissociation constant (K_{ow}), adsorption coefficients K_d, K_{oc} and K_f (affinity for soil, the concentration within soil-water phases) and aqueous solubility. Generally, the concentrations of veterinary medicines detected within surface and groundwaters is low at ng/L to µg/L (Table 1.3 and 1.4), however, their presence still have effects towards non-target organisms as well as the potential formation of antimicrobial resistance (Zou et al., 2021; Kolar and Finizio, 2017; Andrade et al., 2020). Interestingly longer-term leaching has been documented due to the persistence and desorption of some veterinary medicines in soils, for example, Spielmeyer et al., (2017) demonstrated the tendency for sulfamethazine to leach for up to tfour years at a field site in Lower Saxony (92 to 24 ng/L). Currently there is no responsibility for detecting veterinary medicines within surface and groundwater bodies within the EU/UK, however, the critical value for pesticides and

biocides which is often accepted as the quality standard for veterinary medicines is 0.1 µg/L (Hamscher et al., 2005). There is now compelling evidence that this value is not conservative and will not protect the complex and sensitive groundwater ecosystems (Finizio, 2017). This highlights the requirement for the risk assessment and future research to focus on the effects that lower concentrations may have towards these lesser-known species/taxa.

Table 1.3: Collated literature regarding the occurrence and concentrations of veterinary medicines detected in surface water.

Chemical	Location	Water source	Detection	Reference
Chlorfenviphos (anti-patristic)	United Kingdom – Unspecific		0.355-30.8 µg/L	Boxall et al., (2004)
Ciprofloxacin	Chin Kiangsu	River and pond	5.93 and 2.10 µg/L	Wei et al., (2012)
Diclofenac	UK	Estuary	250.8 ng/L	Letsinger et al., 2019
	Germany	River	1030 ng/L	Heberer, (2002)
Difloxacin	China	Yellow sea	20.65 ng/L	Na, et al., (2011)
Emamectin	United Kingdom	Surface water	1.06 µg/L	Boxall et al., (2004)
Enrofloxacin	Ireland	River Tullow	97.8 ng/L	Frenech et al., (2013),
	India	Lake	35 µg/L	Fick et al., (2009)
Fenchlorphos (antiparasitic)	United Kingdom	Surface water	0.777µg/L	Boxal et al., (2004)
Florfenicol	Florfenicol	China Kiangsu	2.4-2.84 µg/L	Wei et al., (2012)
Flubendazole (anthelmintic)	Belgium		0.4-20.2 ng/L	Van de Steene et al., (2010)
Flumethrin	United Kingdom		2.19 µg/L	Boxall et al., (2004)

Ivermectin	United Kingdom		0.8 µg/L	Boxall et al., (2006)
Kitasamycin	China	Beijing, Shahe River	8-10 ng/L	Shao et al., (2009)
Levamisole	Spain	Onyar River	6 ng/L	Zrnčić et al., (2014)
Marbofloxacin	China	Yellow sea	22.29 ng/L	Na, et al., (2011)
Meclofenamic acid	Canada		0.1150 µg/L	Sadezky et al., (2008)
Monensin	Canada	Ontario 14 rivers/lakes	810 ng/L	Kleywegt et al., (2011)
Orbfloxacin	China	Yellow sea	2.69 ng/L	Na, et al., (2011)
Oxolnic acid	France	Seine River	23 ng/L	Tamtam et al., (2011)
Propetamphos	United Kingdom		1.2-19200 µg/L	Boxall et al., (2004)
Spiramycin	Italy		0.742 µg/L	Calamari et al., (2003)
	Spain		0.0037 µg/L	Gros et al., (2010)
Sulfachloropyridazine	Canada		0.02 µg/L	Sadezky et al., (2008)
			3 µg/L	Pei et al., (2006)
Sulfadimethoxine	Luxemburg	Mess river	0.044 µg/L	Krein et al., (2013)
Sulfadimidine	Germany	Bavaria	680 µg/L	Weiss et al., (2007)
Sulfamethazine	Canada		0.4080 µg/L	Sadesky et al., (2008)
	Spain	River Ebro	0.0414 µg/L	Gros et al., (2010)
	Germany		0.18 µg/L	Hamscher et al., (2005)
Sulfamethoxypyridazine	Spain	Barcelona Llobregat River	3704 ng/L	Diaz-Cruz et al., (2008)
Sulfapyridine	United Kingdom	Ely River	142 ng/L	Kasprzyk-Hordern et al., (2008)

Sulfaquinoxaline	Japan		8.9 ng/L	Chang et al., (2008)
Thiabendazole	USA	Wood River	20.5 ng/L	Bartelt-Hunt et al., (2011)
Tilmicosin	Spain	Ebro River	87.5-227 ng/L	Lopez-Serna et al., (2011)
Tylosin	USA	Lake	4.3 ng/L	Wang et al., (2011)

Table 1.4: Collated literature regarding the occurrence and concentrations of veterinary medicines detected in groundwater.

Chemical	Location	Water source	Detection	Reference
Chloramphenicol	China	Shunyi, Changping, Jingjing, GW wells and boreholes swine feedlots	152.3-441.9 µg/L	Li et al., (2018)
Chlortetracycline	China	Shunyi, Changping, Ninghe, and Guangyang, GW wells and boreholes	27.5-664 µg/L	Li et al., (2018)
Chlortenvinphos (antiparasitic)	United Kingdom	Groundwater	0.02-0.07 µg/L	Boxall et al., (2004)
Ciprofloxacin	China	GW boreholes and wells near pig facilities	26.7-261.9 µg/L	Li et al., (2018)
	Spain		90 ng/L	Boy-Roura et al., (2018)
Danofloxacin	Spain		70 ng/L	Boy-Roura et al., (2018)
Enrofloxacin	USA	New Jersey	0.02-0.01 µg/L,	Alvarez et al., (2005)
	China	Beijing provinces	0.26-44.5 µg/L	Li et al., (2018)
Flubendazole	Germany		0.0150 µg/L	Weiss et al., (2007)
Flumequine	Spain	Catolnia	10 ng/L	Boy-Roura et al., (2018)
Monensin	USA	Nebraska	0.020-2350 ng/L	Bartelt-Hunt et al., (2011)

Norfloxacin	China	Shunyi, Changping Daxing, Ninghe and Xiqing	35-389.2 µg/L	Li et al., (2018)
Ofloxacin	Spain	Catolnia	2-3 ng/L	Boy-Roura et al., (2018)
Oxolinic acid	Spain	Catolnia	25 ng/L	Boy-Roura et al., (2018)
Oxytetracycline	China	Changping, Daxing, Ninghe, Xiqing and Anping, GW wells and boreholes (swine feedlots)	30-993.8 µg/L	Li et al., (2018)
Prpetamphos	UK		0.4890 µg/L	Boxall et al., (2004)
Sulfadimethoxine	USA	San Joaquin River	0.068 µg/L	Sadezky et al., (2008)
Sulfadimidine	Germany	Bavaria	4 µg/L	Weiss et al., (2007)
			0.16 µg/L	Hirsch et al., (1999)
Sulfamethazine	Germany		0.24 µg/L	Hamscher et al., (2005)
	USA		0.01 µg/L	Alvarez et al., (2005)
	China	Beijing province	30.6-3494.1 µg/L	Li et al., (2018)
Sulfathiazole	China	Shunyi, Xiqing, and Ninghe	2.7-9.7 µg/L	Li et al., (2018)
Thiabendazole	USA	Nebraska	11 ng/L	Alvarez et al., (2005)
			133 ng/L	Bartelt-Hunt et al., (2011)
Tiamulin	USA	Nebraska, Beef and swine facilities	0.029-0.133 ng/L	Bartelt-Hunt et al., (2011)
Tylosin	Germany		0.42 µg/L	Boxall et al., (2004)
	USA	Assunpink Greek	0.020 µg/L	Alvarez et al., (2005)

1.3 Agricultural management

1.3.1 Manure management and agricultural practices

Manure management practices will ultimately govern the fate of veterinary medicines in the environment, and therefore it is critical to understand these in order to form a comprehensive fate assessment. The use of animal manures as an organic fertilizer and as a form of waste disposal is not a new phenomenon and can be dated back 8,000 years within Europe (Potter et al., 2010; Defra, 2010; Bogaard et al., 2013). Their use is known to improve soil health through increasing or improving the cycling of, organic matter, carbon, nutrients (phosphorus and nitrate), microbial populations, salts and essential metals (i.e., copper and zinc) (Bulluck et al., 2002; Bulluck, 2010; Lee, 2010; Martínez et al., 2019). Increased microbial populations have been reported to improve the cycling of nutrients, bulk densities of soils and cation exchange capacity (Bulluck et al., 2002).

Concern of excess nutrient and metal contamination has been known for some time, nitrogen, phosphorus and metal contamination are ubiquitous surrounding arable farming. The implications of which are the formation of eutrophicated waters and dead hypoxic zones (Kumar et al., 2013; Smith et al., 2001). For example, agricultural runoff which is rich in nutrients has caused the formation of the “dead zone” within the Gulf of Mexico (Esler and Bennett, 2011). Moreover, Nicholson et al., (1999) quantified metals present in swine manures within the UK, the authors reported concentrations ranging from 1.68 to 500 mg/kg for Cu, Zn and As. Hereby presenting the global issue of contaminants present within animal manures and their potential to accumulate within agricultural soils (Ogiyama et al., 2005). Directives were established to reduce the environmental burden of agricultural practices these were, the Nitrate Directive (91/676, EEC 2000/60/EC), the Water Framework Directive (WFD 2000/60/EC) and EQS for metals (EC, 2001).

To reduce the environmental burden of nutrient contamination the nitrate directive set out restrictions on the timings of manure applications as well as the quantities (EC, 2001). The permissible application rate was 170 kg/N/ha and 250 kg/N/ha for arable soils and

pastures respectively (Defra, 2010). During colder periods the ground becomes saturated or frozen, therefore closed application periods were devised these were from the 1st August to 31st December for sandy soils, and from the 1st of October to the 31st January for all other soil types (see SI Table A1) (Komiskey et al., 2011 and Klausner et al., 1976). Moreover, a nitrogen risk map of the EU was devised to highlight nitrogen vulnerable zones (NVZs), this set out further restrictions in regions of high risk (EA, 2017; Defra, 2011). The closed period for NVZs within the UK starts from the 1st of September or 15th October to the 15th of January or 31st December, (Defra, 2013).

In 1992 the Rural Development Programme was established in conjunction with the Common Agricultural Policy (1992) (Science for Environment Policy, 2017). Within this was the establishment of riparian buffer zones to reduce the quantities of agricultural contaminants reaching water sources via surface water runoff. These buffer zones typically contain vegetation which effectively reduce the load of nutrients, metals, pesticides and herbicides reaching surface waters (Muscutt et al., 1993; Raymond et al., 2002; Lin et al., 2010; Chu et al., 2013). They also pose a large service to the ecosystem in that they promote species diversity and abundance (McCracken et al., 2012 and Benton et al., 2003). Their implementation has helped but ultimately not prevented the occurrence of agricultural contaminants within surface waters.

Advanced manure application techniques are now routinely used within the EU, in doing so nutrients losses from volatilization, runoff, and leaching are reduced (Webb et al., 2010; Xu et al., 2021; and Xu et al., 2020). This process is beneficial for farmers to improve soil health and crop yields, but also on an environmental perspective in minimizing the effects that directives 91/676/EEC and 2001/81/EC were set out to minimize (Maguire et al., 2011; Webb et al., 2010; and Powell et al., 2011). Moreover, improved nutrient retentions in soils reduces the requirement of synthetic fertilizers which are expensive due to rapidly depleting sources (Xu et al., 2021; and Xu et al., 2020). For example, Rotz et al., (2011) demonstrated the capability for injection methods to reduce nitrogen and phosphorus losses by 48% and 70% respectively. However, due to efficiency, suitability and cost, conventional broadcasting is still the most popular

application method in arable farming (Webb et al., 2010; Smith, 2001; Rotz et al., 2011; Hujimans et al., 2001).

1.4 The environmental risk assessment for veterinary medicines and regulatory framework

Within the EU, America, Australasia and Japan the risk assessment for veterinary medicines in the environment was implemented under directives 2004/28/EC (previously known as 2001/82/EC), EC/726/2004, and 92/18/EEC (VICH, 2000; VICH, 2003). Members of the Veterinary International Conference on Harmonization (VICH) are the EU, UK, Japan and the US, the principal parties of which are comprised of the European Commission and European Medicines Agency (EMA), US Food and Drug Administration, Japanese Ministry of Agriculture and the Forestry and Fisheries (MAFF). The requirement for a detailed environmental risk assessment for veterinary medicines only includes pharmaceuticals that are attempting to be sold on the market after 1995, hitherto this authorisation was only conducted at national level (Fabrega and Carapeto, 2020 MAFF, 1998). From 1995-2005 risk assessments were based on the applicant's judgement. However, Regulators, environmental agencies and stakeholders are now sceptical of this regulatory work and are now calling for identified risks to be further assessed for environmental efficacy, this is set to be implemented by 2022 under (EU) 2019/6 (Fabrega and Carapeto, 2020).

The environmental risk assessment is comprised of two phases. Phase I encompasses comparisons between the initial Predicted Environmental Concentration (PEC) and terrestrial/aquatic effect data. During this phase the risk assessment considers a total residue approach where the total administered dose to the herd is excreted and applied to land. To generate a PEC (manure, soil, surface water and groundwater) details of administration dose and duration are inputted into the Spaepen, (1997) model, the details of which are provided within the Summary of Product Characteristics (SPC). Should the calculated PEC adhere to the threshold value of ⩽100 µg/kg in soils, the risk assessment stops on the basis that the environmental exposure would be negligible ('no exposure = no risk'), should this value be exceeded a phase II risk assessment is required.

Phase II entails a two-tier system (A and B), tier A generally considers the ecotoxicological effects of the veterinary product towards non-target organisms (Montforts, 1999; VICH 2003). Typically, these are organisms that are likely to be present in the terrestrial and aquatic ecosystems, some of which include plants, earthworms, dung fly's, algae, cyanobacteria as well as aquatic invertebrates such as Daphnids and fish. The initial PEC values are compared to terrestrial and aquatic effect endpoints to generate a Risk Quotient (RQ), should the RQ be ≤1 no further assessments are required. Within tier A there is the option to refine the soil, surface water and groundwaters PECs based on environmental fate data such as, metabolism, degradation during manure storage, soil degradation and adsorption (EMA, 2011; OECD 2000; OECD, 2002). The refined PEC is then compared again to ecotoxicological effect data to assess the risk considering these environmental processes which may influence the concentrations in the environment.

Refinement using manure degradation data can be conducted in the following equations (1a and 1b). These assessments are the most influential within the refinement process given the biodegradability of veterinary medicines. Manure degradation assessments are generally utilized to better assess the concentration that would be applied to land following manure storage. Veterinary medicines are organic compounds and are susceptible to degradation processes such as microbial mineralization, photolysis/hydrolysis, and the formation of bound residues (EMA, 2011; Lamshöft et al., 2010; EMA, 2016; Teeter and Meyerhoff, 2003; Loke et al., 2000; Kühne et al., 2000 and Kolz et al., 2005). One noticeable flaw in manure degradation assessments is that anaerobic digesters, manure composting and manure processing are not considered within the risk assessment (Selvam and Wong, 2017). Soil degradation and adsorption studies are generally utilized to better predict the exposure of veterinary medicines to aquatic compartments. These are done in accordance with OECD, (2000) and OECD, (2002) which require the assessment to be conducted on a range of soil types with differing properties and textures (OECD, 2002). Sophisticated modelling suites (FOCUS: PEARL and SWASH) which utilize the derived adsorption and soil degradation data are used to refine the $PEC_{surface\ water}$ and $PEC_{groundwater}$ better estimate

the concentrations likely to be exposed to surface and groundwaters (FOCUS, 2021; and 2015). Should the RQ value still be ≥ 1, then metabolism and excretion data are used to refine the PEC further (equation 2). Here the percentage of active present within the manure is then used, the available guidance documents are VICH, (2016), EFSA, (2017) and VICH, (2009).

Equation 1a: $$Mt = Mi \times e^{\left(\frac{(-\ln(2)\times\left(\frac{Tst}{2}\right))}{DT50}\right)}$$

Equation 1b: $$\text{PEC}_{\text{soil refined}} = \frac{Mt\times 170}{1500\times 10000\times 0.05\times Ns}$$

Equation 2: $$\text{PEC}_{\text{soil refined – met}} = \text{PEC}_{\text{soil initial}} \times \text{Fa}$$

Where: Mt = mass of active in manure/slurry after the mean storage time (mg), Mi = Mass of active in manure/slurry (mg), Tst = length of time manure is stored (days), DT_{50} = half-life of active in manure (days), 170 = EU nitrogen spreading limit, 1500 = bulk density of dry soil (kg/m^3), 10,000 = area of 1 hectare (m^2/ha), Ns = nitrogen produced during storage time (kg/N), Fa = fraction of the dose considered to be active.

Should the RQ still be ≥ 1 post refinement, the ERA moves to Phase II tier B where the additional studies are used to derive the partition coefficient, bioaccumulation factor and additional taxonomic ecotoxicological effects are used to assess the risk. If the log K_{ow} was found to be ≥4 then a bioconcentration is conducted on fish, for which if the BCF exceeds the threshold (BCF ≥ 1000), or effect is greater than 25% for microbial populations then reconsideration of the chemical compound is suggested. Furthermore, the EQS for pesticides and biocidal compounds in surface water and Groundwater is 0.1 µg/L irrespective of chemical classification (biocidal/pesticidal/veterinary pharmaceuticals), or their properties and environmental effect (2000/60/EG; 2006/118/EC; Montforts et al., 2003).

1.5 The environmental fate of veterinary medicines

1.5.1 Veterinary antibiotic degradation

During the on-farm storage of animal manures veterinary medicines maybe susceptible to various degradation processes, similarly their exposure to soils also results in degradation processes (Table 1.5 and 1.6). Such processes might include microbial mineralization, adsorption and hydrolytic transformation (Berendsen et al., 2018; Lamshöft et al., 2010; Mitchel et al., 2015; Junker et al., 2016). Animal manures are highly microbially active and during anaerobic storage these microbes will use organic compounds as a carbon source and mineralize them into CO_2 (Junker et al., 2020; Lamshöft et al., 2010). The remaining percentage of veterinary medicines and their transformation products are then transferred to soils via the application of manure to land (Kreuzig and Höltge, 2005; Kemper, 2008; Huer et al., 2008). These compounds are then susceptible to soil degradation processes which primarily consists of adsorption but also includes microbial mineralization and to a lesser extent hydrolysis (Table 1.5) (Tolls, 2001; Thiele-Bruhn, 2003). Although regulatory risk assessments for veterinary medicines are confidential and are not available to the public, a wealth of academic/scientific interest has resulted in an understanding of their fate within the environment.

Given that veterinary medicine degradation is a large aspect within the presented thesis it is sensible to explain some context into the terminologies. Firstly degradation of a compound refers to its removal over time within an environmental matrix, this can occur via microbial mineralization which is the process where microbes feed of the compound as a carbon source or hydrolysis which is predominantly the reaction of a compound with water which can alter the compounds structure (Junker et al., 2016). Dissipation is often referred to within the literature, this term relates to the removal of a compound via unknown processes which can include adsorption, microbial mineralization and hydrolysis (Blackwell et al., 2007).

The rate of which veterinary medicines are degraded is compound specific and related to their physical-chemical properties and chemical structure. For example, antibiotics belonging to the penicillin group are readily degradable in manures and soils. They contain a beta lactam ring which is known to be susceptible to cleavage via hydrolytic action and microbial transformation (Li et al., 2011; Mitchell et al., 2014). Whereas tetracyclines are generally considered moderately persistent within pig slurries and soils (DT_{50}/half-life = 55 – 127 d), this is a consequence of their strong chemical structure and affinity for organic matter or carbon which renders the antibiotics non-bioavailable (Berendsen, 2018; Bansal, 2012; Ling-ling. 2010). There are 18 sulphonamides which are considered as relatively mobile and moderately persistent in both pig slurries and soils with DT_{50} values ranging from 0.7 to 22 d and 2.1 to 14 d respectively (Berendsen et al., 2018; Salvia et al., 2014). Macrolide antibiotics are characteristic of a complex but weak chemical structure which is susceptible to microbial transformation and adsorption, they are generally considered nonpersistent (Yuan et al., 2022). However, there has been varied reports of persistence in the environment for example, in liquid manures tylosin, roxithromycin and erthryomycin have reported DT_{50} values of 12 h, 41 d and 120 d respectively (Kolz et al., 2005; Schlüsener et al., 2006a). Interestingly the DT_{50} for erthryomycin and roxithromycin in soils is considerably lower due to the susceptibility of these to photodegradation and adsorption (DT_{50} 20 d and 2.76 d respectively) (Schlüsener et al., 2006b).

Very little literature exists for the degradation of the amphenicol antibiotic class in manures or soils, of the published literature is information regarding florfenicol which has a reported range of 0.16-0.53 h within pig slurries and 7.35 d in soils (Junker et al., 2020; Nightingale et al., 2022; Qiu et al., 202; FDA, 2013). Similarly, there is little literature regarding the degradation rates of aminoglycosides in the environment. However, there is sufficient evidence that lincomycin is persistent within pig slurries, Kutcha and Cessna, (2009) observed minimal degradation over 154 d. Spectinomycin however was identified to degrade much more rapidly with concentrations falling below half the dosing concentration at 8d, this was suspected to be caused by the smaller chemical structure (Kutcha and Cessna. 2009). The fluoroquinolone antibiotic class

contains eight antibiotics, a wide range of elimination rates has been documented within the literature, enrofloxacin was identified to have a DT_{50} of 5 d within solid pig manure, ciprofloxacin was observed to degrade by 67-83% in 56 d within composted pig manures, difloxacin was observed to degrade by 7-15% in 150 d indicating persistence within the environment (Berendsen et al., 2018, Kutcha and Cessna., 2009 and Lamshöft et al., 2010).

Basic guidance and regulations are provided for the manure degradation experimental procedure, however, there is currently no specific OECD or EU level of testing available, hereby contributing to differences within the reported findings (EMA, 2011, OECD, 2002, Junker et al., 2020 and Wohde et al., 2016). For example, two studies assessing the degradability of oxytetracycline within pig slurries, Blackwell et al., (2005) reported a DT_{50} of 79 d and Wang et al., (2015) calculated a DT_{50} range of 9.05 – 9.65 d. Moreover, a comprehensive ring testing report was published by Junker et al., (2016), which investigated variability within the degradation rates for florfenicol (pig slurry) and imidacloprid (cattle slurry) from differing laboratories and manures. The author's reported DT_{50} values to range from, 0.17-0.41 d for florfenicol and 7.4-40 d for imidacloprid. As can be seen imidacloprid degradation was variable, demonstrating that variability exists within manure degradation studies, this was not the case for florfenicol however, it could be criticised that the stability of this analyte is too rapid for such assessments. This assessment was furthered via Junker et al., (2020), who demonstrated an attempt to harmonise such laboratory procedures; stricter experimental procedures. The interlaboratory variability for salicylic acid and paracetamol degradation within pig and dairy slurries were still between 9-12% and 3-46% respectively. As can be seen harmonisation of such testing procedures is complex and variability will most likely always exist due to differences within manure properties (see section 6.0).

Currently other manure storage and treatment processes such as, composting and anaerobic digesters are assessed within the environmental risk assessment for

veterinary medicines, however, specific scientific interest has arisen regarding their suitability as a mitigation measure (Dolliver et al., 2008; De Liguoro et al., 2003; Zhan et al., 2018; Mohring et al., 2009; and Shi et al., 2012; Selvam and Wong, 2017). Throughout the EU manure heaps are abundant and it is important to consider their influence on veterinary medicine fate also. Generally, these systems have greater removal efficiencies over that of slurries due to their, lower moisture contents, microbial diversity, thermophilic temperatures, abundant fungi populations as well as elevated carbon or organic matter contents (Youngquist et al., 2016). For example, doxycline has a DT_{50} of 120 d in pig slurry but under composting conditions this is only 25.7 d (Szatmari et al., 2011; and Widyasari-Mehta et al., 2016).

Mesophilic and thermophilic temperatures are achieved within manure heaps due to high microbial activity and mineralization of organic matter/carbon (Bernal et al., 2009). Elevated temperatures have been identified to increase the adsorption of chlortetracycline enhancing its removal (Kim et al., 2012; Li et al., 2011). This is to be expected given temperature dependent reaction rates are well known to influence organic compounds. As a result, the Arrhenius equation is often utilized within the risk assessment to correct for differences between laboratory and in-field temperatures (Laidler. 1984). However, recent compelling research conducted by Keighley et al., (2021) has demonstrated its poor representativeness of this in estimating the biodegradation rate of metaldehyde in soils.

1.5.2 Advanced storage mechanisms

Intensification within animal husbandry has resulted in the increased usage of anaerobic digesters, these advanced systems offer improved nutrient cycling, odour reductions, pathogen control and biogas production (Frear et al., 2011; Mitchell et al., 2013; Madsen et al., 2011; Subair et al., 2020; Möller and Müller., 2012; Vanotti et al., 2002). Increasing usage of these systems raises questions regarding the fate of veterinary medicines during this timeframe. However, research has demonstrated these systems to be better suited in the removal of veterinary medicines and antibiotic resistant genes (Carballa et al., 2007; Alvarez et al., 2010; Ma et al., 2011; and Pruden et al., 2013).

Anaerobic digesters are complex systems and the control of temperature, moisture, fermentation, additives and homogenization have all been identified to increase biochemical reactions (Turker et al., 2018; Arikan, 2008; and Akyol et al., 2016). When comparing two studies investigating tetracycline elimination it is evident that removal rates are greater in AD over that of conventional slurry storage, for example Shi et al., (2011) reported a DT_{50} of 12 h whilst Winckler and Grafe, (2001) calculated a DT_{50} of 55d in pig slurries. The influence of veterinary medicines on AD performance is now topical within research, although their effect has been summarized as being minimal (i.e., 9% reduction in CH_4 production) (Guo et al., 2012).

Table 1.5: Collated degradation rates of veterinary medicines within anaerobic animal waste.

Veterinary medicine	Matrix and storage condition	DT_{50} or percentage removed	Reference
Amoxicillin	Pig manure (compost containing straw)	0.66 d	Liu et al., (2014)
Bactaricin	Pig slurry	1.9 d	Joy et al., (2014)
Ceftiofur	Recycled dairy water	0.00058-0.00118 d (25°C)	Li et al., (2011)
Chlortetracycline	Pig slurry	7 d (38°C)	Varel et al., 2012
	Pig manure	19 d (19-21°C)	Berendsen et al., 2018
	Pig manure compost	86.6 d	Bao et al., (2009)
Ciprofloxacin	"Rizosediment" (compost)	21 d 67-83% (56 d)	Alexandrino et al., 2017
	Pig manure		Selvam et al., (2012)
Difloxacin	Pig slurry	7-15% (150 d)	Lamshoft et al., 2010

Doxycline	Pig manure (compost)	25.7 d	Szatmari et al., (2011)
	Pig slurry	120 d	Widyasari-Mehta et al., (2016)
Enrofloxacin	Pig manure	5 d	Chen et al., (2018)
	Pig manure	6 d	Berendsen et al., (2018)
			Slana et al., (2017)
Erythromycin	Pig slurry	41 d	Schulsner et al., (2006)
	Pig manure	52 d	Berendsen et al., (2018)
	Pig manure (compost)	1 d	Chen et al., (2018)
Florfenicol	Pig slurry	0.023 d	Junker et al., (2018)
	Dairy manure (compost)	0.5 – 14 d	Mitchel et al., (2015)
Flumequine	Pig manure	46% (30 d)	Bousek et al., (2018)
Lincomycin	Pig slurry	37.7% (35 d)	Kutcha et al., (2009)
Metronidazole	Manured soil (10% pig)	26.9 d	Ingerslav and Halling-sorensen., (2001)
Monensin	Dairy slurry	100% (140 d)	Pei et al., (2007)
	Horse manure (compost)	11 – 39.8 d	Kim et al., (2012)
Norfloxacin	Broiler manure	2.1 d	Ho et al., (2013)
Olaquindox	Manured soil (10% pig)	5.8-8.8 d	Ingerslav and Halling-sorensen (2001)
Oxytetracycline	Pig manure	9.05 d	Wang et al., (2015)
	Calve manure	87 d (DT_{90})	Berendsen et al., (2018)
	Pig slurry	79 d	Blackwell et al., (2005)
	Pig (moisture dependent)	7.8 – 9.3 d	Wang et al., (2008)
Progestrone	Broiler manure (compost)	2.4 d	Ho et al., 2013

Salinomycin	Broiler manure and straw	1.3 d	Ramaswamy et al., (2010)
	Pig slurry	6 d	Schlüsener et al., (2006)
Spectinomycin	Pig slurry	79% (6 d)	Kutcha and Cessna, (2009)
Sulfadiazine	Pig slurry	20 d	Lamshöft et al., (2010)
	Broiler manure (compost)	1.4 d	Ho et al., (2013)
Sulfadiomethoxine	Dairy manure	<7 d	Mitchel et al., 2015
	Calve manure (straw)	64 d	De liguoro et al., (2007)
	Steer manure (varying moistures)	2.34 - 4.49 d	Wang et al., (2006)
Sulfamethazine	Cattle slurry (AD)	>60 d	Mitchel et al., (2015)
	Pig manure (compost)	>35d	Kim et al., (2012)
Sulfamethoxazole	N/A (AD)	8 d	Mohring et al., (2009)
	Dairy waste water (AD)	60 d	Pei et al., (2007)
	Pig manure	1.6-5.7 d	Berendesen et al., (2018)
Sulfamethoxydiazine	Pig manure	>12 h	Shi et al., (2012)
Sulfamethoxypyridazine	N/A (AD)	14 d	Mohring et al (2009)
Sulfamethizole	Pig manure	0.6d-11 d	Berendsen et al., (2018)
Tetracycline	Pig manure (compost)	6 d	Chu et al., (2017)
	Pig slurry	9.5 d	Kühne et al., (2000)
	Pig slurry	0.5 d	Shi et al., (2012)
	Pig slurry	55 d	Wincklet and Grafe, (2001)
Tilmicosin	Compost	2 d	Ho et al., (2013)
Tiamulin	Pig manure	>200 d	Schlusener et al., (2006)
Trimethoprim	Broiler manure (compost)	3.7 d	Ho et al., (2013)
		<8 d	Mohring et al., (2009)

	N/A		
Tylosin	Pig slurry	7.6 d	Teeter and Meyerhoff., (2003)
	Pig manure (compost)	<2 d	Loke et al., (2002)
	Pig slurry	12.9 d	Kolz et al., (2005)
Doramectin	Sheep manure (compost)	12.2 – 20% (21 d)	Gobec and Ivan (2007)
Flubendazole	Pig slurry	28% (102 d)	Kreuzig et al., (2007)
Fenbendazole	Pig slurry	20% (102 d)	Kreuzig et al., (2007)
Ivermectin	Horse manure (compost)	1.6 d	Shwarz and Bonhotal, 2016

Footnote: *Table summarised using the data presented in tables A2-5 which contains the manure properties also. Anaerobic digestion indicated via AD.*

Table 1.6:Collated degradation rates of veterinary medicines within soils.

Veterinary medicine	Soil type	DT_{50}	Reference
Amoxycillin	Clay loam pH 8.2	0.43 d	Braschi et al., (2013)
	Sandy loam pH 5.0	0.57 d	
Ciprofloxacin	Silty clay loam	1.5 d	Cui et al., 2014
		50 d	Giradi et al., (2011)
Chlortetracycline	Silty loam pH 5.7	20 d	Li et al., (2010)
	Clay loam pH 5.7	55% (21 d)	Liu et al., (2012)
	Sandy loam pH 6.1	25 d	
	Sand	34 d	Halling-Sørensen et al., 2005
Doxycline	Silty clay loam pH 7	N/A	Wang et al., (2016)
	Sandy clay loam pH 5.6	533-578 d	Walters et al (2010)
Enrofloxacin	Sandy loam	10 d	Martens et al., (1996)

Monensin	Sand	1.3 d	Sassman and Lee, (2007)
	Clay loam	2 d	
Norfloxacin	Silty clay loam	10	Wang et al., (2016)
	Clay loam	31 – 62 d	Yang et al., (2012)
Oxytetracycline	Sandy loam pH 6.2	21.7 d	Blackwell et al., (2007)
	Silt loam pH 6.24	95.4%	Ma et al., (2016)
Sulfachloropyradazine	Silt loam	20-22 d	Accinelli et al., (2007)
	Sandy loam	3.5 d	Blackwell et al., (2007)
	Clay loam	28 d	Kay et al., (2004)
Sulfadimethoxine	Silt loam pH 5.4	11 d	Wang et al., (2006)
Sulfadiazine	Silt loam pH 7.2	1-8.5 d	Hammesfahr et al., (2008)
	Loamy sand pH 5.7	8.6 d	Sittig et al., (2014)
Sulfamethoxazole	Sandy loam pH 9.23	9 – 15.3 d	Lin and Gan, (2011)
	Silt loam pH 5.7	2-5 d	Liu et al., (2012)
	Clay loam	4.3-13 d	Srinvasan and Sarmah, (2014)
Sulfamethazine	Silt loam	1.3 d	Topp et al., (2013)
	Clay loam	24.8 d	Pan and Chu, (2016)
Tetracycline	Sandy clay loam pH 5.6	578 d	Walters et al., (2010)
	Clay loam	14.1 – 69.3 d	Pan and Chu, (2016)
Trimethoprim	Silt loam pH 5.7	2-5 d	Liu et al., (2009)
Tylosin	Loamy sand pH 6.1	67 d	Halling-Sørensen et al., 2005
	Sandy loam	5.7 d	Ingerslav and Halling-Sørensen, (2001)

1.5.3 Veterinary medicines and sorption

Veterinary medicines and other organic contaminants can bind to the organic matter and carbon present in manures and soils where residuals are sequestered into micro and nano-pores (Table 1.7). The soil-water partition coefficient (K_d, K_{oc}/K_{om} and K_f) is a

means to quantify this, for which batch sorption experiments are employed (OECD, 2000; Tolls, 2001). Generally, the purpose of this is to quantity the ratio of the assessed compound to partition between the water and the solid phase.

It is critical to quantify the partition coefficients for veterinary medicines in order to understand their mobility and bioavailability in the environment (Chen et al., 2017). Therefore, this chemical property is essential in estimating their risk and effect in the environment given it defines their mobility and availability to microbial degraders (Wu et al., 2009). For example, ivermectin was shown to persist for 265 d within soils (Sanderson et al., 2007), thus reducing the bioavailability towards micro-organisms, however there are still concerns regarding direct ingestion of the organic matter (OM) bound pharmaceuticals by earthworms (Mäenpää and Kukkonen, 2006; and Mougin et al., 2003). Soil adsorption coefficients are vital parameters when modelling the risk of veterinary medicines towards groundwater and surface water, this parameter is utilized within numerous environmental fate models such as, FOCUS_PEARL and FOCUS_SWASH (FOUCS, 2014; Wegst-Uhrich et al., 2014; and Ter Laak et al., 2006a).

Veterinary medicines will form various interactions with organic matter/carbon present in soils and manures, this is a compound specific effect that is controlled via the physiochemical properties (log K_{ow} and pKa) and chemical structure; adsorption mechanisms such as, ionic bonding (cation exchange), hydrogen bonding, van der waals forces and hydrophobic partitioning have been reported for various veterinary medicines (Jeckahlke et al., 2014; Gevao et al., 2000; and Ter Laak et al., 2006b; Jones et al., 2005; and Sassman and Lee 2005). Veterinary medicines with a high K_{ow} values are generally considered hydrophobic, indicating an affinity for binding to soils/manures, because of this relationship K_{ow} values are often used in order to predict rates of adsorption (K_{oc} and K_d) within modelling suites such as EPI-Suite (Kanazawa, 1989; and Berthod, 2015). Although the correlation between these values has often been scrutinized due to compound specific differences. Octanol-water coefficients are not a good descriptor of ionisable chemical sorption, other parameters/models are required

for such modelling such as cation exchange and pKa (Droge and Goss, 2013). For example, oxytetracycline has a low K_{ow} (-1.22) value however it has a high adsorption coefficient (K_{oc} 42500 L/kg (Loke et al., 2002; Rabølle and Spliid, 2000; Song et al., 2014).

Research conducted via Chandler et al., (2005) demonstrated the capability of tylosin and tetracycline bound residues to remain bioactive and still inhibit the growth of *Escherichia coli* and *Salmonella sp*. Furthermore, Subbiah et al., (2011), found florfenicol to remain bioactive whilst adsorbed into the soils while ciprofloxacin, tetracycline and neomycin were neutralized. This highlights the complications in regarding adsorbed chemicals as benign or without an environmental risk, but also demonstrates the difficulty in assessing such risk within an ERA.

Various factors are known to influence the rates of adsorption within soils and manures, these are generally OM/OC content, matrix age, pH and ionic strength (ter Laak et al., 2006; Parolo et al., 2008; and Gevao et al., 2000). The adsorption of sulfamethoxazole has been discovered to be mediated by pH, due to sulfamethoxazole's pKa acidic pH's result in the majority of the analyte being uncharged resulting in hydrophobic interactions with OM/OC and cationic complexes with lipds and hydroxyl groups (Jia et al., 2017; Srinivasn et al., 2013; and Chen et al., 2017). Moreover, it has been reported that the presence of slurry/manure decreases sulphonamide adsorption to soil particulates due to the elevation in pH (Thiele-Bruhn, and Aust., 2004; and Thiele-Bruhn 2003). Ionic strength is correlated with pH due to the displacement of protons from cation-exchange sites resulting in shifts in pH, moreover, ionic strength decreases electrostatic processes on negatively charged surfaces such as soils, this in return has been observed to increase oxytetracycline adsorption from 31-77% however the ionic strengths assessed were deemed environmentally unrealistic (ter Laak et al., 2009).

Table 1.7: Collated adsorption coefficients from the available literature.

Veterinary Medicine	Adsorption coefficient K_d (L/kg)	Matrix	Reference
Sulfamethazine	0.27-0.77	Sandy loam	Srinivasan et al., (2010)
	16.55	Clay loam	Srinivasan et al., (2010)
	3.1-17	Loam	Kurwadkar et al., (2007)
Sulphanilamide	0.57	Silt loam	Thiele-Bruhn, and Aust. (2004)
	0.59	Soil-slurry mixture 50:1 (w/w)	
Sulphapyridine	1.02	Silt loam	Thiele-Bruhn, and Aust. (2004)
	1.22	Soil-slurry mixture 50:1 (w/w)	
Sulphadimidine	0.79	Silt loam	Thiele-Bruhn, and Aust. (2004)
	0.74	Soil-slurry mixture 50:1 (w/w)	
Sulphadiazine	2	Silt loam	Thiele-Bruhn, and Aust. (2004)
	1.18	Soil-slurry mixture 50:1 (w/w)	
Sulphadimethoxine	0.73	Silt loam	Thiele-Bruhn, and Aust. (2004)
	0.62	Soil-slurry mixture 50:1 (w/w)	
Tylosin	58.1-148	Sandy loam	Rabolle M, Spliid (2000)
	65	Clay loam	Gupta et al., (2001)
	5.77-21.4	Loam	Zhang et al., (2011)
Oxytetracycline	680	Sandy loam	Rabolle M, Spliid (2000)

	670	Sand	Rabolle M, Spliid (2000)
	2580	Argonaut (gravel loam soil)	Popova et al., (2013)
	1040	Yolo (silt loam)	Popova et al., (2013)
Chlortetracycline	280	Argonaut (gravel loam soil)	Popova et al., (2013)
	440	Yolo (silt loam)	Popova et al., (2013)
Metronidazole	0.57-0.67	Loamy sand and Sand	Rabolle M, Spliid (2000)
Fenbendazole	0.84-0.91	Silt loam	Thiele and Leinweber., (2000)
Enrofloxacin	260-5610	Sandy soils (pH 4.9, 5.3 and 7.5)	Nowara et al., (1997)
	496-3037	Five soils from European countries	Nowara et al., (1997)
	3548-6310	Pure clay (Kaolinite, illite, vermiculite and montmorillonite)	Nowara et al., (1997)
	40,656	Clay silt loam	Leal et al., (2012)
	38	Poultry litter	Leal et al., (2012)
	40,595	Poultry litter and clay silt loam	Leal et al., (2012)
Ciprofloxacin	427	Sandy soil (pH 5.3)	Nowara et al., (1997)
	40,783	Clay silt loam	Leal et al., (2012)
	34.1	Poultry litter	Leal et al., (2012)
	40,728	Poultry litter and clay silt loam	Leal et al., (2012)
Norfloxacin	40,772	Clay silt loam	Leal et al., (2012)
	65.1	Poultry litter	Leal et al., (2012)

	40,716	Poultry litter and clay silt loam	Leal et al., (2012)
Tetracycline	3102–312447	Soil	Sassman and Lee (2005)
	2.2–6.5E4	Clay	Pils and Laird (2007)
Ivermectin	63,020	Argonaut (gravel loam soil)	Popova et al., (2013)
	21,520	Yolo (silt loam)	Popova et al., (2013)
Avermectin	80.2, 17.4, 147	Silt loam, Sand and clay loam	Gruber et al., (1990)
Florfenicol	0.07-0.59		FDA. (2013)

1.6 Current understanding of the influence of matrix properties and experimental conditions on the degradation, adsorption and hydrolysis of veterinary medicines

The properties of environmental matrices can influence the rate of removal for veterinary medicines. Currently our understanding of how soil and water properties can affect veterinary medicine fate is well known (Srinivasan et al., 2013; Conde-Cid et al., 2019; and An et al., 2021). However, research has seldom assessed the influence of manure properties on the degradation-elimination of veterinary medicines, despite the requirement for a comprehensive understanding for the veterinary medicine risk assessment. Animal manures are highly heterogenic and large differences in properties exist between, animal species, farms, and animal ages (see SI Table A2-5). The properties of manures are influenced by a number of factors such as, processing (acidification, aeration or separation), husbandry practice, animal feed, environmental conditions, animal bedding, water type, and biocidal usage (Canh, 1998a; Canh, 1998b; Montforts and Tarazona Lafarga, 2003; Béline et al., 2004; Powell et al., 2006; and Fanguiero et al., 2015). There has been some interest into the influence of manure properties on the degradation of veterinary medicines, but further research is required (Wang and Yates, 2008; wohde et al., 2016; Junker et al., 2020). For example, Kreuzig, (2010) suggested that manure pH and moisture would be important parameters governing the fate of veterinary medicines, but it is expected other parameters will be of equal importance.

1.6.1 Moisture

The moisture of environmental matrices is well known to influence the fate of agricultural contaminants; however, little published research is available regarding its influence towards veterinary medicines. Perhaps this is because this research aspect was thoroughly investigated for pesticides and herbicides (Ou et al., 1982; Mate et al., 2015; and Cycoń et al., 2019). For example, Anderson, (1981) demonstrated that ^{14}C diallate and ^{14}C trillate degradation rates increased as soil moisture increased, the respective DT_{50} values were 17.8 d and 49 d at a moisture content of 9% and 9 d and 34 d at 19%. This relationship was explained by the compound's bioavailability to

microbial mineralization; however, the opposite effect may occur in instances where dissipation is governed by adsorption (Castillo and Torstensson, 2007). Similarly, moisture plays a crucial role for veterinary medicine degradation in manures due to sorption, bioavailability and hydrolysis. Of the published literature for manures, Wang et al., (2007) demonstrated that oxytetracycline degradation was enhanced in elevated moistures, for example the degradation rate constant (*k*) increased from 0.095 to 0.102 at moistures of 60-80%. Due to the known effect of moisture on degradation rates manure degradation assessments are conducted in a harmonized approach in that the dry matter contents are kept to 5%, 60% and 10% in pig, poultry and dairy assessments (CVMP, 2011). The separation of slurries is becoming an increasingly popular practice at farm scale to reduce slurry volume and improve nutrient management, within the EU 7.8% of slurries are now subject to this process (Hjorth et al., 2011; SARGA, 2015). The influence of this practice towards veterinary medicine fate is only now being investigated, although further research is required to fully assess the risk (Marti et al., 2020).

1.6.2 pH

The pH of environmental matrices has numerous influences on the fate of agricultural contaminants. The most influential is its effect on chemical speciation (ionized state and polarity) and adsorption mechanisms (i.e., cation exchange or electrostatic forces) (Mackay and Caterbury, 2005; Hu et al., 2008; Conde-Cid et al., 2019). The influence of soil pH on veterinary medicine fate is well established, for example Sassman and Lee (2005), demonstrated that the DT_{50} of tetracycline was lower within acidic soils over that of neutral. Tetracyclines are zwitterionic compounds meaning they have multiple ionizable functional groups at differing pH's (3 x pKa), at lower pH's tetracyclines exist in a cationic form and therefore their affinity for negatively charged soils is greater (Conde-Cid et al., 2019). This phenomenon is compound specific and has been identified for numerous other compounds such as sulfamethoxazole, sulfadiazine, sulfachloropyridazine and tylosin via differing adsorption mechanisms (Boxall et al., 2002; ter Laak et al., 2006; Yang et al., 2009; Srinivasan et al., 2013; and Chu et al., 2013).

Soil pH is an important parameter governing the fate of contaminants in soils, it is expected that slurry pH will have an equal or greater influence on veterinary medicine fate due to alterations to sorbitive processes, microbial populations and hydrolysis (Thiele-Bruhn and Aust. 2004; Yang et al., 2009; Ali et al., 2013; and Chen et al., 2017;). Ali et al., (2013), conducted a comprehensive assessment on the degradation of tylosin within dairy cattle manure at various pHs, the results revealed that alkaline conditions promoted degradation whilst acidic inhibited degradation. Interestingly, the report later elaborated that this rapid elimination under alkaline conditions was attributed to the formation of an unknown breakdown product. Moreover, a similar trend was identified for pirlimycin where alkaline dairy slurry promoted degradation and acidic reduced degradation (Li et al., 2020).

The hydrolysis of veterinary medicines is known to be influenced via pH and therefore the current experimental guidance requires these experiments to be conducted at a range of pH values (OECD, 2004). Loftin et al., (2008) demonstrated that the transformation of oxytetracycline and tetracycline was increased as water pH increased. The DT_{50} values were 559 and 682 h at pH 5 and 682 h and 1150 h at pH 9 for oxytetracycline and tetracycline respectively. Kune et al., (2000) revealed this trend to be related to the transformation of tetracycline into *iso*-tetracycline a product which is easily broken down into smaller fragments (Hlavka and Boothe 1985; and Halling - Sørensen, 2002). It is to be expected that pH mediated hydrolysis will also influence veterinary medicine fate within slurries due to their high moisture contents.

1.6.3 Redox potential

Redox potential is often used as a parameter to indicate the oxidative – reductive state of environmental matrices (CVMP, 2011; Jang et al., 2021). The oxygen content of environmental matrices directly influences the microbial community and therefore the elimination of organic contaminants (YSI, 2008). Recently researchers have investigated this relationship for various contaminants present in sludges, soil solutions and wastewater. A comprehensive evaluation of which was conducted by Alvarino et al., (2016), where the degradation of ^{14}C-sulfamethoxazole in sludges under varying

redox conditions was assessed and revealed that degradation was greatest under anaerobic over that of aerobic nitrifying conditions. This was found to be related to biodegradation as well as adsorption. Ouyang et al., (2019), reported similar findings in soil solutions where the degradation of sulfamethoxazole was increased in anaerobic (methanogenic or sulphate reducing conditions) over that of aerobic (nitrate-reducing). The authors accredited this towards the increased abundance of specific degraders such as *Desulfovibrio Vulgaris*.

Wastewater treatment is now employing Biological Nutrient Removal (BNR) a stepwise process that involves anaerobic, aerobic and anoxic phases to control nutrient concentration. Recent research has investigated the influence of this process towards the fate of pharmaceutical contaminants (Estrada-Arriaga et al., 2016; Lakshminarasimman et al., 2018). Typically, these systems utilized a redox potential control mechanism and research has demonstrated that differing phases can result in differing rates of contaminant removal. Smook et al., (2008) reported 95% of ibuprofen was removed in the aerobic stage, whereas Inyang et al., (2016) demonstrated trimethoprim removal to be greatest in the anaerobic phase.

Very little information currently exists regarding the influence of redox potentials on the degradation of veterinary medicines in manures despite a wide range of values being reported in the literature (Kolz et al., 2005; Kreuzig, 2010; Weinfurtner, 2011; Wohde et al., 2016; Richter et al., 2016; and Junker et al., 2020). Ali et al., (2013) did however investigate the degradation of tylosin under aerobic – reduced conditions within dairy lagoon sediment. They reported aerobic conditions (+350 mV) to have greater removal efficiencies over that of reduced (-100 mV). However, there is still a lack of research concerning the influence of commonly used anaerobic redox potentials (-100 mV to -400 mV) on veterinary medicine degradation. This information is critical considering that this range is considered acceptable in routine manure degradation assessments within the risk assessment for veterinary medicines (CVMP, 2011).

1.6.4 Compound concentration and mixtures

Typically, environmental fate and effect studies are conducted using singular compound assessments, however, there are concerns regarding if this is representative of the natural environment (Polianciuc et al., 2020). Agricultural soils are likely containing a wide array of contaminants and as a result their fate and effects may differ to that of singular compound assessments. For example, UBA, (2019) outlined that some organic wastes can contain up to 20 differing contaminants and soil have been identified to contain up to 13 antibiotic compounds. It is expected that this will reduce the degradation of agricultural contaminants in manures and soils via inhibited microbial populations and competition for binding sites to organic matter and carbon. There is currently little literature investigating this phenomenon in environmental matrices. Those that have investigated have demonstrated a significant difference in degradation rate, for example Monteiro and Boxall, (2008) identified a soil DT_{50} range of 3.1 – 10.2 d for naproxen whilst this range was extended to 4 – 17.7 d in the presence of carbamazepine, fluoxetine, and sulfamethazine. Moreover, Yang et al., (2016) investigated this phenomenon in sludges and reported DT_{50} values for both individual and synergistic assessments to be 1.3 < 2.7 d, 2.2 < 3.9 d, and 3.2 < 5d for sulphamethoxazole, sulphadimethoxine, and sulphamethazine respectively. A recent review by Marx et al., (2015) demonstrated that antibiotic mixtures increased the toxicological effect towards bacteria communities and algae by up to 50-200% when compared to singular compound assessments. Interestingly Drzymała and Halka, (2020) highlighted a mixture of sulfamethoxazole and diclofenac did not drive any differences within the effects towards *Aliivibrio fischeri, Daphnia Magana or Lemna minor.* This phenomenon demonstrates the compound specific effects and the requirement for future assessments of risks to address this.

1.7 Ecotoxicological effects of veterinary medicines in the environment

Veterinary medicines are bioactive and consequently they may have a plethora of effects towards non-target species in the environment. The risk associated with veterinary medicines is dependent on their usage, exposure to environmental compartments, persistence, pharmacokinetics and bioavailability (Boxall et al., 2003). Therefore, their risks are considered compound specific, some of the literature regarding ecotoxicological effects of veterinary medicines in aquatic and terrestrial systems is compiled in Table 1.8. For this reason, routine effect studies are conducted within the risk assessment to investigate their risk towards soil microorganisms (nitrogen transformation test (OECD 216), plants (OECD 208), earthworms (OECD 207), freshwater plants (OECD 201) and aquatic invertebrates (OECD 211 and 203) (OECD, 2000; OECD 2006; OECD, 1984; OECD, 2011; OECD; 2012; OECD, 2019).

In reviewing the literature, it is apparent that certain veterinary medicine classes pose a greater risk towards terrestrial and aquatic organisms. Parasiticides are generally considered some of the most toxic veterinary medicines and as a result a Phase II risk assessment is required regardless of the outcome during the Phase 1 exposure assessment (Koolz et al., 2008). Antiparasitic pharmaceuticals in the macrocylic lactone group are comprised of, avermectin, ivermectin, doramectin, moxidectin, abamectin and eprimomectin. They are typically administered to treat gastrointestinal roundworm, lungworms, scabies and mites (Lumerat et al, 2012; Jacobson et al., 2000). Their persistence and mode of action results in toxic effect towards dung beetles, horn-flies, earthworms, daphnids, and fish (Strong, 1996; Svendsen et al., 2005; Langford et al., 2014; Bundschuch et al., 2016; and Mackenzie et al., 2021). There is now a great concern regarding the use of antiparasitic compounds towards dung fauna, Ambrožová et al., (2021) conducted a comprehensive field assessment on ivermectin and reported that sites using this compound had a 35% reduction in dung beetle communities. Interestingly these compounds are characteristic of, large molecular masses, hydrophobicity (log K_{ow}), and a strong affinity to OM, so their potential for plant uptake or bioaccumulation is very low (Boxall et al., 2006). There has however been reports

of high risk in aquatic compartments (*Daphnia magna*, and *Oncorhynchus mukiss*) (Liebig et al., 2010; Lumaret et al., 2012).

Coccidiostats are an antiprotozoal routinely used in the protection of poultry against coccidiosis. Literature regarding their effects towards terrestrial and aquatic organisms is sparse. Of the available literature, Schwaiger et al., (2004) demonstrated the toxic effect of diclofenac to aquatic invertebrates (Daphnids and fish) and reported a chronic LOEC of 5 µg/L in *Oncorhynchus mykiss*. The authors stated that at this concentration distinct histopathological alterations in the kidney and the gills were observed. Ionophores are a sub-group of coccidiostats; Hansen et al., (2009) conducted a desk-based risk assessment and outlined that for monensin and salinomycin there were identified risks towards both aquatic and terrestrial organisms during a phase II risk assessment (PEC refinement based on excretion and dissipation).

Numerous publications have detailed the effects of antibiotics in the terrestrial and aquatic environment (Halling-Sørensen et al., 1998; Thiele-Bruhn, 2003). The majority of antibiotics are primarily toxic at high concentrations (Table 1.8), however, there are some exceptions of this. For example, florfenicol has been identified to be toxic at low levels to plants, EC_{50} biomass values were reported to range from 0.32 - 2.59 mg/kg in assessment conducted on six standardized plant assays (Richter et al., 2016). Generally, the most sensitive species to antibiotic compounds are bacteria and cyanobacteria, this is expected for bacteria given their mode of action whilst cyanobacteria contain receptors which are inhibited in a similar means to that of bacteria (Kümmerer, 2003; Guo et al., 2012; and EMA, 2018). The greatest risk of antibiotic contamination in the environment is the formation of antimicrobial resistance.

Antibiotics in the environment result in a selective pressure towards microbial populations, in that the bacteria that survive in the presence of antibiotics evolve and become resistant (Davies, 1994; Baquero et al., 2008; and Allen et al., 2010). A process now known as horizontal gene transfer has also been demonstrated where resistance

can be spread across numerous microbial species which exacerbates the phenomenon (Andam et al., 2011). The promotion of antimicrobial resistance in the environment poses a great threat towards human and veterinary pharmaceutical treatment, currently 15,000 deaths per year in Germany are now attributed to this phenomenon (Aries and Murray, 2009; Klatte et al., 2017). Interestingly this process has been identified to be promoted where lower antibiotic concentrations exist; a direct result of low mortality and increased gene transfer (Klatte et al., 2017). Animal husbandry has now been identified to contribute towards antimicrobial resistance via the usage of antibiotics in the treatment of livestock. For example, Joy et al., (2013) identified tylosin resistant strains to be prevalent in manure storage even following the dissipation of the compound. Moreover, Binh et al., (2010) detected *sul1, sul2, and* bla_{TFM} *strains* in manures from 11 differing farms. In early research conducted via Koike et al., (2007) the ability for resistant genes to be present in shallow groundwater below a storage lagoon was identified. The authors reported the presence of *tet0, tetC tetQ, tetW, tetM, tetS, tetH, tetZ* and *tetT* genes. In a comprehensive review conducted by Andrade et al., (2020) it was demonstrated that of 8,741 investigations 7157 reported the presence of antimicrobial resistance in various matrices. Of those in groundwater, 31.4% of the bacteria were identified to contain antimicrobial resistant genes and were resistant to various antimicrobials.

Table 1.8: Collated literature on the ecotoxicological effect of veterinary medicines in the environment.

Veterinary medicine	Species (most sensitive)	Endpoint	Concentration	Reference
Ceftiofur	Microbes	NOEC	0.25 mg/kg	Boxall et al., (2004)
Chlortetracycline	Green algae (M. aeruginosa)	$EC5_0$	0.05 mg/L	Halling-Sørenson (1999)
	Bacteria (V.fischeri)	EC_{50} (luminescence)	13 mg/L	Park and Choi, (2008)
	Daphnia magna	EC_{50} (24 h immobilization)	380.1 mg/L	Park and Choi, (2008)

	Fish (Oryzias latipes)	LC_{50} (24 h)	88.4 mg/L	Park and Choi, (2008)
	Bean plant (Phaseolus vulgaris)	Root dry weight reduced 66-94%	160 mg/L	Batchelder, (1981)
	Radish (Raphnus sativas.L)	Growth stimulation and N uptake	~160 mg/L	Batchelder, (1982)
Doramectin	Daphnia	PNEC	0.001 µg/L	Boxall et al, (2006)
Enrofloxacin	Bacteria (V.fischeri)	EC_{50} (immobilization 24h)	326.8 mg/L	Park and Choi, (2008)
	Daphnia magna	EC_{50}	131.7 mg/L	Park and Choi, (2008)
Erythromycin	Algae (P.subcapitata)	EC_{50} (72 h growth inhibition)	0.02 mg/L	Isidori et al., (2005)
	Lemna minor	EC_{50}	5.62 mg/L	Pomati et al., (2004)
	Fish (Oryzias latipes)	LC_{50} (96 h mortality)	>100 mg/L	Kim et al., (2009)
Florfenicol	Microorganisms (Aeromonas salmonicida)	MIC	0.3-2.5 mg/L	FDA, (2013)
	Duckweed (Lemna gibba)	EC_{50} (7 d yield)	0.76 mg/L	FDA, (2013)
	Daphnia magna	EC_{50} (24 h)	<100 mg/L	FDA, (2013)
	Fish (Oncorhynchus mykiss)	LC_{50}	>780 mg/L	FDA, (2013)
	Onion (Allium cepa)	EC_{50} (biomass)	0.75 mg/kg	Richter et al., (2016)
	Rapeseed (Brassica napus)	EC_{50} (biomass)	>5 mg/kg	Richter et al., (2016)
Ivermectin	Daphnia	PNEC	0.00025 µg/L	Boxall et al, (2006)
Lincomycin	Earthworms	NOEC	1000 mg/kg	Boxall et al., (2004)
	Daphnia	PNEC	379.4 µg/L	Boxall et al, (2006)

Monensin	Earthworms	NOEC	10 mg/kg	Boxall et al, (2004)
Oxytetracycline	Green algae (M. aeruginosa)	PNEC	0.045 mg/L	Boxall et al, (2006)
	Lemna Minor	EC_{50}	3.26 mg/L	Kołodziejska et al., (2013)
	Daphnia magna	EC_{50}	114 mg/L	Kołodziejska et al., (2013)
	Fish (Oryzias latipes)	LC_{50} (24 h)	215.4 mg/L	Park and Choi, (2008)
	Springtails	LC_{10}	>5000 mg/kg	Bauger et al., (2000)
	Earthworms	LC_{10}	1954 mg/kg	Bauger et al., (2000)
Sulfachloropyridazine	Bacteria (V.fischeri)	EC_{50}	26.4 mg/L	Kim et al., (2007)
	Daphnia magna	EC_{50} (48 h immobilization)	375.3 mg/L	Kim et al., (2007)
	Lemna minor	EC_{50} (48 h)	2.33 mg/L	Pro et al., (2003)
	Fish (Oryzias latipes)	LC_{50} (48 h)	589.3 mg/L	Kim et al., (2007)
Sulfadiazine	Green algae	PNEC	34.9 µg/L	Boxall et al, (2006)
	Daphnia magna	LOEC (24 h immobilization)	150 mg/L	Eguchi et al., (2004)
Sulfadimethoxine	Algae (S. capricornutum)	EC_{50} (growth)	2.3 mg/L	Eguchi et al., (2004)
	Daphnia magna	EC_{50} (96 h immobilization)	204.5 mg/L	Kim et al., (2007)
	Fish (Oryzias latipes)	LC_{50} (48 h)	>100 mg/L	Kim et al., (2007)
Sulfamethoxazole	*E.fetida*	LC_{50}	>4000 mg/kg	Pino et al., (2015)
Sulfamethazine	Bacteria (V.fischeri)	EC_{50}	344.7 mg/L	Kim et al., (2007)
	Freshwater polyp (Hydr vulgaris)	LC_{50}	100 mg/L	Quinn et al., (2008)

	Daphnia magna	EC_{50} (48 h immobilization)	174.4 mg/L	Kim et al., (2007)
	Fish (Oryzias latipes)	LC_{50} (48 h)	>100 mg/L	Kim et al., (2007)
	Oat	EC_{50} (germination)	37 mg/L	Liu et al., (2009)
	Rice	EC_{50} (germination)	45 mg/L	Liu et al., (2009)
	Cucumber	EC_{50} (germination)	>300 mg/L	Liu et al., (2009)
Spiramycin	Green algae (M. aeruginosa)	EC_{50}	0.005 mg/L	Halling-Sørenson (1999)
Streptomycin	Green algae (M. aeruginosa)	EC_{50}	0.007mg/L	Halling-Sørenson (1999)
Tetracycline	Luminescent bacteria		10 mg/L	Jiao et al., (2008)
	Green algae (M. aeruginosa)	EC_{50}	0.09 mg/L	Halling-Sørenson (1999)
	Daphnia magna	EC_{50} (48 h immobilization	340 mg/L	Wollenberger et al., (2000)
	E.fetida	LC_{50}	>2000 mg/kg	Pino et al., (2015)
Tiamulin	Wheat	No effect	No effect	Boxall et al., (2004)
	Lettuce	No effect	No effect	Boxall et al., (2004)
Trimethoprim	Bacteria (V.fischeri)	EC_{50}	176.7 mg/L	Kim et al., (2007)
	Green algae	PNEC	1.6 µg/L	Boxall et al, (2006)
	Daphnia magna	EC_{50} (48 h immobilization)	167.4 mg/L	Kim et al., (2007)
	Fish (Oryzias latipes)	LC_{50} (48 h)	>100 mg/L	Kim et al., (2007)
	Oat	EC_{50} (germination)	86 mg/kg	Liu et al., (2009)
	Rice	EC_{50} (germination)	118 mg/kg	Liu et al., (2009)
	Cucumber	EC_{50} (germination)	>300 mg/kg	Liu et al., (2009)

	E.fetida	LC_{50}	>2000 mg/kg	Pino et al., (2015)
Tylosin	Green algae (M. aeruginosa)	EC_{50}	0.034 mg/L	Halling-Sørenson (1999)
	Daphnia magna	LOEC (24 h immobilization)	700 mg/L	Wollenberger et al., (2000)
	Springtails	LC_{10}	149 mg/kg	Bauger et al., (2000)
	Earthworms	LC_{10}	3306 mg/kg	Bauger et al., (2000)
	Onion (Allium cepa)	EC50 (biomass)	41.3 mg/kg	Richter et al., (2016)
	Rapeseed (Brassica napus)	EC50 (biomass)	61.9 mg/kg	Richter et al., (2016)

1.8 Summary

Research regarding the fate and effects of veterinary medicines in the environment is well established. However in reviewing the literature regarding their fate and effects it is apparent there are currently fundamental gaps in our scientific knowledge which presents uncertainty regarding current assessments of risk. For example current fate and effect assessments are routinely conducted on singular compounds, whereas this is unrepresentative of reality. Typically they will exist synergistically with other organic compounds (i.e., other veterinary medicines, biocides, pesticides and herbicides), which is often hypothesized to influence their fate and risk. Environmental fate assessments typically utilize manure degradation trials to assess the degradability of veterinary medicines within the environment. In doing so the concentrations that would be exposed to land upon manure application can be predicted. However, variability exists between DT_{50} values reported in the literature, indicating our understanding of their exposure is inadequate. This could be related to differences in heterogenic manure properties. Currently these assessments only utilize one manure per animal type but this is not representative of manures regionally or globally. Manures have been reported to

have large differences within their, moisture, pH, redox potential, organic matter content, and microbial populations.

Another criticism of current environmental fate assessments and our scientific understanding is that such experiments are routinely conducted under controlled laboratory conditions. This is expected given the nature of science and the difficulties already faced in controlling external variables. However, this outlines that these assessments do not fully achieve their aim in assessing the fate or effects of veterinary medicines in the environment. Moreover, modern agriculture is continuously evolving, for example manure application has shifted from conventional techniques to advanced injection technologies. The literature regarding their influence on the fate of veterinary medicines is sparse and currently highly variable. In order to assess the exposure of veterinary medicines to environmental compartments it is essential to have an in-depth understanding of these mechanisms.

1.9 Aims and Objectives

Therefore, the aims of the presented research chapters was to investigate whether environmentally realistic conditions and scenarios are driving variability within environmental fate assessments.

The objectives of the presented research therefore were:

- O1 To summarise existing findings regarding the environmental fate of veterinary medicines within the environment
- O2 To investigate if commonly reported pig slurry properties have a significant effect on veterinary medicine degradation and whether these are resulting in poor predictability of exposure within the environmental risk assessment
- O3 Distinguish whether pig slurry pH alters veterinary medicine degradation rates via biotic or abiotic mechanisms

- O4 Evaluate whether anaerobic redox potentials are contributing to variability seen within manure degradation assessments
- O5 Assess whether advanced manure application techniques can be used to reduce the exposure of veterinary medicines into the environment
- O6 Monitor the fate of veterinary medicines at field-scale to elaborate on our understanding of their risk but also validate the sophisticated fate modelling suite FOCUS_PEARL

2.0 Thesis structure

Within the presented thesis the following objectives were achieved within the following chapters. O1 was addressed within a review of the literature which can be found in Chapter 1. This contained a summary and evaluation of the available literature (peer-reviewed articles). Specifically these included details regarding veterinary medicines, occurrence in the environment, environmental exposure, degradation (manures, soils, and groundwaters), sorption, effects to non-target organisms as well as factors affecting their fate in the environment. Objectives 2 and 3 which concerned an investigation to whether manure properties were contributing towards variability within veterinary medicine manure degradation rates were met in Chapter 2. Specifically this was focussed on pig slurry pH. The latter was also elaborated within Chapter 3, where the effect of anaerobic redox potentials on the degradation of veterinary medicines was investigated. These chapters brought specific attention to the risk assessment and whether such variability is contributing to a misunderstanding of environmental exposure and therefore risk. Chapter 4 was concerned in achieving O5 via a semi-field study that investigated the influence of manure application techniques on veterinary medicine fate. O6 was met within Chapter 5 where a field monitoring study was conducted to assess the fate of veterinary medicines at field-scale. This Chapter also provides a thorough investigation to the applicability of sophisticated veterinary medicine leaching models which are routinely used within the environmental risk assessment. Chapter 6 provides an overarching synthesis of the presented research whilst elaborating on the findings scientific implications and conclusions.

References

98/8/EC. 1998. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market.

Aris, A.Z., Shamsuddin, A.S. and Praveena, S.M., 2014. Occurrence of 17α-ethynylestradiol (EE2) in the environment and effect on exposed biota: a review. *Environment International*, 69, pp.104-119.

Adeli, A., Bolster, C.H., Rowe, D.E., McLaughlin, M.R. and Brink, G.E., 2008. Effect of long-term swine effluent application on selected soil properties. *Soil Science*, 173(3), pp.223-235.

Aga, D.S., Lenczewski, M., Snow, D., Muurinen, J., Sallach, J.B. and Wallace, J.S., 2016. Challenges in the measurement of antibiotics and in evaluating their impacts in agroecosystems: a critical review. *Journal of Environmental Quality*, *45*(2), pp.407-419.

Akyol, Ç., Turker, G., Ince, O., Ertekin, E., Üstüner, O. and Ince, B., 2016. Performance and microbial community variations in thermophilic anaerobic digesters treating OTC medicated cow manure under different operational conditions. *Bioresource Technology*, 205, pp.191-198.

Alexandrino, D.A., Mucha, A.P., Almeida, C.M.R., Gao, W., Jia, Z. and Carvalho, M.F., 2017. Biodegradation of the veterinary antibiotics enrofloxacin and ceftiofur and associated microbial community dynamics. *Science of The Total Environment*, 581, pp.359-368.

Ali, M., Wang, J.J., DeLaune, R.D., Seo, D.C., Dodla, S.K. and Hernandez, A.B., 2013. Effect of redox potential and pH status on degradation and adsorption behavior of tylosin in dairy lagoon sediment suspension. *Chemosphere*, 91(11), pp.1583-1589.

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J. and Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology*, *8*(4), p.251.

Alvarez, D.A., Stackelberg, P.E., Petty, J.D., Huckins, J.N., Furlong, E.T., Zaugg, S.D. and Meyer, M.T., 2005. Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream. *Chemosphere*, *61*(5), pp.610-622.

Álvarez, J.A., Otero, L., Lema, J.M. and Omil, F., 2010. The effect and fate of antibiotics during the anaerobic digestion of pig manure. *Bioresource Technology*, 101(22), pp.8581-8586.

Alvarino, T., Nastold, P., Suarez, S., Omil, F., Corvini, P.F.X. and Bouju, H., 2016. Role of biotransformation, sorption and mineralization of 14C-labelled sulfamethoxazole under different redox conditions. *Science of the Total Environment*, *542*, pp.706-715.

An, B., Xu, X., Ma, W., Huo, M., Wang, H., Liu, Z., Cheng, G. and Huang, L., 2021. The adsorption-desorption characteristics and degradation kinetics of ceftiofur in different agricultural soils. *Ecotoxicology and Environmental Safety*, *222*, p.112503.

Andam, C.P., Fournier, G.P. and Gogarten, J.P., 2011. Multilevel populations and the evolution of antibiotic resistance through horizontal gene transfer. *FEMS microbiology reviews*, *35*(5), pp.756-767.

Anderson, J.P.E., 1981. Soil moisture and the rates of biodegradation of diallate and triallate. *Soil Biology and Biochemistry*, *13*(2), pp.155-161.

Andrade, L., Kelly, M., Hynds, P., Weatherill, J., Majury, A. and O'Dwyer, J., 2020. Groundwater resources as a global reservoir for antimicrobial-resistant bacteria. *Water Research*, *170*, p.115360.

Arikan, O., Rice, C., & Codling, E. (2008). Occurrence of antibiotics and hormones in a major agricultural watershed. *Desalination*, 226(1-3), 121-133.

Arikan, O.A., 2008. Degradation and metabolization of chlortetracycline during the anaerobic digestion of manure from medicated calves. *Journal of Hazardous Materials*, 158(2-3), pp.485-490.

Awad, Y.M., Kim, S.C., El-Azeem, S.A.A., Kim, K.H., Kim, K.R., Kim, K., Jeon, C., Lee, S.S. and Ok, Y.S., 2014. Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility. *Environmental earth sciences*, *71*(3), pp.1433-1440.

Baquero, F., Martínez, J.L. and Cantón, R., 2008. Antibiotics and antibiotic resistance in water environments. *Current opinion in biotechnology*, *19*(3), pp.260-265.

Bartelt-Hunt, S., Snow, D.D., Damon-Powell, T. and Miesbach, D., 2011. Occurrence of steroid hormones and antibiotics in shallow groundwater impacted by livestock waste control facilities. *Journal of contaminant hydrology*, *123*(3-4), pp.94-103.

Batchelder, A.R., 1982. Chlortetracycline and oxytetracycline effects on plant growth and development in soil systems. *Journal of Environmental Quality*. 11,675–678.

Bauger, A.J., Jensen, J., Krogh, P.H., 2000. Effects of the antibiotics oxytetracycline and tylosin on soil fauna. *Chemosphere* 40, 751–757.

Benton, T.G., Vickery, J.A. and Wilson, J.D., 2003. Farmland biodiversity: is habitat heterogeneity the key?. *Trends in Ecology & Evolution*, 18(4), pp.182-188.

Bernal, M.P., Alburquerque, J.A. and Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology*, 100(22), pp.5444-5453.

Berthod, L.M.C., 2015. Mechanistic Approach to Predicting the Sorption Characteristics of Pharmaceuticals (Doctoral dissertation, University of Portsmouth).

Binh, C.T.T., Heuer, H., Gomes, N.C.M., Kaupenjohann, M. and Smalla, K., 2010. Similar bacterial community structure and high abundance of sulfonamide resistance genes in field-scale manures. *Manure: management, uses and environmental impacts. Nova Science Publishers, Hauppauge, NY*, pp.141-166.

Blackwell, P. A., Kay, P., and Boxall, A. B. A. (2007). The dissipation and transport of veterinary antibiotics in a sandy loam soil. *Chemosphere* 67, 292–299. doi: 10.1016/j.chemosphere.2006.09.095.

Blackwell, P., Kay, P., Ashauer, R., & Boxall, A. (2009). Effects of agricultural conditions on the leaching behaviour of veterinary antibiotics in soils. *Chemosphere*, 75(1), 13-19.

Blackwell, P.A., Boxall, A.B., Kay, P. and Noble, H., 2005. Evaluation of a lower tier exposure assessment model for veterinary medicines. *Journal of Agricultural and Food Chemistry*, 53(6), pp.2192-2201.

Blackwell, P.A., Kay, P., Ashauer, R. and Boxall, A.B., 2009. Effects of agricultural conditions on the leaching behaviour of veterinary antibiotics in soils. *Chemosphere*, *75*(1), pp.13-19.

Blackwell, P.A., Lützhøft, H.C.H., Ma, H.P., Halling-Sørensen, B., Boxall, A.B. and Kay, P., 2004. Ultrasonic extraction of veterinary antibiotics from soils and pig slurry with SPE clean-up and LC–UV and fluorescence detection. *Talanta*, 64(4), pp.1058-1064.

Bloom RA, Matheson JC. Environmental assessment of aver-mectins by the US food and drug administration. *Veterinary Parasitology*. 1993;48(1-4):281–294.

Bogaard, A., Fraser, R., Heaton, T.H., Wallace, M., Vaiglova, P., Charles, M., Jones, G., Evershed, R.P., Styring, A.K., Andersen, N.H. and Arbogast, R.M., 2013. Crop manuring and intensive land management by Europe's first farmers. *Proceedings of the National Academy of Sciences*, *110*(31), pp.12589-12594.

Boxall, A., Fogg, L., Kay, P., Blackwel1, P., Pemberton, E., & Croxford, A. (2003). Prioritisation of veterinary medicines in the UK environment. *Toxicology Letters*, 142(3), 207-218.

Boxall, A., Kay, P., Blackwell, P., & Fogg, L. (2004). Fate of Veterinary Medicines Applied to Soils. In A. Boxall, P. Kay, P. Blackwell, & L. Fogg, *Pharmaceuticals in the Environment* (pp. 165-180). Berlin, Heidelberg: Springer Berlin Heidelberg.

Boxall, A.B., 2010. Veterinary medicines and the environment. *Comparative and Veterinary Pharmacology*, pp.291-314.

Boxall, A.B., Fogg, L.A., Blackwell, P.A., Blackwell, P., Kay, P., Pemberton, E.J. and Croxford, A., 2004. Veterinary medicines in the environment. *Reviews of Environmental Contamination and Toxicology*, pp.1-91.

Boxall, A.B., Johnson, P., Smith, E.J., Sinclair, C.J., Stutt, E. and Levy, L.S., 2006. Uptake of veterinary medicines from soils into plants. *Journal of Agricultural and Food Chemistry*, *54*(6), pp.2288-2297.

Boxall, A.B.A.; Blackwell, P.; Cavallo, R.; Kay, P.; Tolls, J. The sorption and transport of a sulphonamide antibiotic in soil systems. *Toxicology Letters*. 2002, 131, 19–28.

Boy-Roura, M., Mas-Pla, J., Petrovic, M., Gros, M., Soler, D., Brusi, D. and Menció, A., 2018. Towards the understanding of antibiotic occurrence and transport in groundwater: Findings from the Baix Fluvià alluvial aquifer (NE Catalonia, Spain). *Science of the total environment*, *612*, pp.1387-1406.

Braschi, I., Blasioli, S., Fellet, C., Lorenzini, R., Garelli, A., Pori, M. and Giacomini, D., 2013. Persistence and degradation of new β-lactam antibiotics in the soil and water environment. *Chemosphere*, *93*(1), pp.152-159.

Bundschuh, M., Hahn, T., Ehrlich, B., Höltge, S., Kreuzig, R. and Schulz, R., 2016. Acute toxicity and environmental risks of five veterinary pharmaceuticals for aquatic macroinvertebrates. *Bulletin of environmental contamination and toxicology*, *96*(2), pp.139-143.

Burkhardt, M. and Stamm, C., 2007. Depth distribution of sulfonamide antibiotics in pore water of an undisturbed loamy grassland soil. *Journal of environmental quality*, *36*(2), pp.588-596.

Burkhardt, M., Stamm, C., Waul, C., Singer, H. and Müller, S., 2005. Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. *Journal of Environmental Quality*, 34(4), pp.1363-1371.

Calamari, D., Zuccato, E., Castiglioni, S., Bagnati, R. and Fanelli, R., 2003. Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. *Environmental Science & Technology*, *37*(7), pp.1241-1248.

Canh T. T., A. J. A. Aarnink, M. W. A. Verstegen, and J. W. Schrama.1998a. Influence of dietary factors on the pH and ammonia emis-sion of slurry from growing-finishing pigs. *Journal of Animal Science*.76:1123–1130.

Canh, T. T., A. L. Sutton, A. J. A. Aarnink, M. W. A. Verstegen, J.W. Schrama, and G. C. M Bakker. 1998b. Dietary carbohydratesalter the fecal composition and pH and the ammonia emission from slurry of growing pigs. *Journal of Animal Science*. 76:1887–1895.

Carballa, M., Omil, F., Ternes, T. and Lema, J.M., 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Research*, 41(10), pp.2139-2150.

Castillo, M.D.P. and Torstensson, L., 2007. Effect of biobed composition, moisture, and temperature on the degradation of pesticides. *Journal of Agricultural and Food Chemistry*, *55*(14), pp.5725-5733.

Celik, I., Ortas, I. and Kilic, S., 2004. Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. *Soil and Tillage Research*, *78*(1), pp.59-67.

Chen H, Dong YH, Wang H (2008) Residual characteristics of sulfanilamide in animal feces in Jiangsu Province. *Journal of Agriculture and Environmental Sciences* 27(1):385–389.

Chen, S., Li, X., Sun, G., Zhang, Y., Su, J. and Ye, J., 2015. Heavy metal induced antibiotic resistance in bacterium LSJC7. *International Journal of Molecular Sciences*, *16*(10), pp.23390-23404.

Chen, K.L., Liu, L.C. and Chen, W.R., 2017. Adsorption of sulfamethoxazole and sulfapyridine antibiotics in high organic content soils. *Environmental Pollution*, 231, pp.1163-1171.

Chen, Z., Wang, Y. and Wen, Q., 2018. Effects of chlortetracycline on the fate of multi-antibiotic resistance genes and the microbial community during swine manure composting. *Environmental Pollution*, 237, pp.977-987.

Chepete, J.H., Xin, H. and Li, H., 2011. Ammonia emissions of laying-hen manure as affected by accumulation time. *The Journal of Poultry Science*, *48*(2), pp.133-138.

Chiu, S.H.L., Green, M.L., Baylis, F.P., Eline, D., Rosegay, A., Meriwether, H. and Jacob, T.A., 1990. Absorption, tissue distribution, and excretion of tritium-labeled ivermectin in cattle, sheep, and rat. *Journal of Agricultural and Food Chemistry*, 38(11), pp.2072-2078.

Chu, B., Goyne, K.W., Anderson, S.H., Lin, C.H. and Lerch, R.N., 2013. Sulfamethazine sorption to soil: vegetative management, pH, and dissolved organic matter effects. *Journal of Environmental Quality*, *42*(3), pp.794-805.

Chu, Y., Fang, C., Wang, H., Wu, X., Gu, Y. and Shu, J., 2017. Effects of anaerobic composting on tetracycline degradation in swine manure. *Chinese Journal of Chemical Engineering*, 25(10), pp.1505-1511.

Conde-Cid, M., Ferreira-Coelho, G., Nú˜nez-Delgado, A., Fern´andez-Calvi˜no, D., Arias-Est´evez, M., Alvarez-Rodríguez, E., Fern´andez-Sanjurjo, M.J., 2019. Competitive adsorption of tetracycline, oxytetracycline and chlortetracycline on soils with different pH value and organic matter content. *Environmental Research*. 178, 108669.

Conde-Cid, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Fernández-Calviño, D. and Arias-Estévez, M., 2020. Tetracycline and sulfonamide antibiotics in soils: presence, fate and environmental risks. *Processes*, *8*(11), p.1479.

Cui, H., Wang, S.-P., Fu, J., Zhou, Z.-Q., Zhang, N., and Guo, L. (2014). Influence of ciprofloxacin on microbial community structure and function in soils. *Biology and Fertility of Soils* 50, 939–947. doi: 10.1007/s00374-014-0914-y.

CVMP., 2011. Reflection paper on risk mitigation measures related to the environmental risk assessment of veterinary medicinal products Medicinal Products, 2000. EMEA/CVMP/151/99-

final.

Cycoń, M., Mrozik, A. and Piotrowska-Seget, Z., 2019. Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity. *Frontiers in Microbiology*, *10*, p.338.

Davies, J., 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science*, *264*(5157), pp.375-382.

De Liguoro, M., Cibin, V., Capolongo, F., Halling-Sørensen, B. and Montesissa, C., 2003. Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. *Chemosphere*, *52*(1), pp.203-212.

De Liguoro, M., Poltronieri, C., Capolongo, F. and Montesissa, C., 2007. Use of sulfadimethoxine in intensive calf farming: evaluation of transfer to stable manure and soil. *Chemosphere*, 68(4), pp.671-676.

DEFRA (2011). Controls on Animal By - Products. Guidance on Regulation (EC) 1069/2009 and accompanying implementing Regulation (EC) 142/2011, enforced in England by the Animal By - Products (Enforcement) (England) Regulations 2011: Version 4. [online]. Available:https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/69458/pb13688-animal-by-products-controls-111130.pdf [Accessed 7 Dec. 2018].

DEFRA, 2010. Fertiliser Manual (RB209). 8th Edition. [online] Available at: <http://file:///C:/Users/jnigh/AppData/Local/Temp/Defra%20Fertiliser%20manual%202011-1.pdf> [Accessed 21 July 2021].

Dias, B.O., Silva, C.A., Higashikawa, F.S., Roig, A. and Sánchez-Monedero, M.A., 2010. Use of biochar as bulking agent for the composting of poultry manure: effect on organic matter degradation and humification. *Bioresource Technology*, *101*(4), pp.1239-1246.

Dibner, J.J. and Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. *Poultry Science*, 84(4), pp.634-643.

Dolliver, H. and Gupta, S., 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *Journal of Environmental Quality*, 37(3), pp.1227-1237.

Dolliver, H., Gupta, S. and Noll, S., 2008. Antibiotic degradation during manure composting. *Journal of Environmental Quality*, 37(3), pp.1245-1253.

Du, L. and Liu, W., 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agronomy for Sustainable Development*, *32*(2), pp.309-327.

Drzymała, J. and Kalka, J., 2020. Ecotoxic interactions between pharmaceuticals in mixtures: Diclofenac and sulfamethoxazole. *Chemosphere*, 259, p.127407.

E.G.C. Clarke, A.C. Moffat, Pharmaceutical Society of Great Britain Department of Pharmaceutical Sciences Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-Mortem Material (second ed.), Pharmaceutical Press, London (1986).

Clarke, E.G.C., 1986. Pharmaceutical Society of Great Britain; Department of Pharmaceutical Sciences. Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-Mortem Material, ; Moffat, AC, Ed. EC, 2003. Council and parliament prohibit antibiotics as growth promoters: Commis-sioner Byrne welcomes adoption of Regulation on feed additives. IP/03/1058 (http://europa.eu/rapid/press-release_Id P-03-1058_en.htm?locale=en).

EFSA, 2021. Guidance on the assessment of the safety of feed additives for the consumer. [online] EFSA. Available at: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.5022> [Accessed 23 June 2021].

Eghball, B., 2000. Nitrogen mineralization from field-applied beef cattle feedlot manure or compost. *Soil Science Society of America Journal*, *64*(6), pp.2024-2030.

EMA (2011) Guideline on determining the fate of veterinary medicinal products in manure. EMA/CVMP/ERA/430327/2009.

EMA (2016) Revised Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1).

EMA Committee for Medicinal Products for Veterinary Use (CVMP) and EFSA Panel on Biological Hazards (BIOHAZ), Murphy, D., Ricci, A., Auce, Z., Beechinor, J.G., Bergendahl, H., Breathnach, R., Bureš, J., Duarte Da Silva, J.P., Hederová, J. and Hekman, P., 2017. EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA). EFSA Journal, 15(1), p.e04666.

Ermawati, R., Morimura, S., Tang, Y., Liu, K. and Kida, K., 2007. Degradation and behavior of natural steroid hormones in cow manure waste during biological treatments and ozone oxidation. *Journal of Bioscience and Bioengineering*, *103*(1), pp.27-31.

Estrada-Arriaga, E.B., Cortés-Muñoz, J.E., González-Herrera, A., Calderón-Mólgora, C.G., de Lourdes Rivera-Huerta, M., Ramírez-Camperos, E., Montellano-Palacios, L., Gelover-Santiago, S.L., Pérez-Castrejón, S., Cardoso-Vigueros, L. and Martín-Domínguez, A., 2016. Assessment of full-scale biological nutrient removal systems upgraded with physico-chemical processes for the removal of emerging pollutants present in wastewaters from Mexico. *Science of the Total Environment*, 571, pp.1172-1182.

EU. Directive 81/852/EEC of the Commission of September 28, 1981 as published in the Official Journal of the European Community of November 6, 1981, No. L317, p. 16, amended by: Directive 92/18/EEC of the Commission of March 20, 1992 as published in the Official Journal of the European Community of April 10, 1992, No. L97, p. 1; and Directive 93/40/EEC

of the Council of June 14, 1993 as published in the Official Journal of the European Community of August 24, 1993, No. L214, p. 31.

EU. Directive 81/852/EEC of the Commission of September 28, 1981 as published in the Official Journal of the European Community of November 6, 1981, No. L317, p. 16, amended by: Directive 92/18/EEC of the Commission of March 20, 1992 as published in the Official Journal of the European Community of April 10, 1992, No. L97, p. 1; and Directive 93/40/EEC of the Council of June 14, 1993 as published in the Official Journal of the European Community of August 24, 1993, No. L214, p. 31.

European Commission (2014). Good practices to reduce nutrient loss in the Lombardy region (Italy). Closing the mineral cycles at farm level. [online] Lombardy: European Commission. Available at: https://www.ecologic.eu/11275 [Accessed 26 Mar. 2019].

European Commission Bo. 142.2011 of 25th February 2011, Implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive, [accessed 07/12/2018]. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0142&from=EN

European Commission No. 1069/2009 of 21 October 2009, laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation), [accessed 07/12/2018]. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1069&from=EN

European Commission Regulation, 811/2011, 23rd March 2011, The Animal By-Products (Enforcement) (England), available from: http://www.legislation.gov.uk/uksi/2011/881/introduction/made .

European Commission, 2017. *A European One Health Action Plan against Antimicrobial Resistance (AMR)*. [online] Available at: <https://ec.europa.eu/health/sites/default/files/antimicrobial_resistance/docs/amr_2017_action-plan.pdf> [Accessed 25 October 2021].

European Food Safety Authority, 2013. Conclusion on the peer review of the pesticide risk assessment of the active substance metaflumizone. *EFSA Journal*, *11*(10), p.3373.

European Medicines Agency (2018), European Surveillance of Veterinary Antimicrobial Consumption, 2018. 'Sales of veterinary antimicrobial agents in 30 European countries in 2016'. (EMA/275982/2018).

Fabrega, J. and Carapeto, R., 2020. Regulatory review of the environmental risk assessment of veterinary medicinal products in the European Union, with particular focus on the centralised authorisation procedure. *Environmental Sciences Europe*, *32*(1), pp.1-15.

Fangueiro, D., Hjorth, M. and Gioelli, F., 2015. Acidification of animal slurry–a review. *Journal of Environmental Management*, 149, pp.46-56.

FDA, 2013. Environmental Assessment for Aquaflor (Florfenicol). Aquaflor for Freshwater-reared Finfish in Recirculating Aquaculture Systems. [online] EPA. Available at: <https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/113> [Accessed 7 May 2020].

Feinman, S.E., Matheson III, J.C., 1978. Draft environmental impact statement subtherapeutic antibacterial agents in animal feeds. Bureau of Veterinary Medicine. *Food and Drug Administration*, Rockville, MD, USA.

Fick, J., Söderström, H., Lindberg, R.H., Phan, C., Tysklind, M. and Larsson, D.J., 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. *Environmental Toxicology and Chemistry*, *28*(12), pp.2522-2527.

Filippitzi, M.E., Callens, B., Pardon, B., Persoons, D. and Dewulf, J., 2014. Antimicrobial use in pigs, broilers and veal calves in Belgium. *Vlaams diergeneeskundig tijdschrift*, 83(5), pp.215-224.

FOCUS, 2000, Leaching modelling workgroup. FOCUS General Guidance for Groundwater Scenarios.

FOCUS. (2014), “groundwater scenarios in the EU review of active substances" - The report of the work of the Groundwater Scenarios Workgroup of FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe), Version 1 of November 2000. EC Document Reference Sanco/321/2000 rev.2, 202pp

Franson, J.C., E.J. Kolbe, and J.W. Carpenter. 1985. Famphur toxicosis in a bald eagle. *Journal of Wildlife Diseases* 21(3):318-320

Frear, C., Liao, W., Ewing, T. and Chen, S., 2011. Retraction: Evaluation of Co-Digestion at a Commercial Dairy Anaerobic Digester.

Elmund, G.K., Morrison, S.M., Grant, D.W. and Nevins, M.P., 1971. Role of excreted chlortetracycline in modifying the decomposition process in feedlot waste. Bulletin of Environmental Contamination and Toxicology, 6(2), pp.129-132. Gao, Y., Sun, Z., Liu, Y.,

Sun, X., Li, Y., Bao, Y. and Wang, G., 2007. Effect of albendazole anthelmintics on the enzyme activities of different tissue regions in Eisenia fetida. *European Journal of Soil Biology*, *43*, pp.S246-S251.

García-Galán, M.J., Frömel, T., Müller, J., Peschka, M., Knepper, T., Díaz-Cruz, S. and Barceló, D., 2012. Biodegradation studies of N 4-acetylsulfapyridine and N 4-acetylsulfamethazine in environmental water by applying mass spectrometry techniques. *Analytical and Bioanalytical Chemistry*, 402(9), pp.2885-2896.

Gaskins, H.R., Collier, C.T. and Anderson, D.B., 2002. Antibiotics as growth promotants: mode of action. *Animal Biotechnology*, *13*(1), pp.29-42.

Gelegenis, J., Georgakakis, D., Angelidaki, I., Christopoulou, N. and Goumenaki, M., 2007. Optimization of biogas production from olive-oil mill wastewater, by codirecting with diluted poultry-manure. *Applied Energy*, *84*(6), pp.646-663.

Gevao, B., Semple, K., & Jones, K. (2000). Bound pesticide residues in soils: a review. *Environmental Pollution*, 108(1), 3-14.

Gholamhoseini, M., Ghalavand, A., Khodaei-Joghan, A., Dolatabadian, A., Zakikhani, H. and Farmanbar, E., 2013. Zeolite-amended cattle manure effects on sunflower yield, seed quality, water use efficiency and nutrient leaching. *Soil and Tillage Research*, *126*, pp.193-202.

Grenni, P., Ancona, V. and Caracciolo, A.B., 2018. Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal*, *136*, pp.25-39.

Gros, M., Petrović, M., Ginebreda, A. and Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. *Environment International*, *36*(1), pp.15-26.

Grote, M., Schwake-Anduschus, C., Michel, R., Stevens, H., Heyser, W., Langenkamper, G., Betsche, T. and Freitag, M., 2007. Incorporation of veterinary antibiotics into crops from manured soil. *Landbauforschung Volkenrode*, *57*(1), p.25.

Guo, J., Ostermann, A., Siemens, J., Dong, R. and Clemens, J., 2012. Short term effects of copper, sulfadiazine and difloxacin on the anaerobic digestion of pig manure at low organic loading rates. *Waste Management*, 32(1), pp.131-136.

Halling-Sørensen, B. (2000). Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere*, 40(7), 731-739.

Halling-Sørensen, B., Jacobsen, A.-M., Jensen, J., Sengeløv, G., Vaclavik, E., and Ingerslev, F. (2005). Dissipation and effects of chlortetracycline and tylosin in two agricultural soils: a field-scale study in southern Denmark. *Environment of Toxicology and Chemistry*. 24, 802–810. doi: 10.1897/03-576.1.

Halling-Sørensen, B.N.N.S., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Lützhøft, H.H. and Jørgensen, S.E., 1998. Occurrence, fate and effects of pharmaceutical substances in the environment-A review. *Chemosphere*, *36*(2), pp.357-393.

Hammesfahr, U., Heuer, H., Manzke, B., Smalla, K., and Thiele-Bruhn, S. (2008). Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils. *Soil Biology and Biochemistry*. 40, 1583–1591. doi: 10.1016/j.soilbio.2008.01.010.

Hamscher, G., Pawelzick, H.T., Höper, H. and Nau, H., 2004. Antibiotics in soil: routes of entry, environmental concentrations, fate and possible effects. In *Pharmaceuticals in the Environment* (pp. 139-147). Springer, Berlin, Heidelberg.

Hamscher, G., Pawelzick, H.T., Höper, H. and Nau, H., 2005. Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. *Environmental Toxicology and Chemistry: An International Journal*, *24*(4), pp.861-868.

Hansen, M., Krogh, K.A., Björklund, E., Halling-Sørensen, B. and Brandt, A., 2009. Environmental risk assessment of ionophores. *TrAC Trends in Analytical Chemistry*, *28*(5), pp.534-542.

Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters*, *131*(1-2), pp.5-17.

Henny, C.J., Blus, L.J., Kolbe, E.J. and Fitzner, R.E., 1985. Organophosphate insecticide (famphur) topically applied to cattle kills magpies and hawks. *The Journal of Wildlife Management*, pp.648-658.

Hirsch, R., Ternes, T., Haberer, K. and Kratz, K.L., 1999. Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment*, *225*(1-2), pp.109-118.

Hjorth, M., Christensen, K.V., Christensen, M.L. and Sommer, S.G., 2011. Solid–liquid separation of animal slurry in theory and practice. *Sustainable Agriculture Volume 2*, pp.953-986.

Ho, Y., Zakaria, M., Latif, P., & Saari, N. 2013. Degradation of veterinary antibiotics and hormone during broiler manure composting. *Bioresource Technology*, 131, 476-484.

Ho, Y.B., Zakaria, M.P., Latif, P.A. and Saari, N., 2012. Simultaneous determination of veterinary antibiotics and hormone in broiler manure, soil and manure compost by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1262*, pp.160-168.

Hooda, P.S., Edwards, A.C., Anderson, H.A. and Miller, A., 2000. A review of water quality concerns in livestock farming areas. *Science of the Total Environment*, 250(1-3), pp.143-167.

Höper, H., Kues, J., Nau, H. and Hamscher, G., 2002. Eintrag und Verbleib von Tierarzneimittelwirkstoffen in Böden. *Bodenschutz*, *4*(2), pp.141-148.

Hu, J.T., Zong, H.M., Wang, J.Y., Ma, D.Y., 2008. Adsorption behaviors of florfenicol on sediment in the ocean. *Environmental Chemistry*. 27 (4), 481–484.

Huang, J., Yu, Z., Gao, H., Yan, X., Chang, J., Wang, C., Hu, J. and Zhang, L., 2017. Chemical structures and characteristics of animal manures and composts during composting and assessment of maturity indices. *PloS one*, *12*(6), p.e0178110.

Huang, L., Liu, G., Dong, G., Wu, X., Wang, C. and Liu, Y., 2017. Reaction mechanism of zero-valent iron coupling with microbe to degrade tetracycline in permeable reactive barrier (PRB). *Chemical Engineering Journal*, 316, pp.525-533.

Ingerslev, F. and Halling-Sørensen, B., 2001. Biodegradability of metronidazole, olaquindox, and tylosin and formation of tylosin degradation products in aerobic soil–manure slurries. *Ecotoxicology and Environmental Safety*, 48(3), pp.311-320.

Inyang, M., Flowers, R., McAvoy, D. and Dickenson, E., 2016. Biotransformation of trace organic compounds by activated sludge from a biological nutrient removal treatment system. *Bioresource Technology*, *216*, pp.778-784.

Ishikawa, N.K., Touno, E., Higashiyama, Y., Sasamoto, M., Soma, M., Yoshida, N., Ito, A. and Umita, T., 2018. Determination of tylosin excretion from sheep to assess tylosin spread to agricultural fields by manure application. *Science of the Total Environment*, 633, pp.399-404.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L. and Parrella, A., 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment*, *346*(1-3), pp.87-98.

Qiu, J., Zhao, T., Liu, Q., He, J., He, D., Wu, G., Li, Y., Jiang, C. and Xu, Z., 2016. Residual veterinary antibiotics in pig excreta after oral administration of sulfonamides. *Environmental Geochemistry and Health*, 38(2), pp.549-556. J. Qiu, T.

Jacobsen, A.M., Halling-Sørensen, B., Ingerslev, F. and Hansen, S.H., 2004. Simultaneous extraction of tetracycline, macrolide and sulfonamide antibiotics from agricultural soils using pressurised liquid extraction, followed by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1038*(1-2), pp.157-170.

Jang, Y.N., Hwang, O., Jung, M.W., Ahn, B.K., Kim, H., Jo, G. and Yun, Y.M., 2021. Comprehensive analysis of microbial dynamics linked with the reduction of odorous compounds in a full-scale swine manure pit recharge system with recirculation of aerobically treated liquid fertilizer. *Science of The Total Environment*, 777, p.146122.

Jiao, S.J., Zheng, S.R., Yin, D.Q., Wang, L.H., Chen, L.Y., 2008. Aqueous photolysis of tetracycline and toxicity of photolytic products to luminescent bacteria. *Chemosphere*. 73, 377–382.

Jones AD, Bruland GL, Agrawal SG, Vasudevan D (2005) Factors influencing the sorption of oxytetracycline to soils. *Environmental Toxicology and Chemistry* 24:761–770

Joy, S.R., Bartelt-Hunt, S.L., Snow, D.D., Gilley, J.E., Woodbury, B.L., Parker, D.B., Marx, D.B. and Li, X., 2013. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. *Environmental Science & Technology*, *47*(21), pp.12081-12088.

Junker, T., Atorf, C., Berkner, S., Düring, R.A., Hennecke, D., Herrchen, M., Konradi, S., Merrettig-Bruns, U., Römbke, J., Wagner, J. and Weinfurtner, K., 2020. Development of a test method for transformation of veterinary pharmaceuticals and biocides in anaerobic liquid manure. *Environmental Sciences Europe*, 32(1), pp.1-21.

Junker, T., Hennecke, D., Herrchen, M., Düring, R., Thiele-Bruhn, S., Meinerling, M., Fiebig, S., Topp, E. and Völkel, W., 2016. Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: Ring test for validation of a draft test protocol for studies on transformation in manure. [online] Wörlitzer: Umweltbundesamt. Available at: <https://www.oecd.org/env/ehs/testing/validation-report-anaerobic-transformation-liquid-manure.pdf> [Accessed 23 November 2021].

Kanazawa, J. (1989). Relationship between the soil sorption constants for pesticides and their physicochemical properties. *Environmental Toxicology and Chemistry*, 8(6), 477–484. doi:10.1002/etc.5620080604

Karcı, A. and Balcıoğlu, I.A., 2009. Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. *Science of the Total Environment*, *407*(16), pp.4652-4664.

Kasprzyk-Hordern, B., Dinsdale, R.M. and Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Research*, 42(13), pp.3498-3518.

Kay, P., Blackwell, P., & Boxall, A. (2005). A lysimeter experiment to investigate the leaching of veterinary antibiotics through a clay soil and comparison with field data. *Environmental Pollution*, 134(2), 333-341.

Kay, P., Blackwell, P., & Boxall, A. (2005). Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land. *Chemosphere*, 60(4), 497-507.

Kay, P., Blackwell, P.A. and Boxall, A., (2004). Fate of veterinary antibiotics in a macroporous tile drained clay soil. *Environmental Toxicology and Chemistry*, *23*(5), pp.1136-1144.

Kay, P., Blackwell, P.A. and Boxall, A.B., 2005. Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. *Chemosphere*, *59*(7), pp.951-959.

Kay, P., Blackwell, P.A. and Boxall, A.B., 2005b. Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land. *Chemosphere*, *60*(4), pp.497-507.

Keighley, N., Ramwell, C., Sinclair, C. and Werner, D., 2021. Highly variable soil dissipation of metaldehyde can explain its environmental persistence and mobility. *Chemosphere*, *283*, p.131165.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecological indicators, 8(1), pp.1-13.

Kim, J.W., Ishibashi, H., Yamauchi, R., Ichikawa, N., Takao, Y., Hirano, M., Koga, M. and Arizono, K., 2009. Acute toxicity of pharmaceutical and personal care products on freshwater crustacean (Thamnocephalus platyurus) and fish (Oryzias latipes). *The Journal of Toxicological Sciences*, *34*(2), pp.227-232.

Kim, K.R., Owens, G., Ok, Y.S., Park, W.K., Lee, D.B. and Kwon, S.I., 2012. Decline in extractable antibiotics in manure-based composts during composting. *Waste Management*, 32(1), pp.110-116.

Kim, S.C., Yang, J.E., Ok, Y.S., Jung, D.Y. and Carlson, K., 2012. Degradation kinetics of three veterinary antibiotics in composted and stockpiled manure. *Korean Journal of Soil Science and Fertilizer*, 45(1), pp.43-50.

Kim, Y., Choi, K., Jung, J., Park, S., Kim, P.G. and Park, J., 2007. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environment International*, *33*(3), pp.370-375.

Klausner, S.D., Zwerman, P.J. and Ellis, D.F., 1976. Nitrogen and Phosphorus Losses from Winter Disposal of Dairy Manure 1. *Journal of Environmental Quality*, *5*(1), pp.47-49.

Kleywegt, S., Pileggi, V., Yang, P., Hao, C., Zhao, X., Rocks, C., Thach, S., Cheung, P. and Whitehead, B., 2011. Pharmaceuticals, hormones and bisphenol A in untreated source and

finished drinking water in Ontario, Canada—occurrence and treatment efficiency. *Science of the Total Environment*, 409(8), pp.1481-1488.

Koike, S., Krapac, I.G., Oliver, H.D., Yannarell, A.C., Chee-Sanford, J.C., Aminov, R.I. and Mackie, R.I., 2007. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Applied Environmental Microbiology*, *73*(15), pp.4813-4823.

Kolar, B. and Finizio, A., 2017. Assessment of environmental risks to groundwater ecosystems related to use of veterinary medicinal products. *Regulatory Toxicology and Pharmacology*, *88*, pp.303-309.

Kołodziejska, M., Maszkowska, J., Białk-Bielińska, A., Steudte, S., Kumirsk, J., Stepnowski, P., Stolte, S., 2013. Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry. *Chemosphere*. 92 (9), 1253–1259.

Kolz, A.C., Moorman, T.B., Ong, S.K., Scoggin, K.D. and Douglass, E.A., 2005. Degradation and metabolite production of tylosin in anaerobic and aerobic swine-manure lagoons. *Water Environment Research*, 77(1), pp.49-56.

Komiskey, M.J., Stuntebeck, T.D., Frame, D.R. and Madison, F.W., 2011. Nutrients and sediment in frozen-ground runoff from no-till fields receiving liquid-dairy and solid-beef manures. *Journal of Soil and Water Conservation*, *66*(5), pp.303-312.

Krein, A., Keßler, S., Meyer, B., Pailler, J.Y., Guignard, C. and Hoffmann, L., 2013. Concentrations and loads of dissolved xenobiotics and hormones in two small river catchments of different land use in Luxembourg. *Hydrological Processes*, *27*(2), pp.284-296.

Kreuzig R (2010) The reference manure concept for transformation tests of veterinary medicines and biocides in liquid manure. *Clean Soil Air & Water* 38(8):697–70

Kreuzig, R. and Höltge, S., 2005. Investigations on the fate of sulfadiazine in manured soil: laboratory experiments and test plot studies. *Environmental Toxicology and Chemistry: An International Journal*, 24(4), pp.771-776.

Kreuzig, R., Blümlein, K. and Höltge, S., 2007. Fate of the benzimidazole antiparasitics flubendazole and fenbendazole in manure and manured soipotterls. *CLEAN–Soil, Air, Water*, 35(5), pp.488-494.

Kreuzig, R., Höltge, S., Brunotte, J., Berenzen, N., Wogram, J. and Schulz, R., 2005. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. *Environmental Toxicology and Chemistry: An International Journal*, 24(4), pp.777-781.

Kuchta, S.L. and A.J. Cessna. 2009. Lincomycin and spectinomycin concentrations in liquid swine manure and their persistence during simulated manure storage. *Archives of Environmental Contamination and Toxicology*. 57:1–10. DOI 10.1007/s00244-008-9229-z.

Kumar, R.R., Park, B.J. and Cho, J.Y., 2013. Application and environmental risks of livestock manure. *Journal of the Korean Society for Applied Biological Chemistry*, *56*(5), pp.497-503.

Kümmerer, K. (2009). The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges. *Journal of Environmental Management,* 90(8), 2354–2366. doi:10.1016/j.jenvman.2009.01.023

Kümmerer, K., 2003. Significance of antibiotics in the environment. *Journal of Antimicrobial Chemotherapy*, *52*(1), pp.5-7.

Kurwadkar, S.T., Adams, C.D., Meyer, M.T. and Kolpin, D.W., 2007. Effects of sorbate speciation on sorption of selected sulfonamides in three loamy soils. *Journal of Agricultural and Food Chemistry*, 55(4), pp.1370-1376.

Lakshminarasimman, N., Quiñones, O., Vanderford, B.J., Campo-Moreno, P., Dickenson, E.V. and McAvoy, D.C., 2018. Biotransformation and sorption of trace organic compounds in biological nutrient removal treatment systems. *Science of the Total Environment*, *640*, pp.62-72.

Lamshöft, M., Sukul, P., Zühlke, S. and Spiteller, M., 2010. Behaviour of 14C-sulfadiazine and 14C-difloxacin during manure storage. *Science of the Total Environment*, *408*(7), pp.1563-1568.

Langford, K.H., Øxnevad, S., Schøyen, M. and Thomas, K.V., 2014. Do antiparasitic medicines used in aquaculture pose a risk to the Norwegian aquatic environment?. *Environmental Science & Technology*, *48*(14), pp.7774-7780.

Larkin, R.P., Honeycutt, C.W. and Griffin, T.S., 2006. Effect of swine and dairy manure amendments on microbial communities in three soils as influenced by environmental conditions. *Biology and Fertility of Soils*, *43*(1), pp.51-61.

Leal, R.M.P., Figueira, R.F., Tornisielo, V.L. and Regitano, J.B., 2012. Occurrence and sorption of fluoroquinolones in poultry litters and soils from São Paulo State, Brazil. *Science of the Total Environment*, *432*, pp.344-349.

Lee, J., 2010. Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. *Scientia Horticulturae*, 124(3), pp.299-305.

Lekagul, A., Tangcharoensathien, V. and Yeung, S., 2019. Patterns of antibiotic use in global pig production: a systematic review. *Veterinary and Animal Science*, 7, p.100058.

Letsinger, S., Kay, P., Rodríguez-Mozaz, S., Villagrassa, M., Barceló, D. and Rotchell, J.M., 2019. Spatial and temporal occurrence of pharmaceuticals in UK estuaries. *Science of the Total Environment*, *678*, pp.74-84.

Li, L.-L., Huang, L.-D., Chung, R.-S., Fok, K.-H., and Zhang, Y.-S., 2010. Sorption and dissipation of tetracyclines in soils and compost. *Pedosphere* 20, 807–816. doi: 10.1016/S1002-0160(10)60071-9.

Li, X., Liu, C., Chen, Y., Huang, H. and Ren, T., 2018. Antibiotic residues in liquid manure from swine feedlot and their effects on nearby groundwater in regions of North China. *Environmental Science and Pollution Research*, *25*(12), pp.11565-11575.

Li, Y., Park, S.Y. and Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews*, *15*(1), pp.821-826.

Lin, C.H., Lerch, R.N., Goyne, K.W. and Garrett, H.E., 2011. Reducing herbicides and veterinary antibiotics losses from agroecosystems using vegetative buffers. *Journal of Environmental Quality*, *40*(3), pp.791-799.

Lin, K., and Gan, J. (2011). Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. *Chemosphere* 83, 240–246. doi: 10.1016/j.chemosphere.2010.12.083.

Ling-Ling, L.I., Huang, L.D., Chung, R.S., Ka-Hang, F.O.K. and Zhang, Y.S., 2010. Sorption and dissipation of tetracyclines in soils and compost. *Pedosphere,* 20(6), pp.807-816.

Lithourgidis, A. S., Matsi, T., Barbayiannis, N., & Dordas, C. A. (2007). Effect of Liquid Cattle Manure on Corn Yield, Composition, and Soil Properties. *Agronomy Journal*, 99(4), 1041. doi:10.2134/agronj2006.0332

Liu, F., Wu, J., Ying, G.-G., Luo, Z., and Feng, H. (2012). Changes in functional diversity of soil microbial community with addition of antibiotics sulfamethoxazole and chlortetracycline. *Applied Microbiology and Biotechnology.* 95, 1615–1623. doi: 10.1007/s00253-011-3831-0.

Liu, F., Ying, G.-G., Tao, R., Zhao, J.-L., Yang, J.-F., & Zhao, L.-F. (2009, 5 1). Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. *Environmental Pollution*, 157(5), 1636-1642.

Liu, J., Kleinman, P.J., Aronsson, H., Flaten, D., McDowell, R.W., Bechmann, M., Beegle, D.B., Robinson, T.P., Bryant, R.B., Liu, H. and Sharpley, A.N., 2018. A review of regulations and guidelines related to winter manure application. *Ambio*, pp.1-14.

Liu, J., Xu, X.H., Li, H.T. and Xu, Y., 2011. Effect of microbiological inocula on chemical and physical properties and microbial community of cow manure compost. *Biomass and Bioenergy*, *35*(8), pp.3433-3439.

Lamshöft, M., Sukul, P., Zühlke, S. and Spiteller, M., 2007. Metabolism of 14C-labelled and non-labelled sulfadiazine after administration to pigs. *Analytical and Bioanalytical Chemistry*, 388(8), pp.1733-1745.

Ma, Y., Wilson, C.A., Novak, J.T., Riffat, R., Aynur, S., Murthy, S. and Pruden, A., 2011. Effect of various sludge digestion conditions on sulfonamide, macrolide, and tetracycline

resistance genes and class I integrons. *Environmental Science & Technology*, 45(18), pp.7855-7861.

MacKay, A.A., Canterbury, B., 2005. Oxytetracycline sorption to organic matter by metal-bridging. *Journal of Environmental Quality*. 34 (6), 1964–1971.

Mackenzie, S.L., Hall, G., Schaerf, T.M. and Andrew, N.R., 2021. Impacts of macrocyclic lactones on larval survival, growth and development of three dung beetle species (Coleoptera: Scarabaeidae). *Austral Entomology*.

Madsen, M., Holm-Nielsen, J.B. and Esbensen, K.H., 2011. Monitoring of anaerobic digestion processes: A review perspective. *Renewable and Sustainable Energy Reviews*, 15(6), pp.3141-3155.

Mäenpää KA, Sormunen AJ, Kukkonen JVK (2003) Bioaccumulation and toxicity of sediment associated herbicides (ioxynil, pendimethalin, and bentazone) in Lumbriculus variegatus (Oligochaeta) and Chironomus riparius (Insecta). *Ecotoxicology and Environmental Safety* 56:398–410 .

MAFF, Code of Good Agricultural Practice for the Protection of Water ; Ministry of Agriculture, Fisheries and Food: London, U.K., 1998.

Maguire, R. O., Kleinman, P. J. A., Dell, C. J., Beegle, D. B., Brandt, R. C., McGrath, J. M., & Ketterings, Q. M. (2011). Manure Application Technology in Reduced Tillage and Forage Systems: A Review. *Journal of Environment Quality*, 40(2), 292. doi:10.2134/jeq2009.0228

Mahimairaja, S., Bolan, N.S., Hedley, M.J. and Macgregor, A.N., 1994. Losses and transformation of nitrogen during composting of poultry manure with different amendments: an incubation experiment. *Bioresource Technology*, *47*(3), pp.265-273.

Montforts, Tarazona, L. (2003). Environmental Risk Assessment for Veterinary Medicinal Products Part 4. Exposure assessment scenarios. Retrieved from RIVM: https://www.researchgate.net/publication/27452012_Environmental_Risk_Assessment_for_Veterinary_Medicinal_Products_Part_4_Exposure_assessment_scenarios .

Martens, R., Wetzstein, H.-G., Zadrazil, F., Capelari, M., Hoffmann, P., and Schmeer, N. (1996). Degradation of the fluoroquinolone enrofloxacin by wood-rotting fungi. *Applied Environmental Microbiology*. 62, 4206–4209.

Marti, E., Gros, M., Boy-Roura, M., Ovejero, J., Busquets, A.M., Colón, J., Petrovic, M. and Ponsá, S., 2020. Pharmaceuticals removal in an on-farm pig slurry treatment plant based on solid-liquid separation and nitrification-denitrification systems. *Waste Management*, 102, pp.412-419.

Martínez-Carballo, E., González-Barreiro, C., Scharf, S. and Gans, O., 2007. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environmental Pollution*, *148*(2), pp.570-579.

Mate, C.J., Mukherjee, I. and Das, S.K., 2015. Persistence of spiromesifen in soil: influence of moisture, light, pH and organic amendment. *Environmental Monitoring and Assessment*, *187*(2), pp.1-12.

McCracken, D.I., Cole, L.J., Harrison, W. and Robertson, D., 2012. Improving the farmland biodiversity value of riparian buffer strips: Conflicts and compromises. *Journal of Environmental Quality*, 41(2), pp.355-363.

Metcalfe C. et al., (2008) Exposure Assessment Methods for Veterinary and Human-Use Medicines in the Environment: PEC vs. MEC Comparisons. In: Kümmerer K. (eds) Pharmaceuticals in the Environment. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-74664-5_11v.

Miller, J.J., Beasley, B.W., Yanke, L.J., Larney, F.J., McAllister, T.A., Olson, B.M., Selinger, L.B., Chanasyk, D.S. and Hasselback, P., 2003. Bedding and seasonal effects on chemical and bacterial properties of feedlot cattle manure. *Journal of Environmental Quality*, *32*(5), pp.1887-1894.

Mitchell, S., Ullman, J., Teel, A., & Watts, R. (2015, 9 1). Hydrolysis of amphenicol and macrolide antibiotics: Chloramphenicol, florfenicol, spiramycin, and tylosin. *Chemosphere*, 134, 504-511.

Mitchell, S.M., Ullman, J.L., Bary, A., Cogger, C.G., Teel, A.L. and Watts, R.J., 2015. Antibiotic degradation during thermophilic composting. *Water, Air, & Soil Pollution*, 226(2), pp.1-12.

Mitchell, S.M., Ullman, J.L., Teel, A.L. and Watts, R.J., 2014. pH and temperature effects on the hydrolysis of three β-lactam antibiotics: Ampicillin, cefalotin and cefoxitin. *Science of the Total Environment,* 466, pp.547-555.

Möller, K. and Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences,* 12(3), pp.242-257.

Montforts, M.H.M.M. and Verschoor, A.J., 2003. Environmental Risk Assessment for Veterinary Medicinal Products Part 3. Validation of environmental exposure models.

Montforts, M.H.M.M., 2008. Methodological aspects concerning the environmental risk assessment for medicinal products; research challenges. *In Pharmaceuticals in the Environment* (pp. 393-408). Springer, Berlin, Heidelberg.

Mougin C, Kollmann A, Dubroca J, Ducrot PH, Alvinerie M, Galtier P (2003) Fate of the veterinary medicine ivermectin in soil. *Environmental Chemistry Letters* 1:131–134 .

Muscutt, A.D., Harris, G.L., Bailey, S.W. and Davies, D.B., 1993. Buffer zones to improve water quality: a review of their potential use in UK agriculture. *Agriculture, Ecosystems & Environment*, *45*(1-2), pp.59-77.

Ndayegamiye, A. and Cote, D., 1989. Effect of long-term pig slurry and solid cattle manure application on soil chemical and biological properties. *Canadian journal of soil science*, *69*(1), pp.39-47.

Nicholson FA, Chambers BJ, Williams JR, and Unwin RJ (1999) Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresource Technology* 70, 23–31.

Nowara, A., Burhenne, J. and Spiteller, M., 1997. Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. *Journal of Agricultural and Food Chemistry*, 45(4), pp.1459-1463.

OECD (1984), Test No. 207: Earthworm, Acute Toxicity Tests, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264070042-en.

OECD (2000), Test No. 106: Adsorption -- Desorption Using a Batch Equilibrium Method, OECD Guidelines for the Testing of Chemicals, Section 1, OECD Publishing, Paris, https://doi.org/10.1787/9789264069602-en.

OECD (2000), Test No. 216: Soil Microorganisms: Nitrogen Transformation Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264070226-en.

OECD (2002), Test No. 307: Aerobic and Anaerobic Transformation in Soil, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070509-en.

OECD (2006), *Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264070066-en.

OECD (2011), Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264069923-en.

OECD (2012), Test No. 211: Daphnia magna Reproduction Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264185203-en.

OECD (2019), *Test No. 203: Fish, Acute Toxicity Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264069961-en.

Ogiyama, S., Sakamoto, K., Suzuki, H., Ushio, S., Anzai, T. and Inubushi, K., 2005. Accumulation of zinc and copper in an arable field after animal manure application. *Soil Science & Plant Nutrition*, *51*(6), pp.801-808.

Ou, L.T., Gancarz, D.H., Wheeler, W.B., Rao, P.S.C. and Davidson, J.M., 1982. Influence of soil temperature and soil moisture on degradation and metabolism of carbofuran in soil*s* (Vol. 11, No. 2, pp. 293-298). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Ouyang, W.Y., Su, J.Q., Richnow, H.H. and Adrian, L., 2019. Identification of dominant sulfamethoxazole-degraders in pig farm-impacted soil by DNA and protein stable isotope probing. *Environment International*, *126*, pp.118-126.

Pan, M. and Chu, L.M., 2016. Adsorption and degradation of five selected antibiotics in agricultural soil. *Science of the Total Environment*, 545, pp.48-56.

Pan, M. and Chu, L.M., 2017a. Leaching behavior of veterinary antibiotics in animal manure-applied soils. *Science of the Total Environment*, *579*, pp.466-473.

Pan, M., and Chu, L. M. (2017b). Fate of antibiotics in soil and their uptake by edible crops. *Science of the Total Environment*. 599–600, 500–512. doi: 10.1016/j.scitotenv.2017.04.214

Park, S. and Choi, K., 2008. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. *Ecotoxicology*, *17*(6), pp.526-538.

Parolo, M.E., Savini, M.C., Valles, J.M., Baschini, M.T. and Avena, M.J., 2008. Tetracycline adsorption on montmorillonite: pH and ionic strength effects. Applied Clay Science, 40(1-4), pp.179-186.

Paul, J., & Beauchamp, E. (1989). Relationship between volatile fatty acids, total ammonia, and pH in manure slurries. *Biological Wastes*, 29(4), 313-318.

Pei, R., Cha, J., Carlson, K.H. and Pruden, A., 2007. Response of antibiotic resistance genes (ARG) to biological treatment in dairy lagoon water. *Environmental Science & Technology*, 41(14), pp.5108-5113.

Pei, R., Kim, S.C., Carlson, K.H. and Pruden, A., 2006. Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research*, *40*(12), pp.2427-2435.

Peng, P.C., Wang, Y., Liu, L.Y., Liang, J.B. and Wu, Y.B., 2016. The excretion and environmental effects of amoxicillin, ciprofloxacin, and doxycycline residues in layer chicken manure. *Poultry Science*, 95(5), pp.1033-1041.

Petersen, S.O., Andersen, A.J., Eriksen, J., 2012 Effects of cattle slurry acidification on ammonia and methane evolution during storage. *Journal of Environmental Quality*. DOI: 10.2134/jeq2011.0184.

Petric, I., Šestan, A. and Šestan, I., 2009. Influence of wheat straw addition on composting of poultry manure. *Process Safety and Environmental Protection*, *87*(3), pp.206-212.

Pils JRV, Laird DA (2007) Sorption of tetracycline and chlortetracycline on K- and Ca-saturated soil clays, humic substances, and clay-humic complexes. *Environmental Science and Technology* 41:1928–1933

Pino, M.R., Val, J., Mainar, A.M., Zuriaga, E., Español, C. and Langa, E., 2015. Acute toxicological effects on the earthworm Eisenia fetida of 18 common pharmaceuticals in artificial soil. *Science of the Total Environment*, *518*, pp.225-237.

Polianciuc, S.I., Gurzău, A.E., Kiss, B., Ştefan, M.G. and Loghin, F., 2020. Antibiotics in the environment: causes and consequences. *Medicine and Pharmacy reports*, *93*(3), p.231.

Pomati, F., Netting, A.G., Calamari, D. and Neilan, B.A., 2004. Effects of erythromycin, tetracycline and ibuprofen on the growth of Synechocystis sp. and Lemna minor. *Aquatic Toxicology*, *67*(4), pp.387-396.

Popova, I.E., Bair, D.A., Tate, K.W. and Parikh, S.J., 2013. Sorption, leaching, and surface runoff of beef cattle veterinary pharmaceuticals under simulated irrigated pasture conditions. *Journal of Environmental Quality*, 42(4), pp.1167-1175.

Potter, P., Ramankutty, N., Bennett, E.M. and Donner, S.D., 2010. Characterizing the spatial patterns of global fertilizer application and manure production. *Earth Interactions*, 14(2), pp.1-22.

Powell, J.M., Jokela, W.E. and Misselbrook, T.H., 2011. Dairy slurry application method impacts ammonia emission and nitrate leaching in no-till corn silage. *Journal of Environmental Quality*, *40*(2), pp.383-392.

Powell, J.M., Wattiaux, M.A., Broderick, G.A., Moreira, V.R. and Casler, M.D., 2006. Dairy diet impacts on fecal chemical properties and nitrogen cycling in soils. *Soil Science Society of America Journal*, 70(3), pp.786-794.

Pro, J., Ortiz, J.A., Boleas, S., Fernández, C., Carbonell, G. and Tarazona, J.V., 2003. Effect assessment of antimicrobial pharmaceuticals on the aquatic plant Lemna minor. *Bulletin of Environmental Contamination and Toxicology*, *70*(2), pp.0290-0295.

Pruden, A., Larsson, D.J., Amézquita, A., Collignon, P., Brandt, K.K., Graham, D.W., Lazorchak, J.M., Suzuki, S., Silley, P., Snape, J.R. and Topp, E., 2013. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. *Environmental Health Perspectives*, 121(8), pp.878-885.

Qiu, M., Hu, A., Yu-ming, M.H., Zhao, Y., He, Y., Xu, J. and Lu, Z., 2021. Elucidating degradation mechanisms of florfenicol in soil by stable-isotope assisted nontarget screening. *Journal of Hazardous Materials*, *403*, p.123974.

Quinn, B., Gagné, F. and Blaise, C., 2008. An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, Hydra attenuata. *Science of the Total Environment*, 389(2-3), pp.306-314.

Quiroga, G., Castrillón, L., Fernández-Nava, Y. and Marañón, E., 2010. Physico-chemical analysis and calorific values of poultry manure. *Waste management*, *30*(5), pp.880-884.

Herberg, R., Manthey, J., Richardson, L., Cooley, C. and Donoho, A., 1978. Excretion and tissue distribution of [14C] monensin in cattle. *Journal of Agricultural and Food Chemistry*, 26(5), pp.1087-1090.

Singh, R., Singh, A.P., Kumar, S., Giri, B.S. and Kim, K.H., 2019. Antibiotic resistance in major rivers in the world: a systematic review on occurrence, emergence, and management strategies. *Journal of Cleaner Production*, 234, pp.1484-1505. R

Rabølle, M. and Spliid, N.H., 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere,* 40(7), pp.715-722.

Ramaswamy, J., Prasher, S.O., Patel, R.M., Hussain, S.A. and Barrington, S.F., 2010. The effect of composting on the degradation of a veterinary pharmaceutical. *Bioresource Technology*, 101(7), pp.2294-2299.

Richter, E., Berkner, S., Ebert, I., Förster, B., Graf, N., Herrchen, M., Kühnen, U., Römbke, J. and Simon, M., 2016. Results of extended plant tests using more realistic exposure scenarios for improving environmental risk assessment of veterinary pharmaceuticals. *Environmental Sciences Europe*, 28(1).

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.Á., Prados-Joya, G. and Ocampo-Pérez, R., 2013. Pharmaceuticals as emerging contaminants and their removal from water. A review. *Chemosphere*, 93(7), pp.1268-1287.

Rotz, C.A., Kleinman, P.J.A., Dell, C.J., Veith, T.L. and Beegle, D.B., 2011. Environmental and economic comparisons of manure application methods in farming systems. *Journal of Environmental Quality*, 40(2), pp.438-448.

Kuchta, S.L. and Cessna, A.J., 2009. Lincomycin and spectinomycin concentrations in liquid swine manure and their persistence during simulated manure storage. *Archives of Environmental Contamination and Toxicology*, 57(1), pp.1-10.

Sadezky, A., Löffler, D. and Ternes, T., 2008. Proposal of an environmental indicator and classification system of pharmaceutical product residues for environmental management. *ProjetEuropéen KNAPPE, Deliverable D*, *12*, p.p92.

Salvia, M.V., Experton, J., Geandel, C., Cren-Olivé, C. and Vulliet, E., 2014. Fate of pharmaceutical compounds and steroid hormones in soil: study of transfer and degradation in soil columns. *Environmental Science and Pollution Research*, 21(17), pp.10525-10535.

SARGA, 2015. Evaluation of manure management systems in Europe. LIFE09 ENV/ES/000453. [online] Available at: <https://core.ac.uk/download/pdf/46606176.pdf> [Accessed 25 June 2021].

Sassman SA, Lee LS (2005) Sorption of three tetracyclines by several soils: assessing the role of pH and cation exchange. *Environmental Science and Technology* 39:7452–7459.

Sassman, S. A., and Lee, L. S. (2007). Sorption and degradation in soils of veterinary ionophore antibiotics: Monensin and lasalocid. *Environmental Toxicology and Chemistry* 26, 1614–1621. doi: 10.1897/07-073R.1.

Schlüsener, M.P. and Bester, K., 2006a. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. *Environmental Pollution,* 143(3), pp.565-571.

Schlüsener, M.P., Bester, K. and Spiteller, M., 2003. Determination of antibiotics such as macrolides, ionophores and tiamulin in liquid manure by HPLC–MS/MS. *Analytical and bioanalytical chemistry*, *375*(7), pp.942-947.

Schlüsener, M.P., Von Arb, M.A. and Bester, K., 2006b. Elimination of macrolides, tiamulin, and salinomycin during manure storage. *Archives of Environmental Contamination and Toxicology*, 51(1), pp.21-28.

Schwaiger, J., Ferling, H., Mallow, U., Wintermayr, H. and Negele, R.D., 2004. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquatic Toxicology*, *68*(2), pp.141-150.

Science for Environment Policy (2017), Agri-environmental schemes: how to enhance the agriculture-environment relationship. Tematic Issue 57. Issue produced for the European Commission DG Environment by the Science Communication Unit, UWE, Bristol. Available at: http://ec.europa.eu/science-environmentpolicy .

Selvam, A. and Wong, J.W.C., 2017. Degradation of antibiotics in livestock manure during composting. *Current Developments in Biotechnology and Bioengineering*, pp.267-292.

Selvam, A., Xu, D., Zhao, Z. and Wong, J.W., 2012. Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. *Bioresource Technology*, *126*, pp.383-390.

Shan, S., Wang, H., Fang, C., Chu, Y., & Jiang, L. (2018). Effects of adding biochar on tetracycline removal during anaerobic composting of swine manure. *Chemistry and Ecology*, 34(1), 86-97.

Shao, B., Chen, D., Zhang, J., Wu, Y. and Sun, C., 2009. Determination of 76 pharmaceutical drugs by liquid chromatography–tandem mass spectrometry in slaughterhouse wastewater. *Journal of Chromatography A*, *1216*(47), pp.8312-8318.

Shepherd, M., Philipps, L., Jackson, L. and Bhogal, A., 2002. The nutrient content of cattle manures from organic holdings in England. *Biological Agriculture & Horticulture*, *20*(3), pp.229-242.

Shi, J.C., Liao, X.D., Wu, Y.B. and Liang, J.B., 2011. Effect of antibiotics on methane arising from anaerobic digestion of pig manure. *Animal Feed Science and Technology*, 166, pp.457-463.

Sittig, S., Kasteel, R., Groeneweg, J., Hofmann, D., Thiele, B., Köppchen, S. and Vereecken, H., 2014. Dynamics of transformation of the veterinary antibiotic sulfadiazine in two soils. *Chemosphere*, *95*, pp.470-477.

Slana, M. and Dolenc, M.S., 2013. Environmental risk assessment of antimicrobials applied in veterinary medicine—a field study and laboratory approach. *Environmental Toxicology and Pharmacology*, 35(1), pp.131-141.

Slana, M., Žigon, D. and Sollner-Dolenc, M., 2017. Enrofloxacin degradation in broiler chicken manure under field conditions and its residuals effects to the environment. *Environmental Science and Pollution Research*, 24(15), pp.13722-13731.

Smith, K.A., Brewer, A.J., Crabb, J. and Dauven, A., 2001. A survey of the production and use of animal manures in England and Wales. III. Cattle manures. *Soil Use and Management*, *17*(2), pp.77-87.

Smook, T.M., Zho, H. and Zytner, R.G., 2008. Removal of ibuprofen from wastewater: comparing biodegradation in conventional, membrane bioreactor, and biological nutrient removal treatment systems. *Water Science and Technology*, *57*(1), pp.1-8.

Snow, D., Bartelt-Hunt, S., Devivo, S., Saunders, S., & Cassada, D. (2009, 10). Detection, Occurrence, and Fate of Emerging Contaminants in Agricultural Environments. *Water Environment Research*, 81(10), 941-958.

Song, X., Liu, D., Zhang, G., Frigon, M., Meng, X. and Li, K., 2014. Adsorption mechanisms and the effect of oxytetracycline on activated sludge. *Bioresource Technology,* 151, pp.428-431.

Spaepen, K., van Leemput, L., Wislocki, P., & Verschueren, C. (1997, 9 1). A uniform procedure to estimate the predicted environmental concentration of the residues of veterinary medicines in soil. *Environmental Toxicology and Chemistry*, 16(9), 1977-1982.

Spielmeyer, A., 2018. Occurrence and fate of antibiotics in manure during manure treatments: a short review. *Sustainable Chemistry and Pharmacy*, 9, pp.76-86.

Spielmeyer, A., Höper, H. and Hamscher, G., 2017. Long-term monitoring of sulfonamide leaching from manure amended soil into groundwater. *Chemosphere,* 177, pp.232-238.

Srinivasan, P., Sarmah, A.K. and Manley-Harris, M., 2013. Co-contaminants and factors affecting the sorption behaviour of two sulfonamides in pasture soils. *Environmental Pollution*, *180*, pp.165-172.

Srinivasan, P., Sarmah, A.K., Manley-Harris, M. and Wilkins, A.L., 2010. Sorption of sulfamethoxazole, sulfachloropyridazine and sulfamethazine onto six New Zealand dairy farm soils. In 19th World Congress of Soil Solutions for a Changing World.

Sukul, P., Lamshöft, M., Kusari, S., Zühlke, S. and Spiteller, M., 2009. Metabolism and excretion kinetics of 14C-labeled and non-labeled difloxacin in pigs after oral administration, and antimicrobial activity of manure containing difloxacin and its metabolites. *Environmental Research*, 109(3), pp.225-231.

Suresh, A., Choi, H.L., Oh, D.I. and Moon, O.K., 2009. Prediction of the nutrients value and biochemical characteristics of swine slurry by measurement of EC–Electrical conductivity. *Bioresource Technology*, *100*(20), pp.4683-4689.

Svendsen, T.S., Hansen, P.E., Sommer, C., Martinussen, T., Grønvold, J. and Holter, P., 2005. Life history characteristics of Lumbricus terrestris and effects of the veterinary antiparasitic compounds ivermectin and fenbendazole. *Soil Biology and Biochemistry*, *37*(5), pp.927-936.

Tamtam, F., Le Bot, B., Dinh, T., Mompelat, S., Eurin, J., Chevreuil, M., Bonté, P., Mouchel, J.M. and Ayrault, S., 2011. A 50-year record of quinolone and sulphonamide antimicrobial agents in Seine River sediments. *Journal of Soils and Sediments*, *11*(5), pp.852-859.

Tasho, R.P. and Cho, J.Y., 2016. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. *Science of the Total Environment*, *563*, pp.366-376.

Ter Laak TL, Gebbink WA, Tolls J (2006a) Estimation of sorption coefficients of veterinary medicines from soil properties. *Environmental Toxicology and Chemistry* 25:933–941

Ter Laak TL, Gebbink WA, Tolls J (2006b) The effect of pH and ionic strength on the sorption of sulfachloropyridazine, tylosin and oxytetracycline to soil. *Environmental Toxicology and Chemistry* 25:904–911

Thiele-Bruhn, S. and Aust, M.O., 2004. Effects of pig slurry on the sorption of sulfonamide antibiotics in soil. *Archives of Environmental Contamination and Toxicology*, 47(1), pp.31-39.

Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils–a review. *Journal of Plant Nutrition and Soil Science*, *166*(2), pp.145-167.

Tolls, J., 2001. Sorption of veterinary pharmaceuticals in soils: a review. *Environmental Science & Technology*, 35(17), pp.3397-3406.

Topp, E., Chapman, R., Devers-Lamrani, M., Hartmann, A., Marti, R., Martin-Laurent, F., et al., (2013). Accelerated biodegradation of veterinary antibiotics in agricultural soil following long-term exposure, and isolation of a sulfamethazine degrading microbacterium sp. *Journal of Environmental Quality* 42, 173–178. doi: 10.2134/jeq2012.0162

UBA, 2019. *Environmental risks from mixtures of antibiotic pharmaceuticals in soils –a literature review*. [online] Available at: <https://www.umweltbundesamt.de/sites/default/files/medien/1410/publikationen/2019-04-11_texte_32-2019_mixtures-antibiotica-soil.pdf> [Accessed 4 February 2022].

UK-VARSS (2018). UK Veterinary Antibiotic Resistance and Sales Surveillance Report (UK-VARSS 2017). New Haw, Addlestone: Veterinary Medicines Directorate.

UK-VARSS(2017).Veterinary Antibiotic Resistance and Sales Surveillance Report(UK-VARSS2017).New Haw, Addlestone: Veterinary Medicines Directorate.

UK-VARSS(2021).Veterinary Antibiotic Resistance and Sales Surveillance Report(UK-VARSS2020).New Haw, Addlestone: Veterinary Medicines Directorate.

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A. and Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), pp.5649-5654.

Van De Steene, J.C., Stove, C.P. and Lambert, W.E., 2010. A field study on 8 pharmaceuticals and 1 pesticide in Belgium: removal rates in waste water treatment plants and occurrence in surface water. *Science of the Total Environment*, *408*(16), pp.3448-3453.

Van Grinsven, H.J.M., Ten Berge, H.F.M., Dalgaard, T., Fraters, B., Durand, P., Hart, A., Hofman, G., Jacobsen, B.H., Lalor, S.T., Lesschen, J.P. and Osterburg, B., 2012. Management, regulation and environmental impacts of nitrogen fertilization in northwestern Europe under the Nitrates Directive: a benchmark study. *Biogeosciences*, *9*(12), pp.5143-5160.

Vanotti, M.B., Rashash, D.M.C. and Hunt, P.G., 2002. Solid–liquid separation of flushed swine manure with PAM: effect of wastewater strength. Transactions of the ASAE, 45(6), p.1959.

Velthof, G.L., Nelemans, J.A., Oenema, O. and Kuikman, P.J., 2005. Gaseous nitrogen and carbon losses from pig manure derived from different diets. *Journal of Environmental Quality*, *34*(2), pp.698-706.

Veterinary Medicines Directorate. (2002). Sales of antimicrobial products used as veterinary medicines, growth promoters and coccidiostats in the UK in 2000. [Online.] http://www.vmd.gov.uk/general/publications/amrrpt2000v51.htm (27 April 2003, date last accessed) .

VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products), 2004, Environmental Impact Assessment for Veterinary Medicinal Products Phase II Guidance. VICH GL 38 Ecotoxicity Phase II).

VICH, 2009. VICH topic GL48: Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: marker residue depletion studies to establish product withdrawal periods. [online] EMA. Available at: <https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl48-studies-evaluate-metabolism-residue-kinetics-veterinary-drugs-food-producing-animals_en.pdf> [Accessed 23 June 2021].

VICH. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase I. London: CVMP/VICH, 2000. CVMP/VICH/592/98-final.

VICH. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase II Draft Guidance. London: CVMP/VICH, 2003. CVMP/VICH/790/03-Consultation.

Virtue, W.A. and Clayton, J.W., 1997. Sheep dip chemicals and water pollution. *Science of the total environment*, *194*, pp.207-217.

Walters, E., McClellan, K., and Halden, R. U. (2010). Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. Water Res. 44, 6011–6020. doi: 10.1016/j.watres.2010.07.051

Wang, C., Shi, H., Adams, C.D., Gamagedara, S., Stayton, I., Timmons, T. and Ma, Y., 2011. Investigation of pharmaceuticals in Missouri natural and drinking water using high performance liquid chromatography-tandem mass spectrometry. *Water research*, *45*(4), pp.1818-1828.

Wang, J., Lin, H., Sun, W., Xia, Y., Ma, J., Fu, J., et al., (2016). Variations in the fate and biological effects of sulfamethoxazole, norfloxacin and doxycycline in different vegetable-soil systems following manure application. *Journal of Hazardous Materials* 304, 49–57. doi: 10.1016/j.jhazmat.2015.10.038

Wang, Q. and Yates, S.R., 2008. Laboratory study of oxytetracycline degradation kinetics in animal manure and soil. *Journal of Agricultural and Food Chemistry,* 56(5), pp.1683-1688.

Wang, Q., Guo, M., and Yates, S. R. (2006). Degradation kinetics of manure-derived sulfadimethoxine in amended soil. . *Journal of Agricultural and Food Chemistry* 54, 157–163. doi: 10.1021/jf052216w

Watanabe, N., Bergamaschi, B.A., Loftin, K.A., Meyer, M.T. and Harter, T., 2010. Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. *Environmental Science & Technology*, *44*(17), pp.6591-6600.

Webb, J., Pain, B., Bittman, S. and Morgan, J., 2010. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—a review. *Agriculture, Ecosystems & Environment*, 137(1-2), pp.39-46.

Wegst-Uhrich, S.R., Navarro, D.A., Zimmerman, L. and Aga, D.S., 2014. Assessing antibiotic sorption in soil: a literature review and new case studies on sulfonamides and macrolides. *Chemistry Central Journal,* 8(1), pp.1-12.

Wei, R., Ge, F., Chen, M. and Wang, R., 2012. Occurrence of ciprofloxacin, enrofloxacin, and florfenicol in animal wastewater and water resources. *Journal of Environmental Quality*, *41*(5), pp.1481-1486.

Weinfurtner, K., 2011. Matrix parameters and storage conditions of manure.

Weiss, C., Conte, A., Milandri, C., Scortichini, G., Semprini, P., Usberti, R. and Migliorati, G., 2007. Veterinary drugs residue monitoring in Italian poultry: Current strategies and possible developments. *Food control*, *18*(9), pp.1068-1076.

Whalen, J.K., Chang, C., Clayton, G.W. and Carefoot, J.P., 2000. Cattle manure amendments can increase the pH of acid soils. *Soil Science Society of America Journal*, *64*(3), pp.962-966.

Widyasari-Mehta, A., Suwito, H.R.K.A. and Kreuzig, R., 2016. Laboratory testing on the removal of the veterinary antibiotic doxycycline during long-term liquid pig manure and digestate storage. *Chemosphere*, 149, pp.154-160.

Winckler, C. and Grafe, A. 2001. Use of veterinary drugs in intensive animal production: Evidence for persistence of tetracycline in swine slurry. *Journal of Soils and Sediments*., 1: 66–70.

Wohde, M., Berkner, S., Junker, T., Konradi, S., Schwarz, L. and Düring, R.A., 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. *Environmental Sciences Europe*, 28(1), pp.1-25.

Wollenberger, L., Halling-Sørensen, B. and Kusk, K.O., 2000. Acute and chronic toxicity of veterinary antibiotics to Daphnia magna. *Chemosphere*, *40*(7), pp.723-730.

Wu, C., Spongberg, A. L., & Witter, J. D. (2009). Sorption and biodegradation of selected antibiotics in biosolids. *Journal of Environmental Science and Health, Part A*, 44(5), 454–461. doi:10.1080/10934520902719779

XIA, W., GYRD-HANSEN, N., & NIELSEN, P. (1983). Comparison of pharmacokinetic parameters for two oxytetracycline preparations in pigs. *Journal of Veterinary Pharmacology and Therapeutics*, 6(2), 113–120. doi:10.1111/j.1365-2885.1983.tb00387.x

Yang, J.-F., Ying, G.-G., Liu, S., Zhou, L.-J., Zhao, J.-L., Tao, R., et al., (2012). Biological degradation and microbial function effect of norfloxacin in a soil under different conditions. *Journal of Environmental Science and Health*. 47, 288–295. doi: 10.1080/03601234.2012.638886

Yang, Y.-Y., Borch, T., Young, R., Goodridge, L., & Davis, J. (2010). Degradation Kinetics of Testosterone by Manure-Borne Bacteria: Influence of Temperature, pH, Glucose Amendments, and Dissolved Oxygen. *Journal of Environment Quality,* 39(4), 1153.

Yang, C.W., Hsiao, W.C. and Chang, B.V., 2016. Biodegradation of sulfonamide antibiotics in sludge. *Chemosphere,* 150, pp.559-565.

Youngquist, C.P., Mitchell, S.M. and Cogger, C.G., 2016. Fate of antibiotics and antibiotic resistance during digestion and composting: a review. *Journal of Environmental Quality*, *45*(2), pp.537-545.

YSI, 2008. ORP Management in wastewater as an indicator of process efficiency. YSI, Yellow Springs, OH http://www. ysi. com/media/pdfs/A567-ORP-Management-in-Wastewater-as-an-Indicator-of-Process-Efficiency. pdf (accessed on 15.08. 13).

Yuan, Q., Sui, M., Qin, C., Zhang, H., Sun, Y., Luo, S. and Zhao, J., 2022. Migration, Transformation and Removal of Macrolide Antibiotics in The Environment: A Review. *Environmental Science and Pollution Research,* pp.1-18.

Zhai, N., Zhang, T., Yin, D., Yang, G., Wang, X., Ren, G. and Feng, Y., 2015. Effect of initial pH on anaerobic co-digestion of kitchen waste and cow manure. *Waste Management*, *38*, pp.126-131.

Zhang, Q., Yang, C., Dang, Z. and Huang, W., 2011. Sorption of tylosin on agricultural soils. Soil science, 176(8), pp.407-412.

Zhang, Y., Hu, S., Zhang, H., Shen, G., Yuan, Z., & Zhang, W. (2017, 12 31). Degradation kinetics and mechanism of sulfadiazine and sulfamethoxazole in an agricultural soil system with manure application. *Science of The Total Environment*, 607-608, 1348-1356.

Zhao, L., Dong, Y.H. and Wang, H., 2010. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of the Total Environment*, 408(5), pp.1069-1075.

Zou, H., He, J., Guan, X., Zhang, Y., Deng, L., Li, Y. and Liu, F., 2021. Microbial responses underlying the denitrification kinetic shifting exposed to ng/L-and μg/L-level lomefloxacin in groundwater. *Journal of Hazardous Materials*, *417*, p.126093.

Zrnčić, M., Gros, M., Babić, S., Kaštelan-Macan, M., Barcelo, D. and Petrović, M., 2014. Analysis of anthelmintics in surface water by ultra high performance liquid chromatography coupled to quadrupole linear ion trap tandem mass spectrometry. *Chemosphere*, *99*, pp.224-232.

Chapter 2

Assessing the Influence of Pig Slurry pH on the Degradation of Selected Antibiotics and Their Transformation Products

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Abstract

Veterinary medicines are routinely used in animal husbandry and the environment may consequently be exposed to them via manure applications. This presents potential environmental and societal risks such as toxicological effects to aquatic/terrestrial organisms and the spread of antimicrobial resistance. Regulatory studies that assess the degradability of veterinary antibiotics during manure storage currently permit the use of just one manure per animal type although we speculate that heterogenic properties such as pH could be driving significant variability within degradation rates. To bridge this knowledge gap and assess degradation variability with pH, laboratory degradation studies were performed on a broad range of antibiotics (ceftiofur, florfenicol, oxytetracycline, sulfamethoxazole and tylosin) at three different environmentally relevant pH levels (5.5, 7, and 8.5). The effect of pig slurry pH on degradation rates was found to be significant and compound specific. Usually, acidic slurries were found to inhibit degradation when compared to neutral pH, for florfenicol, tylosin, and ceftiofur; the associated changes in DT_{50} (half-life) values were 2–209 h, 35.28–234 h, and 0.98–2.13 h, respectively. In some circumstances alkaline slurries were observed to enhance the degradation rate when compared to those for neutral pH, for tylosin, the respective changes in DT50 values were from 3.52 to 35.28 h. Comparatively, the degradation of sulfamethoxazole was enhanced by acidic conditions compared to neutral (DT_{50} 20.6–31.6 h). Tentative identification of unknown transformation products (TPs) was achieved for sulfamethoxazole and florfenicol for the first time in pig slurries. These results reveal the importance of considering slurry pH when assessing the degradation of antibiotic compounds, which has implications for the acidification of manures and the environmental risk assessment for veterinary medicines.

2.0 Introduction

Livestock are routinely administered veterinary medicines to improve or protect animal health. As a consequence of poor absorption within the gastrointestinal tract of the animal, high percentages of veterinary medicines are often excreted as the parent compound or their metabolites (Thiele-Bruhn, 2003). Excretion rates of pharmaceuticals depend on specific pharmacodynamic and pharmacokinetic processes/properties and vary between 10 and 90% of the administered dose (Hirsch et al., 1999; Montforts et al., 1999; Sukul et al., 2009). The environment is exposed to veterinary medicines via manure applications to land or directly via excretion from animals reared on pasture (Kemper, 2008). The usage of animal manures as a fertilizer is commonplace within agriculture, primarily due to its excellent nutrient content, but also because application to land offers a suitable route for waste disposal (Potter et al., 2010; Lee, 2010; Bogaard et al., 2013; Salgado et al., 2019).

Veterinary medicines that are present within the terrestrial environment can migrate to the aquatic environment via, runoff to surface water or leaching into groundwater (Boxall et al., 2004; Kay et al., 2005; Kreuzig et al., 2005; Li et al., 2018). Common on-farm practice is to store animal slurries, during which veterinary antibiotics are subject to various degradation and dissipation processes such as microbial mineralization, adsorption, hydrolysis, and chemical transformation (Lamshöft et al., 2010). Typically, pig slurry is stored on average for 53 days within the EU, during which time veterinary medicines are subject to varying rates of degradation (EMA, 2016).

Within the environmental risk assessment for veterinary medicines slurry incubation studies are employed to assess the degradability of veterinary medicines during this timeframe. This provides a more accurate estimate of concentrations exposed to land following manure application (EMA, 2011). Presently there is a lot of uncertainty regarding veterinary medicine degradation within manures due to differences in manure properties; despite the fact that a thorough understanding is critical for harmonisation of manure degradation studies (VICH, 2000; VICH, 2003; EMA, 2011). Variation exists between manure degradation studies, for example Blackwell et al., (2005) reported a DT50 of 79 d for oxytetracycline within pig slurries whereas Wang et al., (2015) calculated a range of 9.05–9.65 d. When regulatory soil degradation studies are performed (e.g., OECD 307), the use of four soil types is usually required due to the known influence of soil properties on the degradation rate and route of organic compounds (OECD, 2002; Junker et al., 2020). Soil matrix properties can affect sorption, hydrolysis, microbial mineralization as well as the ionic strength and speciation of contaminants (pKa) via changes in pH (Sassman and Lee, 2005; ter Laak et al., 2006; Chatterjee et al., 2013; Jechalke et al., 2014; Mitchell et al., 2014; Junker et al., 2020). Most regulatory manure degradation studies consider one animal manure per animal type, but we hypothesise varied manure properties such as pH to be of equal or greater importance to those of soils.

The pH of pig slurries is heterogenic and is related to manure management (acidification), animal feed, animal age, water, temperature, redox potential, as well as the aging process. Through these mechanisms and the degradation of organic matter, different ratios of ammonia, ammonium and volatile fatty acids (fulvic and humic acids) form thereby altering slurry pH (Paul and Beauchamp, 1989; M⌀ller et al., 2004; Page et al., 2014; Joubin, 2018). Animal feed is well known to directly influence slurry pH and higher protein diets have been identified to increase pH whilst carbohydrates decrease the pH (Canh et al., 1998a, 1998b). Typically pig slurry is expected to be neutral in pH, however, a wide range of pig slurry pHs are documented within the literature, for example Cooper and Cornforth (1978) noted a range of 6.5–7.5 and Weinfurtner (2011) observed pH values of 13 pig slurries in Germany to be in the range of 5.55–9.14. Within the literature there are publications reporting extremely acidic pig slurries (e.g., 4.8, 5.11) (Choudhary et al., 1996; Thiele-Bruhn, 2003a; Martinez-Suller et al., 2008; Wang et al., 2015; Shan et al., 2018), as well as alkaline pig slurries (e.g., pH 9.14, 8.92, and 8.11). (Sommer and Husted, 1995; Hu et al., 2011; Mroz et al., 2000).

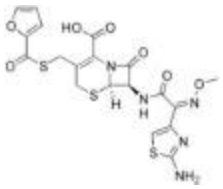
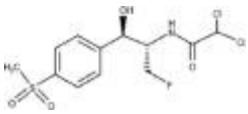
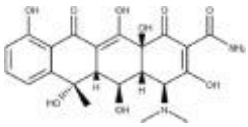
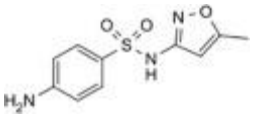
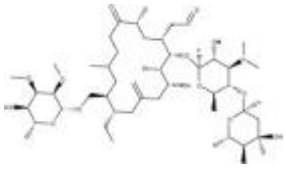
Despite documentation of wide-ranging pH values in pig slurries and the potential impacts on veterinary medicine degradation, studies of the effect of pH are lacking (Ratasuk et al., 2012; Ali et al., 2013). Therefore, the aim of this research was to address this significant knowledge gap and quantify the effect of various slurry pHs on the degradation of a wide range of veterinary antibiotics, including their transformation products. This work has the potential to contribute towards a more robust environmental risk assessment for veterinary medicines.

2.1 Methodology

2.1.1 Chemical compounds and stock preparation

All chemicals and solvents were of the highest available purity. Methanol, orthophosphoric acid, H_2SO_4 (86%), NaOH (98%), Na_2EDTA, citric acid and di-sodium hydrogen orthophosphate were purchased from Fischer Scientific (UK). Analytical grade antibiotics were used (94–98%); oxytetracycline (OTC), ceftiofur (CFT) and tylosin tartrate (TYL) were purchased from Scientific Laboratory Supplies SLS (UK), whilst florfenicol (FLO) and sulfamethoxazole (SMX) were purchased from VWR (UK). The influence of pH on degradation rates will likely be compound specific, therefore, a suite of veterinary antibiotics with differing modes of action were selected to encompass a wide range of physical-chemical properties (Table 2.0) (i.e., pKa, Log Kow, Kd, and molecular weight). Stock solutions were prepared in methanol and fresh matrix matched calibration standards were made following the extraction of pig slurries and aqueous samples. 0.1 M Na_2 McIlvaine buffer pH 4 (50:50) was prepared by mixing 307.25 mL of 0.1 M citric acid, 192.75 mL 0.2 M Na_2HPO4 and 500 mL of 0.1 M Na_2EDTA. Kolthoff and Vleeschouwer buffers were prepared at varying pHs via OECD 111 (OECD, 2004). To obtain pH 5.5 buffer, 68 mL of 0.1 M NaOH and 50 mL 0.1 M citric acid were combined, whilst to obtain a pH of 7, 68 mL of 0.1 M NaOH, 50 mL boric acid and 0.37 g KCl were mixed.

Table 2.0: Physiochemical properties of the veterinary antibiotics utilized in this study.

Antibiotic, Group and Molecular Weight (g/mol)	Chemical Structure	Log K_{ow}	pKa	K_d (mL/g)	K_{oc} (mL/g)
Ceftiofur (Cephalosporin) 523.56 (Yalkowsky and He, 2003)		1.6 (US EPA, 2006)	2.68 (Ribeiro et al., 2018)	14.5–31.72 (An et al., 2021)	755.60–944.66 (An et al., 2021)
Florfenicol (amphenicol) 358.21 (FDA, 2013)		0.37 (FDA, 2013)	9.03 (FDA, 2013)	0.07–0.59 (FDA, 2013)	10 - 27 (FDA, 2013)
Oxytetracycline (Tetracycline) 460.44 (Yalkowsky and He, 2003)		−1.22 (ter Laak et al., 2006)	3.27, 7.32, 9.11 (Stephens et al., 1956)	540 - 1026 (Rabølle and Spliid, 2000)	27792–93317 (Rabølle and Spliid, 2000)
Sulfamethoxazole (Sulphonamide) 253.27 (Yalkowsky and He, 2003)		0.89 (Hansch et al., 1995)	1.44, 5.7 (Srinivasan et al., 2010)	1.13–2.41 (Hu et al., 2019)	12.36–23.99 (Hu et al., 2019)
Tylosin (Macrolide) 916.1 (Yalkowsky and He, 2003)		2.5 (ter Laak et al., 2006)	7.1 (ter Laak et al., 2006)	3 - 156 (ter Laak et al., 2006)	136.36–5032.26 (ter Laak et al., 2006)

Adsorption/desorption details (An et al., 2021): – *Range of a sandy loam, loam and clay soils* (FDA, 2013) – *Range of four standardized soils* (ter Laak et al., 2006), – *Range of loamy sand to clay loam* (Rabølle and Spliid, 2000), – *Range of soils from sandy soil to a loamy sand* (Hu et al., 2019), –*Range of a sandy clay loam and a sandy loam*.

2.1.2 Pig slurry sampling, conditioning and characteristics

10 kg of fresh manure was sampled from a pig farm in Welburn, York (UK), the sample was obtained from pigs that were 12 weeks old. The pigs had received sulfadiazine and trimethoprim during their first 6 weeks of life, which is standard husbandry practice when pigs are within the gestation, farrowing and weaning periods (Filippitzi et al., 2014; Lekagul et al., 2019). However, none of the study compounds had been administered prior to collection. Pig manure was stored under anaerobic conditions at room temperature for no longer than two weeks prior to usage within the study. The manure was homogenised before the moisture content was derived and then adjusted into a slurry using tap water to attain a dry matter content of 5% (d/w). (EMA, 2011).

To characterise the dissolved fraction of pig slurries, the sample was centrifuged at 3250 rpm (2 h) and decanted, the supernatant was then filtered using G/F and 1 PS Whatman papers, followed by a 20 μm G/F and 0.45 μm nylon syringe filter. Analysis of the sample was achieved using an Analytik Jena Multi NC2100 (carbon), Autoanalyzer (nutrients) and ICP-OES (iCAP 7400 Radical) for metals, please refer to Supplementary Information (SI 1.0) for further details. The pig slurry properties were as follows, pH 7.2 ± 0.5, NH_4–N 22.72 mg/L NO_2–N 0.2 ng/L, PO_4–N 24 ng/L, OC 174 ± 6.6 mg/L, inorganic carbon 1087.9 ± 14.3 mg/L, Co 0.05 mg/L, Zn 0.313 mg/L, Cd 0.003 mg/L, Cu 0.032 mg/L and Pb 0.006 mg/L. These properties were found to be comparable to previously published pig slurry characteristics (Weinfurtner, 2011; Sommer et al., 2015).

2.1.3 Biodegradation experiment

Due to a mixture of antibiotics being tested the slurry was dosed with a mixture of five compounds at 20% of the Predicted Environmental Concentration (PEC) which were calculated using the Spaepen (Spaepen et al., 1997) model (SI Table B1). An exception of this was ceftiofur which was dosed at 40% due to the very low PEC and poor analytical sensitivity. The dosage concentrations were 3 mg/kg, 1 mg/kg, 5 mg/kg, 3 mg/kg, and 3.3 mg/kg for CFT, FLO, OTC, SMX and TYL respectively. The

degradation of the five antibiotic compounds was performed at three pig slurry pHs with five replicates and one control per treatment; the selected pH treatments were alkaline (pH 8.5), neutral (pH 7) and acidic (pH 5.5). The pH of the pig slurry was controlled using either 3 M H_2SO_4 or 3 M NaOH; daily checks were conducted, and the required adjustment frequencies varied depending on the pH treatment. The volume of acid/base that was added were recorded and the differences in the moisture contents between the treatments was corrected. The incubation vessels facilitated continuous pH and ORP monitoring and subsampling via permanently installed probes and a detachable lid (Figure B1). The vessel lids contained an inlet and outlet facilitating the purging of the headspace with nitrogen, this helped maintain anaerobic conditions with redox potentials values ranging from −250 mV to −400 mV (EMA, 2011). pH conditions were acclimated for ten days prior to dosing to allow the microbial populations to equilibrate to the manipulated pHs (Fangueiro et al., 2015a).

Dosing of the pig slurries was achieved using a mixed stock solution that was completely dissolved within methanol; the percentage of methanol to pig slurry was ≤2% to avoid inhibition of the acclimated microbial populations (EMA, 2011). Subsequently sub-samples of the slurries were taken using a serological pipette and the concentrations of antibiotics were measured at 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 3 d, 7 d, 14 d, 28 d and 56 d. During the incubation the vessels were kept in the dark at 23 ± 0.5 °C, both temperature and light were controlled during this timeframe using a tinytag datalogger (temperature) and a room with the exclusion of light.

Detection and quantification of veterinary antibiotics was achieved using a singular method on a SCIEX Triple Quad 5500+ LC-MS/MS which utilized High Performance Liquid Chromatography (HPLC) reversed phase methodology. A Phenomenex Kinetics XB-C18 2.6 µm LC column (50 × 2.1 mm) was used and the mobile phases consisted of 0.1% formic acid in methanol and 0.1% formic acid (aqueous). A 15 µl injection volume and a flow rate of 0.4 mL/m was utilized, and the duration of the method was 11 min with the following gradient, 0 min (0% B), 3 min (90% B), 8 min (90% B), 8.1

min (10% B), 11 min (0% B). Analyst 1.6 software (SCIEX) was used to process and quantitate the samples. The LOD's of the assessed analytes in pig slurries were found to range from 0.000025 to 0.05 mg/kg, whilst the LOQ's ranged from 0.006 to 0.073 mg/kg (see SI Table B3 for specific LODs/LOQs and MS methodology). Quality controls were utilized within the analysis of veterinary medicines to ensure precision and accuracy during the data acquisition (please see SI section 2.0 for further details).

2.1.4 Abiotic degradation experiments

In order to assess abiotic processes, sterile slurry degradation and hydrolysis experiments were performed. The sterile manure experiment was performed with the same conditions (pH, temperature and light) as the non-sterile experiment (except for sterilisation). The slurries were moisture corrected and autoclaved at 120 °C for 30 min at 0.206 bar. Formaldehyde was used to maintain sterility during the studies duration; during the acclimation period the starting concentration of formaldehyde was 0.3%, subsequently, 1.42 mL of formaldehyde was added weekly. Sterility of the test system was checked weekly using nutrient broth and swab checks and, if required, further confirmations were achieved using agar plates. Sterile swabs were used to sample the slurry and the nutrient broths were incubated at 30 °C for 12 h. Visual comparisons for turbidity (bacterial growth) were made between the respective controls and the sample. The hydrolysis analysis was also conducted under the same experimental conditions as both manure degradation studies. For hydrolysis, Kolthoff and Vleeschouwer and buffers as well as deionized water were set up at varying pHs as per the OECD 111 guideline (OECD, 2004). These samples were aqueous alone and contained no pig slurry. Buffers containing the antibiotics were stored in 10 mL glass culture tubes and destructively sampled and filtered using a 0.2 μm nylon syringe filter prior to analysis.

2.1.4 Liquid chromatography-high resolution mass spectrometry (LC-HRMS)

A Thermo Scientific Exactive Orbitrap LC-HRMS system was used to analyse samples from the 2 h, 6 h, 24 h, 48 h and 14 d timepoints. An ACE 3Q aqueous (150 mm by 3

mm, 3 μm) LC column was used, the mobile phases consisted of methanol: acetonitrile (50:50) with 0.1% formic acid (mobile phase A) and H_2O with 0.1% formic acid in methanol (mobile phase B). The gradient was 37 min in total and utilized reversed phase chromatography, the details were as follows, 0–5 min (0% B), 5–20 min (100% B), 20–30 min (100% B), 30–35 min (0% B) and 37 min (0% B). A 25 μl injection volume, 0.4 mL flow rate and column oven temperature of 40 °C was utilized. Both positive and negative ionization electron spray conditions were assessed in separate runs. Calibrations were performed prior to ensure the instruments mass accuracy (±5 ppm).

Using Thermo Fisher software Xcalibur 2.2 (SP1) qualitative processing techniques were utilized to identify potential TP peaks. An in-house database of 66 possible phase 1 TPs was used and the processing mass tolerance windows were set at ±5 ppm and ±10 ppm for the positive and negative conditions respectively. Occurrence of a specific TP was confirmed and tentatively identified through respective comparisons to the controls (i.e., presence vs absence). Peak areas were collated, and comparisons were made between pH treatments; due to peak area (Cps) being used, the comparisons made are relative between samples and do not reflect absolute concentration. Unfortunately, standards for all TPs were not available to determine absolute concentrations or confirm identities. Nevertheless, the observed trends, in relative abundance, reveal findings with regards to the TP rate or route assessments.

2.1.5 Degradation kinetics and statistical analyses

Modelling software (CAKE v3.3) was used to derive the kinetic profile; the same kinetic models were used throughout all pH treatments facilitating statistical comparisons. The most appropriate fit for all three pHs was selected, ensuring the residual plots had a chi-squared <15%. In order to obtain the degradation kinetics of the identified TPs, peak areas were plotted against a normalized time (i.e., where degradation initiated). The degradation software was used to derive the DT_{50} values using Single First Order (SFO) and First Order Multi Compartment (FOMC) models (Eq 1 + 2).

Equation 1: $DT50\ (SFO) = \frac{ln}{k}$

Equation 2: $DT50\ (FOMC) = \beta(2\frac{1}{\alpha} - 1)$

Where: k is the degradation rate constant, α is alpha and β is beta.

Statistical analyses were completed using Minitab 18. An analysis of variance (two-way) was conducted on the sample data to statistically compare between the three pH treatments over time for each analyte (concentration = time*pH), a three-way ANOVA was conducted for the analyses of time*pH*treatment. Statistical analysis was conducted using five replicates (n = 5) and statistical significance was reported at the 95% confidence interval (p value = $\leq$ 0.05). Tukey post hoc comparisons were undertaken on the analyses of variance to distinguish comparisons between pHs and treatments.

2.2 Results

2.2.1 Degradation within pig slurries at different pHs

The degradation data was best fit to SFO kinetics for CFT, OTC, FLO and SMX, whereas TYL followed a bi-phasic degradation pattern, therefore the FOMC was the best suited model (Fig. 1). Generally, the persistence of each compound under neutral slurry conditions followed this hierarchy: FLO < CFT < TYL < SMX < OTC. OTC was persistent throughout the experiment and the study's duration (56 d) was not adequate to fully assess the degradability of this compound within the different slurry pHs.

An ANOVA (two-way) revealed the significant effect of non-sterile pig slurry pH on the degradation of FLO ($p \leq 0.05$), TYL ($p \leq 0.05$), SMX ($p \leq 0.05$) and to a lesser extent CFT ($p \leq 0.05$) (Fig. 1 and Table .2). Within acidic pig slurries the degradation rates of FLO, CFT, and TYL were significantly inhibited over that of the neutral treatment ($p \leq 0.05$), whilst alkaline conditions were found to promote degradation ($p \leq 0.05$) (Fig. 1). FLO degradation within acidic slurries exhibited a lag phase of 72 h and the degradation rate was reduced by a factor of over 100 in comparison to that of neutral (k 0.00229 < 0.2451) ($p \leq 0.05$). This resulted in a 100-fold increase in DT50 in comparison to neutral/alkaline conditions (2 h–8.7 d) (Table .2 and Fig. 1). Similarly, TYL degradation within acidic slurries was inhibited and also experienced a lag phase, resulting in a six-fold increase in the DT50 value when compared to pH 7 (1.47–9.75 d) ($p \leq 0.05$). Interestingly, under alkaline conditions TYL exhibited a ten-fold increase in degradation rate over that of neutral (3.52–35.26 h) (Fig. 1 and Table .2) ($p \leq 0.05$). Comparatively, acidic conditions promoted the degradation rate constant for SMX over that of neutral (k 0.3976 < 0.4458) ($p \leq 0.05$), whilst alkaline pH was found to significantly hinder the degradation (Fig. 1), thus driving differences within the calculated DT_{50} values, these were 20.6 h at pH 5.5, 31.6 h for pH 7 and 122.8 h at pH 8.5 (Table 2).

2.2.2 Abiotic degradation in sterile slurry

Of the selected antibiotics, FLO, CFT, TYL and SMX degradation rates in sterile slurry were found to be significantly reduced during the analysis of variance (three-way), when they were compared to the non-sterile treatment ($p \leq 0.05$) (Fig. 1 and Table .2). However, OTC under sterile conditions was observed to degrade faster over that of non-sterile; a 70-fold increase in degradation was noted at a pH of 7 between non-sterile and sterile treatments (Figure 2.0 and Table 2.1).

Degradation in sterile slurry treatments was not significantly affected by the slurry pH for FLO and CFT. However, the analysis of variance (two-way) revealed that pH treatment drove differences for SMX, and to a lesser extent TYL. Faster dissipation (adsorption/degradation) was exhibited for SMX within sterile acidic conditions (DT50 5.04 d) compared to that of neutral (DT50 23.7 d) ($p \leq 0.05$) (Table 2.1). Increased TYL degradation was observed under sterile alkaline conditions at 56 d. Although this trend was deemed insignificant, it revealed some degree of difference to that of neutral ($p = 0.094$); a 700-fold increase in β rate constant was observed (Table 2.1).

2.2.3 Hydrolysis

A three-way ANOVA demonstrated that the rates of degradation for CFT, FLO, SMX and TYL within the hydrolysis experiment were significantly lower than in the slurry system ($p \leq 0.05$) (Table 2.0). For example, at pH 7, FLO persisted for 56 d and the concentration did not change. Comparatively, OTC was found to be hydrolytically unstable which resulted in a DT_{50} of 9.3 h at pH 7. The influence of pH within buffers was very minor or had no significant effect on the elimination of FLO, OTC and TYL. Differences in pH and hydrolytic degradation rates were observed for both CFT and SMX, CFT degradation was increased in alkaline buffers whilst SMX was promoted under acidic conditions ($p \leq 0.05$) (Table 2.0 and Figure B2).

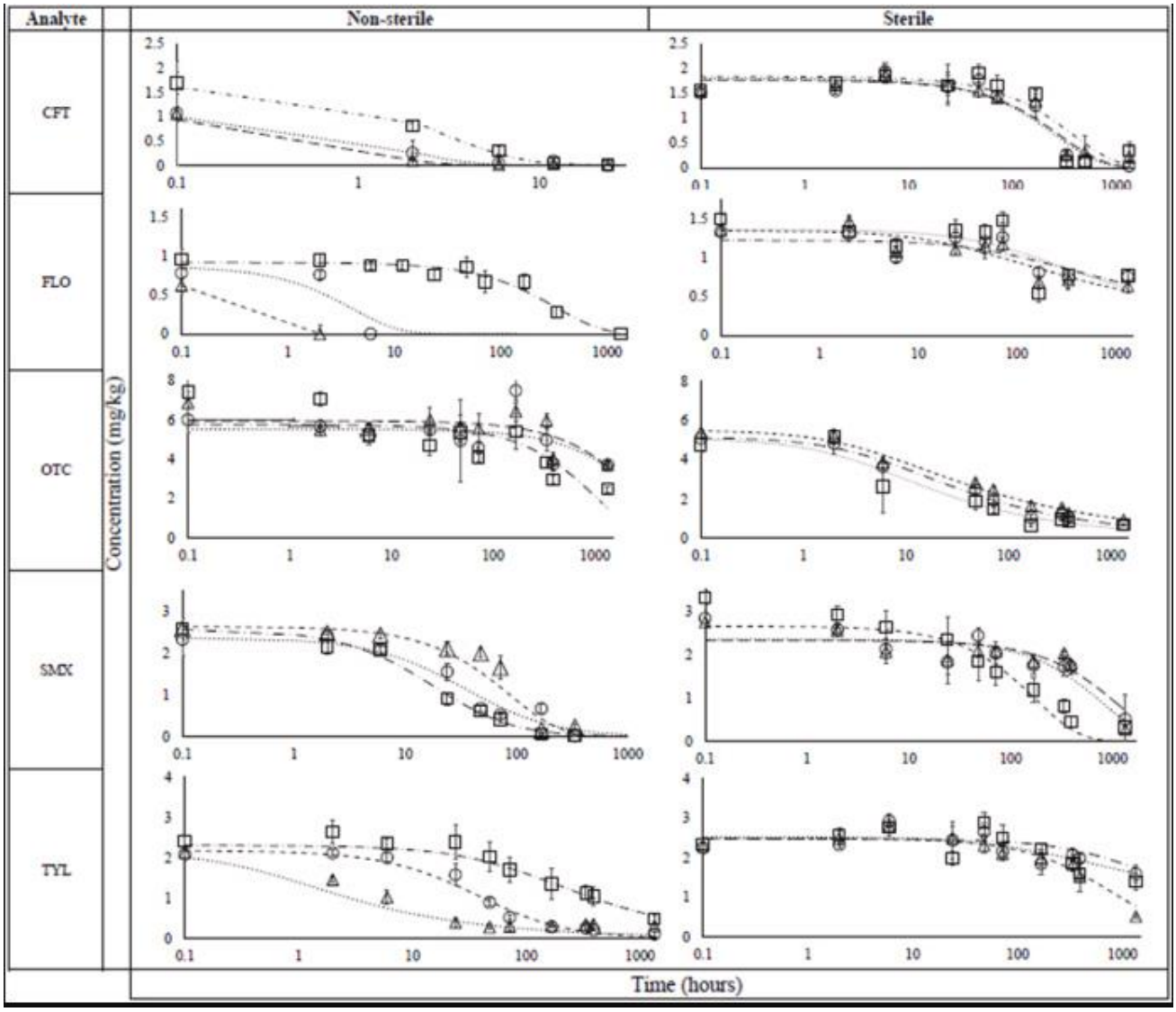


Figure 2.0: Degradation of selected veterinary antibiotics at varying pHs over time within non-sterile pig slurry and sterile pig slurry. The x-axis is presented logarithmically, the error bars represent the standard deviation of five replicates.

Table 2.0: Degradation rates (DT_{50}) of veterinary antibiotics under different pH conditions and treatments, non-sterile slurry, sterile slurry and aqueous buffer (hydrolysis).

Compound	Matrix	pH 8.5	pH 7	pH 5.5
Ceftiofur	*Pig slurry*	0.59 h	0.99 h	2.13 h
	Sterile pig slurry	6.5 d	6.5 d	2.33 d
	Aqueous buffer	19 d	49.16 d	75 d
Florfenicol	*Pig slurry*	<2 h	<2 h	8.7 d
	Sterile pig slurry	30.95 d	79.58 d	45.83 d
	Aqueous buffer	>416 d	>416 d	>416 d
Oxytetracycline	*Pig slurry*	78 d	90 d	29.04 d
	Sterile pig slurry	38.8 h	30.7 h	17 h
	Aqueous buffer	17.8 d	9.4 d	11 d
Sulfamethoxazole	*Pig slurry*	122.8 h	31.6 h	20.6 h
	Sterile pig slurry	32.5 d	23.7 d	5.04 d
	Aqueous buffer	>416 d	>416 d	80.41 d
Tylosin	*Pig slurry*	3.52 h	35.28 h	234 h
	Sterile pig slurry	165 d	245 d	675 d
	Aqueous buffer	>416 d	>416 d	114 d

Footnote – For compounds and assessments which were inherently persistent a DT_{50} value of >416 d has been selected. A consequence of not doing so would result in trends and forced modelled predictions, the stability past this point is unknown.

2.2.4 Identification of transformation products within non-sterile pig slurry

A large spectrum of known and unknown TPs was tentatively identified for all of the study compounds in the non-sterile pig slurry treatments (SI Table B4). Monochloroflorfenicol (MCF) was tentatively identified within pig slurries, this TP is formed via the loss of a chlorine adduct and the exact mass was 323.03942 m/z. Moreover, TPs of FLO were detected for the first time in pig slurries, referred to as FLO_M_338 and FLO_M_271; the structural changes proposed for these TPs were the addition of H2 +O (338.98990 m/z) and the loss of -Cl2, –O (271.06784 m/z). OTC

degraded into metabolites referred to as APO-OTC, A-APO and B-APO OTC which are isomers with the same mass (442.13760 m/z) and could not be differentiated. SMX had a wide range of identified TPs, there were 11 observed in total referred to as SMX_M_215–301. Five of the identified metabolites have also been previously observed and documented, these were SMX_M: 287, 269, 239, 173 and 215, whilst both SMX_M_239 and SMX_259 have not been previously identified.

The transformation products that were identified to have differing rates of formation and degradation within the pH treatments for non-sterile pig slurries are presented in Figure 2.1, please refer to Figures B 3–10, for other TP profiles. The spectrum of TPs for FLO reveals pig slurry pH to affect the timing of TP formation as well as the intensity detected, this resulted in differences in TP degradation rates and routes (Figure 2.1, and Figure 2.3). Generally, under pHs 7–8.5 FLO degraded into MCF rapidly whereas under pH 5.5 FLO was observed to form FLO_M_338 and very little MCF. As a result of faster FLO degradation under alkaline conditions (Figure 2.0), rapid formation of MCF was observed (Figure 2.1). Interestingly the k rate constants were similar (pH 8.5 k 0.0753 and pH 7 k 0.07395), which produced DT_{50} values similar to one another (SI Table B5). Rapid and greater intensity of FLO_M_338 formation was observed within acidic slurries, this resulted in a DT_{50} that was over five times that of the neutral treatment (6.4–33 h) (Fig. 2). TYL transformation into TYL_B was found to be rapid at a pH of 5.5 (Figure 2.1), the calculated DT_{50} was found to be two times higher than that of neutral (13.4–31.4 h). SMX degradation produced a wide spectrum of both known and unknown TPs (Table B4), the formation of these was observed not to be pH dependent (SI Figures B 5–8).

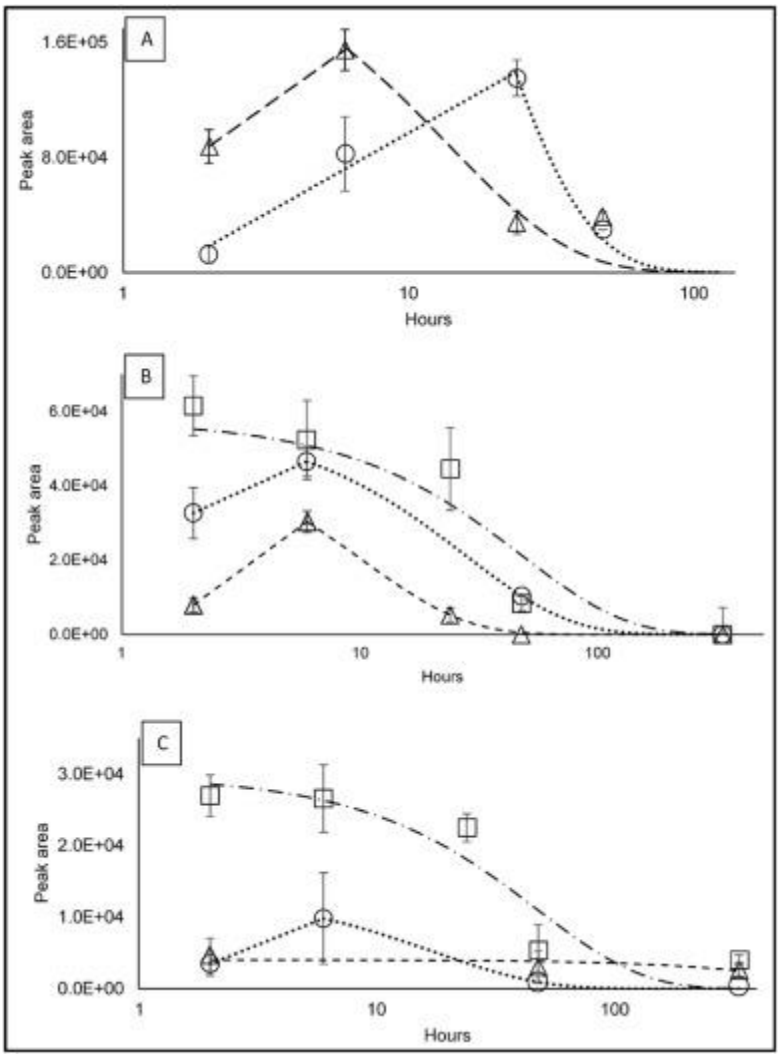


Figure 2.1: Kinetics of identified TPs in non-sterile slurries as per slurry pH treatments over time, metabolite intensity peak area is plotted on the Y-axis and time on the X-axis. A - Monochloroflorfenicol, B – TYL_B, C - FLO_M_338.

Key: pH 8.5 pH 7 pH 5.5 .

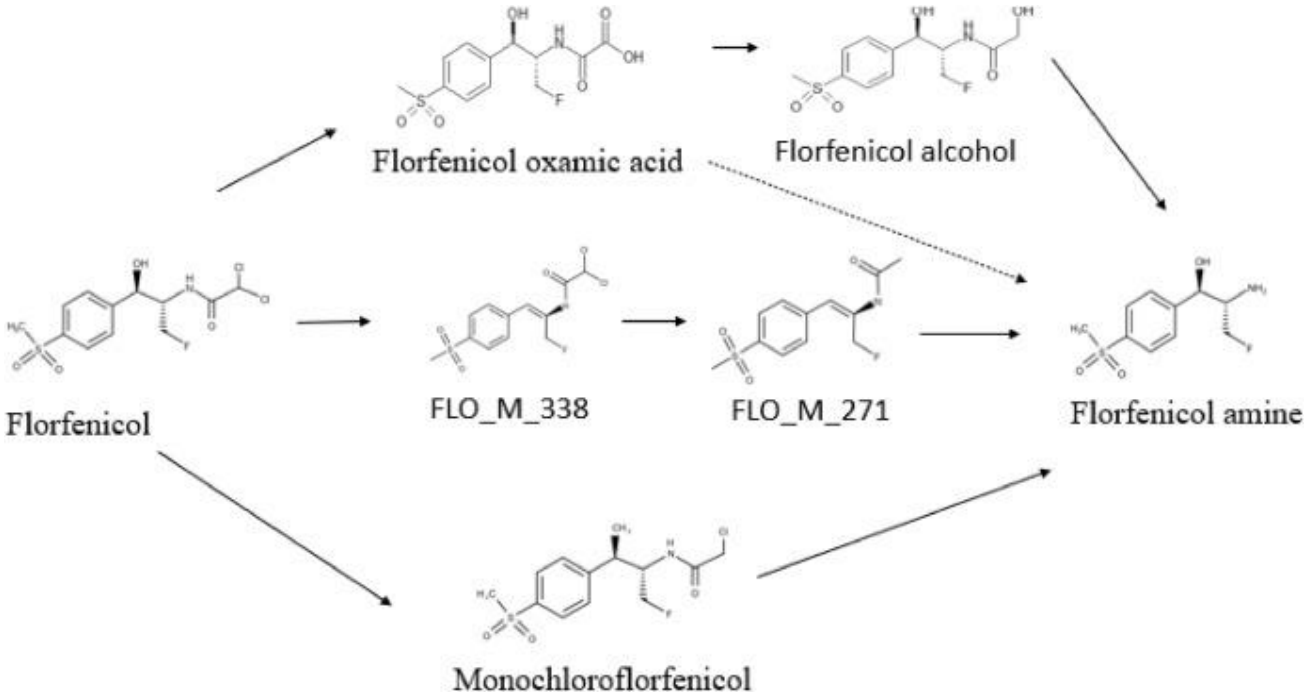


Figure 2.2: Purposed degradation pathway of florfenicol in non-sterile pig slurries.

2.3 Discussion

2.3.1. Antibiotic degradation as a function of pH

CFT, FLO, TYL and SMX were all observed to be impersistent within pig slurries at a pH of 7 and the DT_{50} values were in-line with previous reports of degradation rates within pig slurries/manures and anaerobic digestion (Gilbertson et al., 1990; Hollis, 1991; Mohring et al., 2009; Chatterjee et al., 2013; Berendsen et al., 2018; Junker et al., 2020). Acid or alkaline treatments were observed to increase/decrease degradation rate constants. Although OTC is known to be environmentally persistent (Blackwell et al., 2005; Junker et al., 2020), Wang et al., (2015) reported a DT50 value of 9.05–9.65 d, which we speculate to be related to the acidic pH (pH 5.6) and low moisture content of the pig manure used. The persistence of OTC within pig slurries is likely a result of adsorption mechanisms (cation exchange and electrostatic forces) which render it unavailable to hydrolytic and microbial degradation processes (MacKay and Canterbury, 2005; Blackwell et al., 2005). A similar pH effect for TYL has been observed within dairy lagoon sediment, comparable to those findings presented here, with alkalinity favouring degradation and acidic inhibiting degradation (Ali et al., 2013).

2.3.2 Biotic and abiotic degradation mechanisms

2.3.3 Microbial mineralization

It is evident from the degradation assessments in sterile and nonsterile slurry that biotic degradation is the primary removal mechanism for FLO, TYL and CFT in pig slurries. The differences in degradation rates for these antibiotics at pH 5.5 and 8.5 are speculated to be a result of reduced/increased microbial activity and mineralization. Sommer et al., (2015) reported acidifications of pig slurries reduced methanogen populations by 6–20%, whilst Lin et al., (2015) reported weak alkaline conditions promote anaerobes such as *Porphyromonadaceae* and *Lachnospiraceae*. The sterile-OTC assessment was inconclusive; we speculate that the faster rates of depletion under sterile conditions was attributed to the degradation of formaldehyde into formic acid,

likely further acidifying the surface of the organic matter and further increasing adsorption mechanisms (Suresh et al., 2019).

2.3.4 Adsorption and hydrolysis

It is well known that the pH of soils and sediments influences the rate and mechanisms of adsorption for veterinary medicines, for example publications have detailed an increase in the adsorption coefficients at lower pHs for tetracycline, spectinomycin and florfenicol (Hu et al., 2008; Wang et al., 2014; Conde-Cid et al., 2019). Whilst an adsorption study alone was not conducted, the differences between the abiotic - biotic degradation assessments (non-sterile, sterile and hydrolysis) indicate that adsorption processes could have contributed to the differing rates of degradation that was observed between the pH treatments. These assessments revealed that differences within the degradation rates between pH treatments could be related to adsorption processes for SMX and to a lesser extent TYL (Figure 2.0).

At lower pHs SMX is an uncharged species (pKa^1 1.4 and pKa^2 5.7), increasing its affinity to form hydrophobic interactions with organic matter/carbon as well as cationic complexes with lipids, carboxylic and hydroxyl groups (Boguta and Sokołowska, 2020, Hu et al., 2019; Jia et al., 2017, Srinivasan et al., 2013). Therefore, we speculate that the increased SMX elimination at a pH 5.5 and stunted dissipation at pH 8.5 was found to be related to sorption and to a lesser extent hydrolysis. This phenomenon has previously been identified for SMX within acidic composted manures and acidic manured soils. For example, the adsorption-desorption coefficient (Kd) at pH 4 was 2383.3 L/kg whilst at pH 5.5 this was 24.9 L/kg (Hu et al., 2019; Thiele-Bruhn and Aust, 2004). Moreover, the accelerated hydrolysis rate at lower pHs is a result of increased protonation of SMX when it is predominantly in its cationic form (Manzo and Martinez de Bertorello, 1978; Białk-Bielińska et al., 2012). ter Laak et al., (2006) demonstrated that at pH 8.5 the adsorption coefficient of TYL increases, therefore it is highly likely that the observed differences within the degradation rate under the non-sterile degradation assessment are related to the promotion of adsorption mechanisms.

2.3.4 Transformation products within slurry treatments

It is critical to consider TPs when assessing the degradability of compounds within environmental matrices as in some cases these products can maintain a similar mode of action, as well as having similar or greater ecotoxicological effects, and persistence within the environment (Sinclair and Boxall, 2003; La Farre et al., 2008; Koba et al., 2017). For example, florfenicol alcohol (FOH) has been reported to have similar toxicological effects towards the green algae *Pseudokirchneriella Subcapitata* as FLO; the minimum inhibitory concentrations for these were found to be > 0.98 mg/L (Hoberg, 1991; FDA, 2013). Increasing attention is being given towards TPs within recent publications, however, due to cost and availability of analytical standards and LC-HRMS, our understanding of the behaviour of TPs in the environment is still in its infancy.

Differences within degradation rates for CFT could be related to the formation of desfuroylceftiofur and its analogue deacetyl-cefotaxime. These TPs form through the cleavage of thioester bonds and it has been identified that this occurs more rapidly under alkaline conditions (Koshy and Cazers, 1997; Sunkara et al., 1999). The rapid degradation of FLO under alkaline/neutral conditions was found to be a result of biotic transformation into MCF. MCF is unstable and degrades into the final degradant florfenicol amine (DT50 35 h), although no clear relationship was observed between the pH treatments, MCF and florfenicol amine (FA) (Figure 2.2). Higher intensity detections were observed for both FOA and FLO_M_338 within the acidic slurries, however it remains unclear whether increased formation was observed within these conditions or inhibited degradation provided better detections of these TPs. FLO_M_271 is potentially a degradant of FLO_M_338 and both have previously identified within agricultural soils, but this is the first time they have been identified within pig slurries (Qiu et al., 2021).

Nine metabolites were identified during the degradation of SMX, five of which have previously been identified and detected within sewage sludge and hospital wastes

(Martín de Vidales et al., 2012; Srinivasan et al., 2013). SMX's TP profile is complex but indicates that all pH treatments had the same TPs present (Figures B 5–8). Although higher rates of formation and faster degradation rates were observed at different pHs, there was little compelling evidence to suggest that the acidic slurry treatment increased the rate or altered the route of SMX transformation. TYL_B has been previously detected within animal tissues, slurries and manured soils and is known to form under acidic conditions (Loke et al., 2000; Cherlet et al., 2002). The transformation product profile of TYL did not reveal a suitable explanation to increased removal within the alkaline treatment although we suspect this to be related to unidentified TPs that were not detected during the analyses.

2.3.5 Scientific importance and implications towards the management of manures and the veterinary medicine risk assessment

The processing of animal slurries is often overlooked within veterinary medicine risk assessment even though farmers frequently adjust the properties of slurries (acidification and moisture) in order to improve/retain nutrient content, reduce greenhouse gas emissions and improve compliance with the Nitrate Directive (91/676/EEC) (Kai et al., 2008; Clemens et al., 2002; Sommer et al., 2017; Wang et al., 2014). Slurry acidification is becoming increasingly popular in Holland and Denmark due to the reduced requirement to plough or inject slurries from Dutch authorities; in 2012 10% of slurries were acidified (Hjorth et al., 2013; Fangueiro et al., 2015a).

Until now the influence of acidifying slurries on antibiotic degradation was unknown but the results presented here demonstrate this could lead to an exceedance of permissible limits of antibiotics in the environment deemed as acceptable under current risk assessment paradigms. For example, if the measured fate data (DT_{50}) were used to derive $PEC_{soil\ refined}$, the slurry acidification scenario would result in increased soil concentrations of FLO and TYL (EMA, 2011). The $PEC_{soil\ refined}$ for TYL at a pH of 7 is 45 times lower than that when using the pH 5.5. Similarly, FLO $PEC_{soil\ refined}$ is 1.23E-32 µg/kg at a pH 7, but at pH 5.5 this was calculated to be 138.21 µg/kg. If degradation

was assessed at an acidic pH, the PEC calculation is in an excess of the specified 100 µg/kg threshold (EMA, 2016). Elevated concentrations also present wider ecosystem risks that would not have been considered if degradation was assessed at neutral pH. For example, increased concentrations of TYL within arable soils would decrease post-emergence survival for all of the standardized plants assessed via Simon et al., (2015) (EC_{50} 15–400 mg/kg). Likewise, the elevated FLO soil concentrations have previously been identified to cause 10% decrease in the biomass for *A.cepa, B.napus* and, *S.alba* (EC_{50} 60 to >70 µg/kg) (Richter et al., 2016). Comparatively, the acidification process would be an ideal mitigation measure for removing SMX from pig slurries and soils.

In order to rectify variability within manure degradation trials, we suggest that multiple slurries of the same type with a range of properties should be used, which will provide a more thorough assessment of veterinary medicine degradability. It is probable that similar effects of pH seen in the correct investigation maybe observed in cattle manure and poultry litter, but the impact of pH on degradation rate and route of antibiotics has not been determined yet. To ensure the subsequent environmental risk assessment is comprehensive and precautionary then it is important that manure degradation studies replicate realistic on-farm practices and variability. Therefore, we suggest a range of pig slurries are considered which as a minimum demonstrate a range of realistic pH values.

2.4 Conclusion

The crucial findings of this study demonstrate the influence of pig slurry pH on the dissipation of veterinary antibiotics and could contribute to a more robust risk assessment. A compound specific effect was found for antibiotic degradation and the effect of slurry pH, for example, FLO, TYL and CFT degradation was inhibited under acidic conditions and enhanced under alkaline over that of neutral. SMX degradation was observed to increase under acidic conditions and be inhibited under alkaline over that of neutral. Biotic processes drove differences between pH treatments for FLO, TYL and CFT, whilst abiotic processes contributed to differences for SMX and to a lesser extent TYL. Different TPs were identified associated with particular pH conditions,

revealing differences within biotic processes. The findings presented here demonstrate that current regulatory and manure management practices could result in greater environmental exposure than anticipated.

References

EMA, Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 and GL38. 2016 EMA/CVMP/ERA/418282/ 2005-Rev.1-Corr.1.

EMA, 2011. Guideline on Determining the Fate of Veterinary Medicinal Products in Manure. EMEA/CVMP/ERA/430327/2009.

Ali, M., Wang, J.J., DeLaune, R.D., Seo, D.C., Dodla, S.K., Hernandez, A.B., 2013. Effect of redox potential and pH status on degradation and adsorption behavior of tylosin in dairy lagoon sediment suspension. *Chemosphere* 91 (11), 1583–1589.

An, B., Xu, X., Ma, W., Huo, M., Wang, H., Liu, Z., Cheng, G., Huang, L., 2021. The adsorption-desorption characteristics and degradation kinetics of ceftiofur in different agricultural soils. *Ecotoxicology and Environmental Safety*. 222, 112503.

Berendsen, B.J., Lahr, J., Nibbeling, C., Jansen, L.J., Bongers, I.E., Wipfler, E.L., Van de Schans, M.G., 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. *Chemosphere* 204, 267–276.

Białk-Bielinska, ´ A., Stolte, S., Matzke, M., Fabianska, ´ A., Maszkowska, J., Kołodziejska, M., Liberek, B., Stepnowski, P., Kumirska, J., 2012. Hydrolysis of sulphonamides in aqueous solutions. *Journal of Hazardous Materials*. 221, 264–274.

Blackwell, P.A., Boxall, A.B., Kay, P., Noble, H., 2005. Evaluation of a lower tier exposure assessment model for veterinary medicines. *Journal of Agricultural Food Chemistry*. 53 (6), 2192–2201.

Bogaard, A., Fraser, R., Heaton, T.H., Wallace, M., Vaiglova, P., Charles, M., Jones, G., Evershed, R.P., Styring, A.K., Andersen, N.H., Arbogast, R.M., 2013. Crop manuring and intensive land management by Europe's first farmers. *Proceedings of the National Academy of Sciences*. 110 (31), 12589–12594.

Boguta, P., Sokołowska, Z., 2020. Zinc binding to fulvic acids: assessing the impact of pH, metal concentrations and chemical properties of fulvic acids on the mechanism and stability of formed soluble complexes. *Molecules* 25 (6), 1297.

Boxall, A.B., Fogg, L.A., Blackwell, P.A., Blackwell, P., Kay, P., Pemberton, E.J., Croxford, A., 2004. Veterinary medicines in the environment. *Revisions in Environmental Contamination and Toxicology*. 1–91.

Canh, T.T., Aarnink, A.J., Verstegen, M.W., Schrama, J.W., 1998a. Influence of dietary factors on the pH and ammonia emission of slurry from growing-finishing pigs. *Journal of Animal Sciences* 76 (4), 1123–1130.

Canh, T.T., Sutton, A.L., Aarnink, A.J., Verstegen, M.W., Schrama, J.W., Bakker, G.C., 1998b. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. *Journal of Animal Science* 76 (7), 1887–1895.

Chatterjee, N.S., Gupta, S., Varghese, E., 2013. Degradation of metaflumizone in soil: impact of varying moisture, light, temperature, atmospheric CO2 level, soil type and soil sterilization. *Chemosphere* 90 (2), 729–736.

Cherlet, M., De Baere, S., Croubels, S., De Backer, P., 2002. Quantitation of tylosin in swine tissues by liquid chromatography combined with electrospray ionization mass spectrometry. *Analytical Chemica Acta* 473 (1–2), 167–175.

Choudhary, M., Bailey, L.D., Grant, C.A., 1996. Review of the use of swine manure in crop production: effects on yield and composition and on soil and water quality. *Waste Management Resources* 14 (6), 581–595.

Clemens, J., Bergmann, S., Vandr´e, R., 2002. Reduced ammonia emissions from slurry after self-acidification with organic supplements. *Environmental Technology* 23 (4), 429–435.

Conde-Cid, M., Ferreira-Coelho, G., Núnez-Delgado, ˜ A., Fernandez-Calvino, ˜ D., AriasEst´evez, M., Alvarez-Rodríguez, ´ E., Fernandez-Sanjurjo, M.J., 2019. Competitive adsorption of tetracycline, oxytetracycline and chlortetracycline on soils with different pH value and organic matter content. *Environmental Research*. 178, 108669.

Cooper, P., Cornforth, I.S., 1978. Volatile fatty acids in stored animal slurry. *Journal of the Science of Food and Agriculture* 29 (1), 19–27.

SANTE, 2021. Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes. SANTE/2020/12830.

VICH, 2000. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase I. CVMP/VICH, London. CVMP/VICH/592/98-final.

VICH, 2003. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase II Draft Guidance. London. CVMP/VICH GL 38. CVMP/VICH/790/03-Consultation.

Fangueiro, D., Hjorth, M., Gioelli, F., 2015. Acidification of animal slurry–a review. *Journal of Environmental Management*. 149, 46–56.

La Farre, M., P´erez, S., Kantiani, L., Barcelo, ´ D., 2008. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *Trac. Trends Analytical. Chemistry*. 1 (11), 991–1007, 27.

FDA, 2013. Environmental Assessment for Aquaflor (Florfenicol), Aquaflor for Freshwater-Reared Finfish in Recirculating Aquaculture Systems. FDA [online] EPA.

Filippitzi, M.E., Callens, B., Pardon, B., Persoons, D., Dewulf, J., 2014. Antimicrobial use in pigs, broilers and veal calves in Belgium. *Vlaams Diergeneeskundig Tijdschrift* 83 (5), 215–224.

Gilbertson, T.J., Hornish, R.E., Jaglan, P.S., Koshy, K.T., Nappier, J.L., Stahl, G.L., Cazers, A.R., Nappier, J.M., Kubicek, M.F., 1990. Environmental fate of ceftiofur sodium, a cephalosporin antibiotic. Role of animal excreta in its decomposition. *Journal Agricultural Food Chemistry*. 38 (3), 890–894.

Hansch, C., Leo, A., Hoekman, D., Livingstone, D.J., 1995. Exploring QSAR: Hydrophobic, Electronic, and Steric Constants. American Chemical Society, Washington, DC.

Hirsch, R., Ternes, T., Haberer, K., Kratz, K.L., 1999. Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment*. 225 (1–2), 109–118.

Hjorth, M., Cocolo, G., Jonassen, K., Sommer, S.G., 2013 Jun. Acidifications effect on transformations in and composition of animal slurry. In15th RAMIRAN Int. Conf. 10–13.

Hoberg, J.R., 1991. SCH 45705: Toxicity to the Freshwater Green Alga, Selenastrum Capricornutum. Schering-Plough Report No.: A-25389.

Hollis, J.M., 1991. Mapping the vulnerability of aquifers and surface waters to pesticide contamination at the national/regional scale. Pesticides in Soils and Water. BCPC Monogr. 47, 165 174.

Hu, J.T., Zong, H.M., Wang, J.Y., Ma, D.Y., 2008. Adsorption behaviors of florfenicol on sediment in the ocean. *Environmental Chemistry* 27 (4), 481–484.

Hu, Z., Liu, Y., Chen, G., Gui, X., Chen, T., Zhan, X., 2011 Aug. Characterization of organic matter degradation during composting of manure–straw mixtures spiked with tetracyclines. *Bioresources and. Technology* 102 (15), 7329–7334.

Hu, S., Zhang, Y., Shen, G., Zhang, H., Yuan, Z., Zhang, W., 2019. Adsorption/desorption behavior and mechanisms of sulfadiazine and sulfamethoxazole in agricultural soil systems. *Soil Tillage Research*. 186, 233–241.

OECD, 2004. Hydrolysis as a Function of pH. OECD Guidelines For the Testing of Chemicals.

Jechalke, S., Heuer, H., Siemens, J., Amelung, W., Smalla, K., 2014. Fate and effects of veterinary antibiotics in soil. *Trends in Microbiology* 22 (9), 536–545.

Jia, Y., Khanal, S.K., Zhang, H., Chen, G.H., Lu, H., 2017. Sulfamethoxazole degradation in anaerobic sulfate-reducing bacteria sludge system. *Water Research* 119, 12–20.

Joubin, M., 2018. Animal Slurry Acidification: Effects of Slurry Characteristics Use of Different Acids, Slurry pH Buffering. RISE, Uppsala, Sweden, p. 40.

Junker, T., Atorf, C., Berkner, S., Düring, R.A., Hennecke, D., Herrchen, M., Konradi, S., Merrettig-Bruns, U., Rombke, ¨ J., Wagner, J., Weinfurtner, K., 2020. Development of a test method for transformation of veterinary pharmaceuticals and biocides in anaerobic liquid manure. *Environmental Sciences Europe*. 32 (1), 1–21.

Kai, P., Pedersen, P., Jensen, J.E., Hansen, M.N., Sommer, S.G., 2008. A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emissions. *European Journal of Agronomy*. 28 (2), 148–154.

Kay, P., Blackwell, P.A., Boxall, A.B., 2005. Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. *Chemosphere* 59 (7), 951–959.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological. Indicators*. 8 (1), 1–3.

Koba, O., Golovko, O., Kodesova, R., F´er, M., Grabic, R., 2017. Antibiotics degradation in soil: a case of clindamycin, trimethoprim, sulfamethoxazole and their transformation products. *Environmental Pollution*. 220, 1251–1263.

Koshy, K.T., Cazers, A.R., 1997. Controlled hydrolysis of ceftiofur sodium, a broad spectrum cephalosporin; isolation and identification of hydrolysis products. *Journal of Pharmaceutical Science*. 86 (3), 389–395.

Kreuzig, R., Holtge, ¨ S., Brunotte, J., Berenzen, N., Wogram, J., Schulz, R., 2005. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. *Environmental Toxicology and Chemistry an International Journal.* 24 (4), 777–781.

Lamshoft, ¨ M., Sukul, P., Zühlke, S., Spiteller, M., 2010. Behaviour of 14C-sulfadiazine and 14C-difloxacin during manure storage. *Science of the Total Environment* 408 (7), 1563–1568.

Lee, J., 2010. Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. *Science of Horticulture*. 124 (3), 299–305.

Lekagul, A., Tangcharoensathien, V., Yeung, S., 2019. Patterns of antibiotic use in global pig production: a systematic review. *Veterinary Animal Science* 7, 100058.

Li, X., Liu, C., Chen, Y., Huang, H., Ren, T., 2018. Antibiotic residues in liquid manure from swine feedlot and their effects on nearby groundwater in regions of North China. *Environmental Science and Pollution*. 25 (12), 11565–11575.

Lin, L., Wen, L., Chen, S., Yang, X., Liu, X., Wan, C., 2015. Effect of alkaline treatment pattern on anaerobic fermentation of swine manure. *Process Biochemistry*. 50 (11), 1710–1717.

Loke, M.L., Ingerslev, F., Halling-Sørensen, B., Tjørnelund, J., 2000. Stability of tylosin A in manure containing test systems determined by high performance liquid chromatography. *Chemosphere* 40 (7), 759–765.

MacKay, A.A., Canterbury, B., 2005. Oxytetracycline sorption to organic matter by metal-bridging. *Journal Environmental Quality*. 34 (6), 1964–1971.

Manzo, R.H., Martinez de Bertorello, M., 1978. Isoxazoles. 4. Hydrolysis of sulfonamide isoxazole derivatives in concentrated sulfuric acid solutions. A new treatment of the medium effects on protonation equilibriums and reaction rates. *Journal of Organic Chemistry*. 43 (6), 1173–1177.

Martín de Vidales, M.J., Robles-Molina, J., Domínguez-Romero, J.C., Cañizares, P., Sáez, C., Molina-Díaz, A. and Rodrigo, M.A., 2012. Removal of sulfamethoxazole from waters and wastewaters by conductive-diamond electrochemical oxidation. *Journal of Chemical Technology & Biotechnology*, 87(10), pp.1441-1449.

Martinez-Suller, L., Azzellino, A., Provolo, G., 2008. Analysis of livestock slurries from farms across Northern Italy: relationship between indicators and nutrient content. *Biosystem Engineering*. 99 (4), 540–552.

Mitchell, S.M., Ullman, J.L., Teel, A.L. and Watts, R.J., 2014. pH and temperature effects on the hydrolysis of three β-lactam antibiotics: Ampicillin, cefalotin and cefoxitin. *Science of the total environment*, 466, pp.547-555.

Mohring, S.A., Strzysch, I., Fernandes, M.R., Kiffmeyer, T.K., Tuerk, J., Hamscher, G., 2009. Degradation and elimination of various sulfonamides during anaerobic fermentation: a promising step on the way to sustainable pharmacy? *Environmental Science and Technology*. 43 (7), 2569–2574.

Montforts, M.H., Kalf, D.F., van Vlaardingen, P.L., Linders, J.B., 1999. The exposure assessment for veterinary medicinal products. *Science of the Total Environment* 225 (1–2), 119–133.

Mroz, Z., Moeser, A.J., Vreman, K., van Diepen, J.T., Van Kempen, T., Canh, T.T., Jongbloed, A.W., 2000. Effects of dietary carbohydrates and buffering capacity on nutrient digestibility and manure characteristics in finishing pigs. *Journal of. Animal Sciences*. 78 (12), 3096–3106.

M⊘ller, H.B., Sommer, S.G., Ahring, B.K., 2004. Biological degradation and greenhouse gas emissions during pre-storage of liquid animal manure. *Journal of Environmental Quality*. 33 (1), 27–36.

Page, L.H., Ni, J.Q., Heber, A.J., Mosier, N.S., Liu, X., Joo, H.S., Ndegwa, P.M., Harrison, J.H., 2014. Characteristics of volatile fatty acids in stored dairy manure before and after anaerobic digestion. *Biosystem Engineering* 118, 16–28.

Paul, J.W., Beauchamp, E.G., 1989. Relationship between volatile fatty acids, total ammonia, and pH in manure slurries. *Biological Wastes* 29 (4), 313–318.

Potter, P., Ramankutty, N., Bennett, E.M., Donner, S.D., 2010. Characterizing the spatial patterns of global fertilizer application and manure production. *Earth Interactions*. 14 (2), 1–22.

Qiu, M., Hu, A., Yu-ming, M.H., Zhao, Y., He, Y., Xu, J., Lu, Z., 2021. Elucidating degradation mechanisms of florfenicol in soil by stable-isotope assisted nontarget screening. *Journal of Hazardous Materials*. 403, 123974.

Rabølle, M., Spliid, N.H., 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere* 40 (7), 715–722.

Ratasuk, N., Boonsaner, M., Hawker, D.W., 2012. Effect of temperature, pH and illumination on abiotic degradation of oxytetracycline in sterilized swine manure. *Journal of Environmental Science and Health*. 47 (11), 1687–1694.

Ribeiro, A.R., Lutze, H.V., Schmidt, T.C., 2018. Base-catalyzed hydrolysis and speciation dependent photolysis of two cephalosporin antibiotics, ceftiofur and cefapirin. *Water Research*. 134, 253–260.

Richter, E., Berkner, S., Ebert, I., Forster, B., Graf, N., Herrchen, M., Kühnen, U., Rombke, J., Simon, M., 2016. Results of extended plant tests using more realistic exposure scenarios for improving environmental risk assessment of veterinary pharmaceuticals. *Environmental Science Europe*. 28 (1), 1–7.

Salgado, M.M., Blu, R.O., Janssens, M., Fincheira, P., 2019. Grape pomace compost as a source of organic matter: evolution of quality parameters to evaluate maturity and stability. *Journal of Clean Production*. 216, 56–63.

Sassman, S.A., Lee, L.S., 2005. Sorption of three tetracyclines by several soils: assessing the role of pH and cation exchange. *Environmental Science and Technology*. 39 (19), 7452–7459.

Shan, S., Wang, H., Fang, C., Chu, Y., Jiang, L., 2018. Effects of adding biochar on tetracycline removal during anaerobic composting of swine manure. *Chemical Ecology.* 34 (1), 86–97.

Simon, M., Herrchen, M., Graf, N., Forster, B., Rombke, J., 2015. Concept Development for an Extended Plant Test in the Environmental Risk Assessment of Veterinary Medicinal Products. Federal Environmental Agency, (Germany).

Sinclair, C.J., Boxall, A.B., 2003. Assessing the ecotoxicity of pesticide transformation products. *Environmental Science and Technology*. 37 (20), 4617–4625.

Sommer, S.G., Husted, S., 1995. A simple model of pH in slurry. *Journal of Agricultural Sciences.* 124 (3), 447–453.

Sommer, S.G., Hjorth, M., Leahy, J.J., Zhu, K., Christel, W., Sørensen, C.G., 2015. Pig slurry characteristics, nutrient balance and biogas production as affected by separation and acidification. *Journal of Agricultural Sciences.* 153 (1), 177–191.

Sommer, S.G., Clough, T.J., Balaine, N., Hafner, S.D., Cameron, K.C., 2017. Transformation of organic matter and the emissions of methane and ammonia during storage of liquid manure as affected by acidification. *Journal of Environmental Quality*. 46 (3), 514–521.

Spaepen, K.R., van Leemput, L.J., Wislocki, P.G., Verschueren, C., 1997. A uniform procedure to estimate the predicted environmental concentration of the residues of veterinary medicines in soil. *Environmental Toxicology and Chemistry*.: *An International Journal*. 16 (9), 1977–1982.

Srinivasan, P., Sarmah, A.K., Manley-Harris, M., Wilkins, A.L., 2010. Sorption of Sulfamethoxazole, Sulfachloropyridazine and Sulfamethazine onto Six New Zealand Dairy Farm Soils. In19th World Congress of Soil Solutions for a Changing World.

Srinivasan, P., Sarmah, A.K., Manley-Harris, M., 2013. Co-contaminants and factors affecting the sorption behaviour of two sulfonamides in pasture soils. *Environmental Pollution*. 180, 165–172.

Stephens, C.R., Murai, K., Brunings, K.J., Woodward, R.B., 1956. Acidity constants of the tetracycline antibiotics. *Journal of American Chemical Society*. 78 (16), 4155–4158.

Sukul, P., Lamshoft, ¨ M., Kusari, S., Zühlke, S., Spiteller, M., 2009. Metabolism and excretion kinetics of 14C-labeled and non-labeled difloxacin in pigs after oral administration, and antimicrobial activity of manure containing difloxacin and its metabolites. *Environmental Research*. 109 (3), 225–231.

Sunkara, G., Navarre, C.B., Kompella, U.B., 1999. Influence of pH and temperature on kinetics of ceftiofur degradation in aqueous solutions. *Journal of Pharmaceutical Pharmacology*. 51 (3), 249–255.

Suresh, S., Kante, K., Fini, E.H., Bandosz, T.J., 2019. Combination of alkalinity and porosity enhances formaldehyde adsorption on pig manure-derived composite adsorbents. *Microporous Mesoporous Materials*. 286, 155–162.

ter Laak, T.L., Gebbink, W.A., Tolls, J., 2006. The effect of pH and ionic strength on the sorption of sulfachloropyridazine, tylosin, and oxytetracycline to soil. *Environmental Toxicology and Chemistry*: *an International Journal*. 25 (4), 904–911.

OECD, 2002. Test No. 307: Aerobic and Anaerobic Transformation in Soil. OECD Guidelines for the Testing of Chemicals. Section 3.

Thiele-Bruhn, S., Aust, M.O., 2004. Effects of pig slurry on the sorption of sulfonamide antibiotics in soil. *Archives of Environmental Contamination and Toxicology*. 47 (1), 31–39.

Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils–a review. *Journal of Plant Nutrition and Soil Science*. 166 (2), 145–167.

US EPA, 2006. Estimation program interface (EPI) suite. Ver.3.12. Available from: as of Apr 18. https://www.epa.gov/oppt/exposure/pubs/episuitedl.htm. 2004.

Wang, N., Zhao, W.Y., Huang, W.L., 2014a. Adsorption of streptomycin on soil: effects of pH and the presence of cations. *Advanced Materials Research*. 1010, 10–14.

Wang, K., Huang, D., Ying, H., Luo, H., 2014b. Effects of acidification during storage on emissions of methane, ammonia, and hydrogen sulfide from digested pig slurry. *Biosystems Engineering*. 122, 23–30.

Wang, Y., Chen, G., Liang, J., Zou, Y., Wen, X., Liao, X. and Wu, Y., 2015. Comparison of oxytetracycline degradation behavior in pig manure with different antibiotic addition methods. *Environmental Science and Pollution Research*, 22(23), pp.18469-18476.

Weinfurtner, K., 2011. Matrix Parameters and Storage Conditions of Manure.

Yalkowsky, S.H., He, Yan, 2003. Handbook of Aqueous Solubility Data: an Extensive Compilation of Aqueous Solubility Data for Organic Compounds Extracted from the AQUASOL dATAbASE. CRC Press LLC, Boca Raton, FL, p. 658.

Chapter 3:

Exploring the effect of Anaerobic Pig Slurry Redox Potentials on the Degradation on Veterinary Medicines

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Abstract

Veterinary medicines are frequently used within intensive livestock husbandry and there has been a growing interest regarding their fate in the environment. However, research has seldom assessed the influence of pig slurry properties on the fate of veterinary medicines even though such an understanding is essential for a more robust environmental risk assessment. Changes within manure degradation rates have the potential to alter the concentration of antibiotics applied to land, and the outcome of the risk assessment. The aim of this work was to investigate whether commonly reported redox potentials affect the degradation rates of acetyl-salicylic acid, ceftiofur, florfenicol, oxytetracycline, sulfamethoxazole, and tylosin. The employed redox potentials were − 100 mV (reduced), − 250 mV (anaerobic) and − 400 mV (very anaerobic). A compound specific relationship was observed where the degradation of ceftiofur, florfenicol, oxytetracycline and sulfamethoxazole was inhibited under reduced conditions over that of very anaerobic; the respective DT_{50} values were 0.7–1.84 h, 1.35–3.61 h, 22.2–49.8 h, 131–211 h and 35.4–94 h. In contrast, tylosin was found to degrade faster at reduced conditions over very anaerobic (DT_{50} 6.88–19.4 h). The presented research demonstrates the importance of redox potential on degradation rates and suggests we need stringent and harmonized redox control to improve the environmental risk assessment of veterinary medicines.

3.0 Introduction

Veterinary medicines are routinely used within animal husbandry to improve/protect animal health, moreover, in some regions of the world veterinary antibiotics are also used to promote the growth of livestock (Patel et al., 2020). Often high percentages of administered veterinary medicines and their metabolites are excreted resulting in high concentrations of biologically active chemicals being detected within animal manures and urine (Halling-Sørensen et al., 2001; and Sukul et al., 2009). Typically, animal manures are used as organic fertilizers to enrich soils, improve nutrient contents/cycling as well as this being a suitable method for waste disposal (Potter et al., 2010). A consequence of doing so, however, is the potential spread of veterinary medicines into the environment (Kuchta et al., 2009; Lee, 2010; Kim et al., 2011; Nguyen Dang Giang et al., 2015; Bogaard et al., 2013; Balzer et al., 2016; and Martínez-Carballo et al., 2007). This is of concern given societal impacts such as antimicrobial resistance as well as impacts on terrestrial and aquatic ecosystems (Thiele-Bruhn and Beck, 2005; Liu et al., 2009; and Joy et al., 2014). Due to the aforementioned environmental risk of veterinary medicines, the environmental risk assessment was implemented under directive 2004/28/EC (EMEA, 1997; VICH, 2000; VICH, 2003; and EMA, 2016).

Laboratory manure degradation trials are often conducted to assess the degradability of veterinary medicines during on-farm storage (EMA, 2011). Briefly these studies analyse the concentration of the analyte over time, typically 120 d but this is dependent on the veterinary medicine (EMA, 2011). Such assessments are essential to understand the concentrations of veterinary medicines that are applied to land; during storage veterinary medicines are subject to varying dissipative processes such as microbial mineralization, sorption, and hydrolysis which have the potential to reduce the parent compound concentration (Lamshöft et al., 2010). Currently, there is variability in reported veterinary medicine degradation rates within manures; this is most likely attributed to differences in slurry properties and the unknown effect this has on degradation rates (Kreuzig, 2010; EMA, 2011; and Wohde et al., 2016). For example, degradation rates (DT_{50}) ranging between <2 and 45 d have been reported for tylosin in pig manures (Loke et al., 2000; and Berendsen et al., 2018). Despite reported uncertainty and variability within the

literature, the use of just one manure per animal type is permitted within the risk assessment. As a consequence, such assessments may result in bias and poor environmental representativeness. We speculate that variability in properties such as redox potential is driving this variability.

Manure properties are highly heterogenic due to differences in storage conditions, water content, animal feed, age, usage of biocides as well as physical manure amendments (Canh et al., 1998a, 1998b; Deng et al., 2007; Kreuzig, 2010; and Weinfurtner, 2011). The redox potential of animal manures is highly heterogenic due to differences in microbial processes (methanogenic vs aerobic) and the oxidative-reductive state is governed and correlated with pH, temperature, moisture, and manure age (Singh, 2001). For example, Park et al., (2006) investigated the influence of temperature and moisture on redox and reported lower redox values within summer (− 333 mV) over winter (− 232 mV) as a result of increased microbial activity. Redox potentials of pig slurries are seldom reported in the scientific literature, despite it being a requirement within manure degradation trials under the risk assessment (− 250 mV to − 400 mV) (Weinfurtner, 2011; EMA, 2011; and Wohde et al., 2016). When redox potentials are reported they range from slightly aerobic-reduced values (i.e., +50 to − 189 mV) (Kolz et al., 2005; Kreuzig, 2010; and Widyasari-Mehta et al., 2016) to more commonly reported anaerobic conditions (i.e., − 285 to − 410 mV) (Lamshoft ¨ et al., 2010; Richter et al., 2016; and Junker et al., 2020).

Redox potential of environmental matrices/wastes can be controlled to eliminate contaminants such as nutrients, metals, organic matter, industrial chemicals, phenolic compounds, and endocrine disrupters (Charpentier et al., 1987, 1998; and Dusing et al., 1992; Goncharuk et al., 2010; and Ghernaout, and Elboughdiri, 2020). For example, redox potential control is often utilized to treat wastewaters, with a series of anaerobic, anoxic and aerobic phases being employed to establish biological nutrient removal (BNR) (Akın and Ugurlu, 2005; Luo et al., 2002). During these phases differing microorganisms dominate and result in varying biological processes which take place at specific ORP ranges, which have also been outlined in animal wastes (Ribeiro et al., 2017). At +100 to +350 mV nitrification occurs, at +25 mV to +250 mV sulphuration, at +50 to − 50 mV

denitrification, at − 100 to − 225 mV biological phosphorus release occurs, and from − 175 to − 400 mV methane is produced (YSI, 2008). Moreover, aeration of wastewaters is often utilized within membrane bioreactors to increase the transformation of emerging contaminants (Yoon et al., 2004; and Sun et al., 2016). Given the benefits of redox control, scientific interest has also considered its applicability for nutrient and odor control within animal manures (Pain et al., 1990; Burton, 1992; and Bèline et al., 2004). In the context of animal slurries nutrient control is often considered a desirable process which can be facilitated with aeration (redox control), for example this process has been shown to increase nitrification and reduce methane and ammonia losses (Calvet et al., 2017).

Limited studies have considered redox conditions and veterinary medicine fate, although Ali et al., (2013) demonstrated that the removal of tylosin in dairy lagoon sediment was increased under aerobic (+350 mV) compared to reduced (− 100 mV) conditions and Bachmann et al., (1988) reported that oxic conditions promoted degradation of A/B-Hexachlorocyclohexane. These findings demonstrate the influence of redox potential on veterinary medicine fate, however, very little is known with regards to the effect of a range of anaerobic redox potentials in animal slurries (Wohde et al., 2016). It is essential to understand this relationship to harmonise laboratory assessments and reduce variability within the risk assessment; such an understanding would contribute to more accurate assessments providing better environmental representativeness.

Until now the influence of anaerobic pig slurry redox potentials on veterinary medicine degradation was largely unknown; this work aims to bridge this knowledge gap and improve our understanding of variability within manure degradation trials. Controlling the redox potentials of wastes and other environmental parameters has been troublesome for researchers for some time. Previous redox control methods are available within the literature; however, these methods are often costly, time-consuming, and complex (Patrick et al., 1973; and Chuan et al., 1996). Controlling systems primarily consisted of self-constructed platinum electrode probes and a relay system which is comprised of a complex self-made calomel half-cell, millivolt meter and meter relay (Chuan et al., 1996; Carbonell-Barrachina et al, 2000; Lissner et al., 2003; Hjorth et al., 2012; and Ali et al.,

2013). Moreover, the systems typically utilize a magnetic stirrer to distribute the oxygen into the matrix solution (Yu and Rinklebe, 2013). Here we present a pragmatic and cost-effective means for redox control.

3.1 Methodology

3.1.2 Chemicals

All chemicals and solvents were of the highest available purity (94–98%). NaOH, Na_2EDTA, citric acid and di-sodium hydrogen orthophosphate were purchased from Fischer Scientific (UK). Numerous veterinary medicines with a broad range of physiochemical properties (i.e., sorption parameters, octanol-partition coefficient, pKa and solubility) were selected for use within the experiment (SI Table C1). Florfenicol and sulfamethoxazole were purchased from VWR (UK), whilst acetyl-salicylic acid (ASA), oxytetracycline (OTC), ceftiofur (CFT) and tylosin tartrate (TYL) were purchased from SLS (UK). 0.1 M Na_2EDTA-Mcilvaine buffer 50:50 (pH 4) was prepared by mixing 614.5 mL of 0.1 M citric acid, 385.5 mL of 0.2 M disodium orthophosphate and 500 mL 0.1 M Na_2EDTA. Redox probes were calibrated using a +220 mV checking solution from Mettler-Toledo (Sigma-Aldrich, UK). Matrix matched standards were used to quantify the analytes; these were prepared on the day of the extraction.

3.1.3 Manure sampling and properties

Fresh pig manure was sampled from fattening pigs at a farm in Welburn, York (54°05′31.2″N; 0°53′03.0″W), and then stored at 3 °C for a maximum of two weeks. A low storage temperature was selected to reduce the anaerobic content of pig manures; thus, allowing the desired redox potential to be easily manipulated prior to the acclimation period. This deviates from the guidance document EMA (2011), to rectify this issue the pig slurries were acclimated for 2 weeks at the desired redox potential prior to dosing. The pigs had not received any of the selected veterinary medicines used within this study. The manure was homogenized and moisture was corrected to a dry weight of 5% (EMA, 2011).

In order to characterise the dissolved fraction of the slurry it was centrifuged at 3250 rpm for 2 h and decanted, before the supernatant was collected and sequentially filtered through varying filter grades. The filtering sequence was as follows; G/F Whatman, 1 Ps filter paper, 20 μm GF syringe filter then a 0.45 μm nylon syringe filter. Dissolved carbon and nutrients were then analysed using a Analytik Jena Multi NC2100 (carbon) and Autoanalyzer (nutrients). Manure was sampled twice in order to repeat the experiment (replication), (see Table 3.0 for reported manure characteristics). The properties of the manures utilized within the studies were in-line with one another, an exception of this is redox potential although this is unimportant given that redox was specifically manipulated within the study.

Table 3.0: Pig slurry property data for manures collected for both replicates of the study, the data displayed demonstrates the dissolved available fraction of the slurry.

Slurry Sample	Starting redox (mV)	pH	DOC, DIC (mg/L)	NH_4^+, NO_2^- (mg/L)	PO_4^{3-} (mg/L)
14/01/2021	-278	7.3	2578.71±8.05, 656.92±0.68,	276.44±1.7, 1.27	19.95±0.14
20/02/2021	-412.7	7.4	2838.36±13.41, 267.36±1.68,	523.34±8.1, 0.40±0.033	12.39±0.027

Footnote: *Dissolved organic carbon (DOC) and Dissolved Inorganic carbon (DIC).*

3.1.4 Bioreactor and redox control

In order to control the anaerobic redox potentials of pig slurry bioreactor vessels were designed using 2000 mL wide neck Erlenmeyer flasks which were sealed using 62 mm rubber bungs (Figure 3.0 and Figure 4.0). The bung contained four holes made from food grade Nalgene tubing in the top to facilitate aeration, nitrogen purging, air outlet, and sample retrieval. A OCS. tec GmbH & Co. KG ORP controller containing a platinum electrode was used to maintain the redox values, the specifications of this controller were −1000 mV to +1000 ± 0.2 mV. The ORP controlling system was connected to an air pump via a plug and when the redox potential value dropped below the desired ORP value (mV) (- 100mV or -250 mV) the pump automatically turned on aerating the vessels and increasing the redox potential. Dependent on the treatment both nitrogen and air were fed through a humidifier which consisted of a glass vessel and

porous stone which was used to reduce the sample from drying out. The electrodes were installed into the system at 8 cm depth and a glass rod was used to hold this in place as substantial movement reduced a false redox potential reading. Within the duplicate vessel a WTW xylem platinum redox potential probe was utilized to ensure redox potentials were uniform between the replicates (checks were undertaken twice a day). An aquatic airflow manifold was used to evenly distribute the rate of aeration between both vessels (Figure 3.0). The system was set up on a rotary bed shaker that was used to homogenize the sample and distribute oxygen throughout the entire sample. The rotary bed shaker was set to 30 rpm throughout the study duration, although prior to sample retrieval the sample was homogenized at 120 rpm for 30 s.

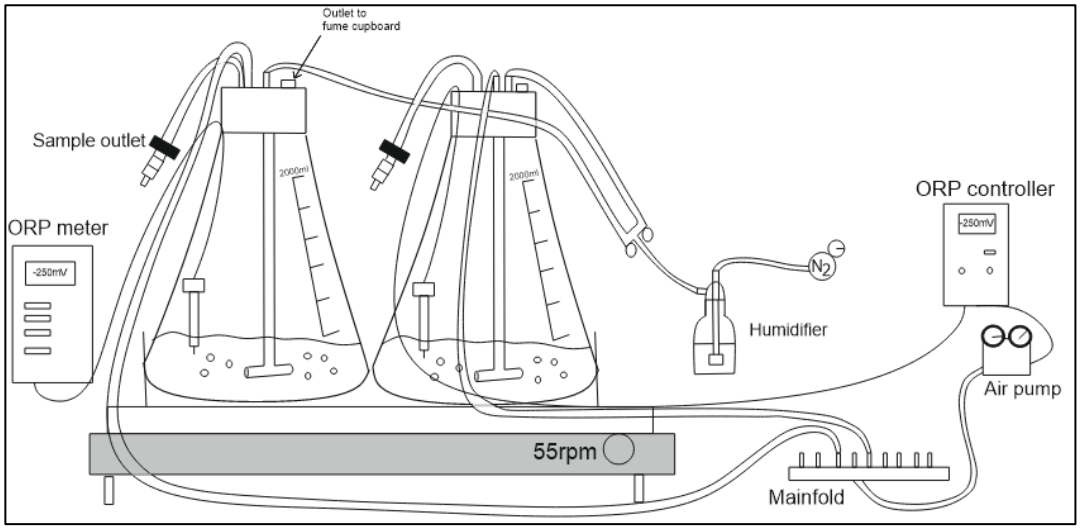
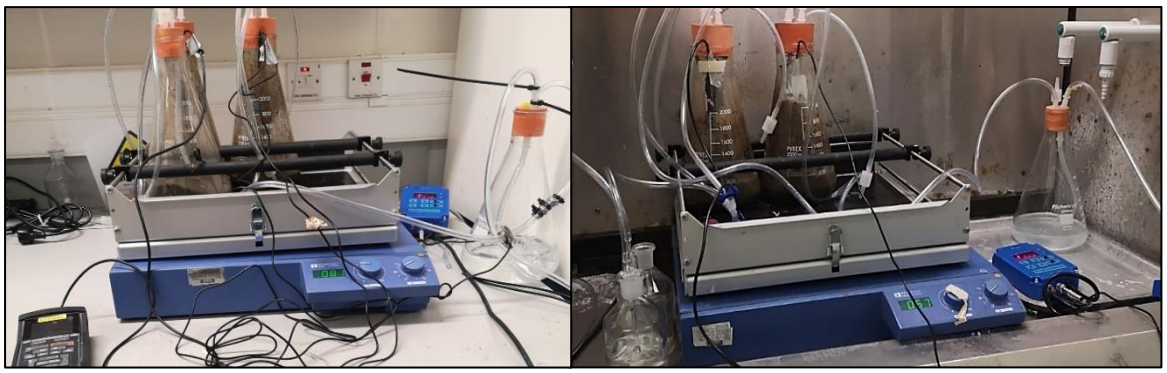


Figure 3.0: Schematic drawing of the experimental rig used to control redox potential. Three treatments of this design were simulated in tandem.



SI Figure 3.1: Picture displaying the experimental system for controlling the redox potential of pig slurry to -100mV (A) -250mV (B).

3.1.5 Experimental conditions

Three treatments of pig slurry were assessed with the selected redox potentials within the anaerobic range defined by OECD 307 and EMA (2011): −100 mV (reduced), −250 mV (anaerobic) and −400 mV (very anaerobic). The tolerance of these redox potentials was ± 50 mV throughout the duration of the study. The treatments were achieved using the following: reduced (−100 mV) was achieved with intermittent aeration, anaerobic (−250 mV) incorporated nitrogen at 8 cc's with a similar aeration system as reduced, and very anaerobic (−400 mV) utilized nitrogen (10 cc's) to purge the system of oxygen. The bioreactors were maintained at the desired redox potentials for two weeks prior to dosing with antibiotics; this acclimation period allowed the microorganisms present to adjust to the conditions. There is strong evidence that the microbial dynamics would have transitioned in this timeframe (Hanajima et al., 2011). The time taken for the redox treatments to reach the desired values was 2–3 d which was not included in the acclimation period as this reflected a time where the redox values were not stable. The moisture content of the slurries were adjusted every 2 days to ensure that the treatments were consistent. The experiment was kept in the dark (foil coating) at 20 ± 2 °C for the duration of the study and repeated twice in order to obtain sufficient replicates for statistical comparisons (n = 4). There was a control per each treatment which was also used to make the blank matrix for the calibration standards. The selected temperature was monitored using a tinytag datalogger and was deemed an appropriate range under the current manure degradation guidance document for pig slurries (EMA, 2011; OECD, n.d.).

Veterinary medicine dosage concentrations were derived using the Spaepen et al., (1997) model. The dosage concentrations selected were at $1/5^{th}$ of the calculated Predicted Environmental Concentration (PEC) to avoid any significant inhibition of the microbial populations within the pig slurry (SI Table C2). Moreover, CFT was dosed at 2/5th of the PEC due to sensitivity issues of this analyte. The study was conducted over 14 d and the given timepoints were 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 3 d, 7 d and 14 d.

3.1.6 Sample extraction and analytical technique

At the given timepoint 12.5 mL of slurry was retrieved from the bioreactor using a pre-installed outlet which was connected to a vacuum pump (Figure 1). After each timepoint 5 mL of deionized water was used to clean the tubing to remove any contaminants. 12.5 mL of 0.1 M Na_2EDTA-Mcilvaine buffer was added to the slurry sample and shaken using a rotary bed shaker at 250 rpm for 20 min, the sample was then centrifuged at 3250 rpm for 20 min at 4 °C. The extraction was repeated twice, and the supernatants were combined. Samples were then filtered to 0.2 µm and stored at −20 °C prior to analysis. Extraction efficiencies of the six analytes at 1%, 10% and 100% of the dosage concentration were calculated (SI Table C3). The majority of the compounds met the SANCO 3029 recovery criteria (<70%) at the 100% dose, an exception to this was CFT which did not meet the requirements at any dosage level. Due to sensitivity issues, TYL failed the criteria at 1%, however still met the 10% criteria.

Veterinary medicines were analysed using a SCIEX Triple Quad 5500+ LC-MS/MS System. The analytical method comprised of using a Phenomenex Kinetics XB-C18 column (50 × 2.1 mm) at a set temperature of 40 °C and the mobile phases consisted of 0.1% formic acid (aqueous) and 0.1% formic acid in methanol. The method had a 30 µl injection volume, the flow rate was set to 0.4 mL/min, and the chromatographic duration was 11 min. The gradient was a reversed phase and consisted of the following organic gradient percentages, 0 min (0%), 3 min (90%), 8 min (90%), 8.1 min (10%) 11 min (0%). Please see the SI Table .4 for additional mass spectrometer details. Analyst 1.6 was utilized to process the data as well as to quantify the concentrations based on a calibration curve (≤ 95%).

3.2 Results

The majority of the assessed veterinary medicines (ASA, CFT, FLO, SMX and TYL) were found to be impersistent within pig slurries (Hollis, 1991). An exception was OTC which was found to be persistent, and the study duration was too short to fully assess the degradability of OTC under varying redox potentials. All of the assessed veterinary

medicines followed Single First Order degradation kinetics. The influence of redox potential on the degradation rates of selected veterinary medicines was found to be compound specific (Figure 3.1 and Table 3.1). Reduced conditions (−100 mV) were found to inhibit the degradation of CFT, FLO, OTC and SMX over that of both anaerobic and very anaerobic (-250 and −400 mV) ($p \leq 0.05$) (Figure 3.1). Inhibited FLO degradation under reduced conditions resulted in a calculated DT_{50} two times that of very anaerobic, the DT_{50} values being 22.22 h at −400 mV and 49.8 h at −100 mV. Similarly, SMX degradation rate was stunted under reduced conditions over that of very anaerobic; the degradation rate constant (*k*) was found to be over 2 times larger (i.e., *k* 0.007377 at 100 mV and 0.01956 at −400 mV). Such inhibition drove differences within the calculated DT_{50} values, these were 35.4 h and 94 h ($p \leq 0.05$). Moreover, a significant effect between very anaerobic and reduced conditions was also observed for CFT and for OTC (Figure 3.1 and Table 3.1) ($p \leq 0.05$). Only a minor difference was observed between the degradation rates of OTC, which would suggest that a longer study duration is required to assess this relationship (*k* 0.005303 at −400 mV and 0.003306 at −250 mV).

Comparatively, TYL degradation rate was stunted by a factor of 2.8 under very anaerobic conditions over reduced, this resulted in DT_{50} values of 6.88 h at −100 mV and 19.4 h at −400 mV ($p \leq 0.05$). No significant differences were observed between the anaerobic-reduced treatments for the majority of the assessed veterinary medicines, an exception of this was FLO for which, under reduced conditions, a DT_{50} of 49.8 h was reported compared to 29.6 h under anaerobic ($p \leq 0.05$). The degradation rate of ASA was unaffected by redox potential and the persistence of this compound was short lived (Figure 3.1).

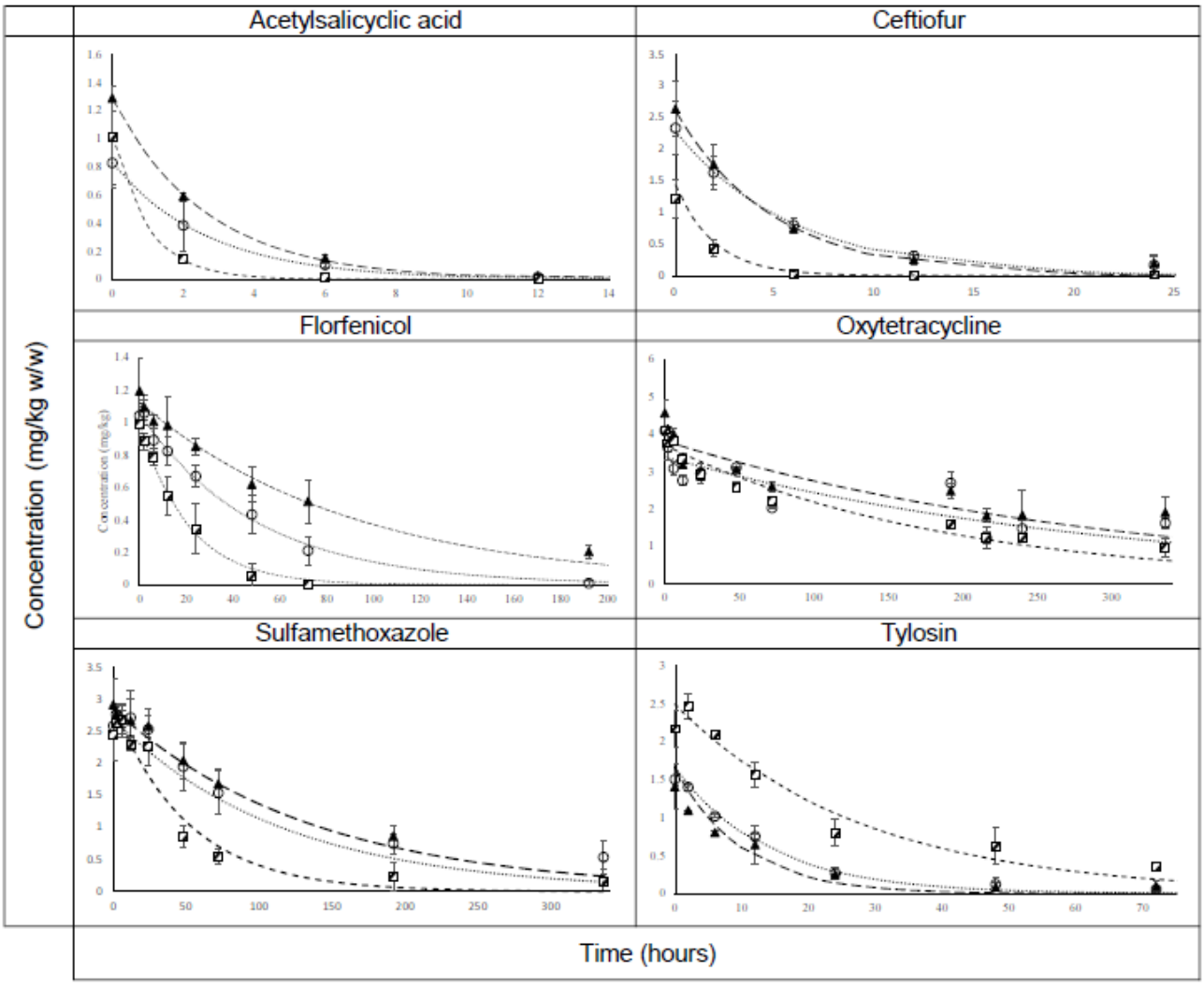


Figure 3.1: Degradation of veterinary medicines under varying anaerobic redox potentials over time.

Key: -100 mV -▲- -250mV ···◯··· -400mV-▨-

Table 3.1: Collated degradation parameters of veterinary medicines within pig slurry under varying anaerobic redox potentials.

Degradation Parameter	Redox potential	Acetylsalicylic acid	Ceftiofur	Florfenicol	Sulfamethoxazole	Tylosin	Oxytetracycline
DT_{50} (hours)	-100 mV (reduced)	1.84	3.61	49.8	94	6.88	211
	-250 mV (anaerobic)	1.87	4.28	29.6	80.5	9.58	210
	-400 mV	0.71	1.35	22.22	35.4	19.4	131

	(very anaerobic)						
k degradation rate constant	-100 mV (reduced)	0.3767	0.2071	0.01392	0.007377	0.1007	0.003288
	-250 mV (anaerobic)	0.3706	0.1717	0.02338	0.008615	0.07233	0.003306
	-400 mV (very anaerobic)	0.9812	0.5132	0.03128	0.01956	0.03574	0.005303

3.3 Discussion

3.3.1 Redox potential control

The test system showed appropriate control throughout the duration of the study as a tolerance of ±50 mV was achieved (28 d) (SI Figure C1); this was also achieved within the replicate study (total duration was 56 d). There are publications detailing experimental details for redox control of environmental matrices, however, these typically utilize a methodology that was devised via Patrick et al., (1973). This system has proved to work and has been adopted for a range of environmental matrices (Willis et al., 1974; Chuan et al., 1996; Carbonell-Barrachina et al., 2000; Lissner et al., 2003; Hjorth et al., 2012; and Ali et al., 2013), however this design is arguably outdated, expensive and requires sufficient knowledge in electrical rewiring. Typically, these utilized a magnetic stirrer to distribute the oxygen throughout the matrix although through a series of developments it was obvious numerous complications can arise with this. For example, the magnetic flea would often get stuck and redox control would be lost (Yu and Rinklebe, 2013), moreover, cheaper magnetic stirrers can also produce heat over long durations of use which is problematic for degradation assessments. There is no evidence within the literature to suggest redox control has been achieved on pig slurries; there is also no defined methodology that would be deemed suitable to control redox potentials of slurries during degradation assessments that meet the EMA, (2011) criteria.

Here we have demonstrated a cheap and effective means to control the redox potential of pig slurries; the presented system design could also be adapted for use in other environmental matrices such as water, wastewater, sludges, and soil solutions. The system was maintained at the desired redox potential throughout the duration of the study, although, to do so it is paramount to take care in the construction and development of the system to obtain an experimental apparatus that remains functional and within tolerance for the duration of the experiment. For example, to replicate redox conditions in two vessels, it was critical to evenly distribute the airflow between the vessels; this was shown to work well using the aquatic air manifold. One of the major issues with the system was the durability of the redox probes, during the development of this system it was apparent false readings can result in excess aeration. To overcome this issue, we would suggest a more robust redox probe that could connect to the controller, which could be achieved using a Bayonet Neill–Concelman (BNC) adapter, or alternatively spare probes and a thorough cleaning process (i.e., fine grain sandpaper and acetone). The position of the ORP probes was at 8 cm depth and secured to the side of each vessel and it was paramount to ensure these positions were the same given what is known regarding the influence of depth on ORP readings (Yu and Bishop, 2001). Moreover, the positioning of the aeration inlet was centred within the vessel, ensuring that aeration did not affect the readings.

3.3.2 Veterinary medicine fate

The degradation rates of SMX, TYL and CFT under very anaerobic conditions were found to be in line with previous assessments within pig manures and pig slurries, for example the following DT_{50} values have been reported, SMX 2.6 d, TYL <2 d and CFT ~2 h (Gilbertson et al., 1990; Loke et al., 2000; and Berendsen et al., 2018). The degradation rates of OTC and FLO under all redox treatments were reported to deviate from previous assessments in pig slurries, Blackwell et al., (2005) reported a DT_{50} of 79 d for OTC and Junker et al., (2016) calculated a DT_{50} of 0.17–0.41 d for FLO. We speculate this to be related to the unique experimental design.

3.3.3 Redox potential and degradation rate

Based on the current literature we can speculate that a number of processes could be contributing to the differences in degradation rates observed under varying redox potentials. The pH values at each redox treatment were similar (8.3–8.5), therefore differences in degradation rates are unlikely to be related to adsorption via ionic charge and the pKa of the chemical. There is however compelling evidence of increased adsorption rates of 2,4,6-Trinitrotoluene and pentachlorophenol to soil sediment suspensions under oxidized conditions (Gambrell and Patrick. 1988; and Pennington and Patrick, 1990). The reasoning for this is generally unknown and suggests further research is required to understand this relationship (Price et al., 2001; Dorival-García et al., 2013). Furthermore, biotic processes are the predominant degradation processes for these analytes, therefore it is unlikely that adsorption mechanisms resulted in the variation of degradation rates with differing redox potentials (Liu et al., 2010; Fan et al., 2019). Future work could be aimed at conducting abiotic assessments to ascertain the influence of microbial mineralization on the degradation of veterinary medicines under varying redox potentials.

It is evident from the dataset and the available literature that redox potential has a compound-specific effect on contaminant fate within the environment. For example, de Souza et al., (2014) found anaerobic activated sludge biomass to promote the degradation of norfloxacin over that of aerobic due to mineralization rates. To further this point, DDT is known to degrade faster under anaerobic conditions whereas kepone and permethrin degrade faster under aerobic/reduced conditions (Gambrell and Patrick, 1988). Given what we know regarding degradation processes of the assessed analytes we consider it sensible to speculate that increases in degradation rates are attributed to the greater mineralization efficiency of methanogenic bacteria. We suggest that reduced conditions have greater microbial diversity as indicated via the biochemical processes that occur at this specific ORP value (denitrifying, sulphate-reducing, and phosphorus degrading bacteria) (YSI, 2008), but overall reduced microbial abundance of specific degraders (methanogens and sulphate-reducing bacteria), thus resulting in poor removal rates for SMX, CFT, and FLO.

Pig slurries with redox values from − 250 mV to − 400 mV are typically comprised of methanogenic and sulphate reducing microbial populations (Jang et al., 2021). Given that microbial mineralization is the primary degradation process for SMX and FLO it is highly likely that these populations are responsible for the differences seen in degradation rates (FDA, 2013; Alvarino et al., 2016). Interestingly anaerobic conditions have previously been observed to promote the degradation of these compounds over that of aerobic (Richter et al., 2016; Ouyang et al., 2019; and Alvarino et al., 2016). Ouyang et al., (2019) reported an increase of SMX degradation in anaerobic sludges over that of aerobic (nitrate-reducing conditions) and attributed this to the presence of sulphate-reducing-bacteria (Desulfovibrio Vulgaris). Moreover, similar findings were reported via Jia et al., (2017) who investigated the degradation of SMX within a Sulphate Reducing Bacterium (SRB) sludge reactor and stated removal rates were enhanced via the presence of *Clostiridum sp*. These bacterial strains are considered SMX degraders and are also abundant within pig slurries (Cook et al., 2008; Cook et al., 2008; and Karnachuk et al., 2021). These findings are further supported via a comprehensive assessment presented via Alvarino et al., (2016) who investigated ^{14}C-SMX degradation within sludges at varying redox potentials. The authors concluded under anaerobic conditions microbial mineralization and to a lesser extent adsorption were greater than that of aerobic nitrifying conditions. The respective $K_{bio\text{-}l}$ and K_d values were 0.08 L/g vss d (volatile suspended solids per day) and 40 L/kg under anaerobic conditions and 0.01 L/g vss d and 7 L/kg under aerobic conditions. Conversely aerobic/oxic conditions have been reported to increase the biodegradation of SMX within granular/suspended activated sludge, wastewater, and soils, although these matrices differ greatly to that of pig slurries (Liu et al., 2010; Poirier-Larabie et al., 2016; and Kang et al., 2018).

Compared to SMX and FLO, TYL is susceptible to greater degradation under reduced conditions, which could be a result of greater microbial mineralization via denitrifying bacteria. Similar findings were reported by Ali et al., (2013) who observed a similar effect of redox potential on the degradation of TYL within dairy lagoon sediment, for example, they found TYL to fully degrade in 4 d under aerobic conditions (+350 mV), whereas

under reduced (− 100 mV) it took 20 d. Moreover, similar trends have been reported in Loke et al., (2000), Kolz et al., (2005), and Seo et al., (2018), where aerobic conditions promoted TYL degradation in pig slurries. Microbial transformation has been suggested to be the predominant process which affects TYL degradation under varying oxygen levels (Ali et al., 2013; and Loke et al., 2000). However, Kolz et al., (2005) stated that adsorption mechanisms may have also contributed. It remains unclear whether adsorption mechanisms contribute towards these differences. Sodium azide was used as a sterilant within these studies although its effectiveness is questionable given the loss of potency that was observed via Ali et al., (2013) when using sodium azide to conduct abiotic-biotic degradation assessments.

3.3.4 Implication of scientific findings

The results clearly demonstrate that redox potential has a significant effect on the degradation rates of veterinary medicines. A compound specific effect was observed, suggesting that aeration of slurries for odour and nutrient control need to be considered in regards to pharmaceutical fate. Moreover, it is clear from the dataset that there were differences in the degradation rates for the assessed veterinary medicines across a range of redox potentials that are deemed acceptable under the current manure degradation guidance documents and OECD guidelines 307/308 (EMA, 2011; OECD, 2002a,b). OECD 307/308 states anaerobic conditions are achieved at redox potentials of < -100 mV, which highlights the implications of greater variability. Thereof presenting the requirement for more stringent redox control during manure degradation assessments or to conduct such experiments on numerous manures with differing properties. The consequence of utilizing such a wide range of redox potentials within manure degradation assessments means that such laboratory assessments may inadequately predict the concentrations applied to land.

In order to assess this relationship a Phase II risk assessment was conducted on the selected antibiotic compounds (VICH, 2003). Differences in the degradation rates of SMX under varying anaerobic redox potentials was found to result in differences in the

identified risk towards terrestrial and aquatic organisms (Table 3.2). The environmental risk assessment for veterinary medicines utilizes a Risk Quotation (RQ) approach (RQ = PEC/PNEC) with an RQ > 1 suggesting there is an environmental risk (VICH, 2003). The differences observed between anaerobic redox potentials and subsequent degradation rates resulted in a range of refined PEC values and thus the risk. For example, under very anaerobic conditions the RQ for both the terrestrial and aquatic assessments was < 1, indicating no environmental risk. However, under anaerobic and reduced conditions within the terrestrial assessment the calculated RQ was > 1, this was also found to be true for the reduced conditions within the groundwater assessment.

Under reduced and anaerobic conditions, the calculated $PEC_{soil\ refined}$ for SMX were 32.06 µg/kg and 14.6 µg/kg respectively, when these were compared to the PNEC of the most sensitive terrestrial species and endpoint (rice germination) a risk was identified (Liu et al., 2009). The FOCUS_PEARL modelling suite was used to predict the concentrations of SMX that would be leached into groundwater following the application of manures that were stored under varying redox potentials. As a result of the inhibited degradation under the reduced condition the $PEC_{groundwater}$ refined was 0.079 µg/L, whilst for anaerobic this value was 0 µg/L (FOCUS, 2014). Due to the greater groundwater exposure a risk (RQ > 1) was identified towards two cyanobacteria species *Synechococcus leopoliensis* and *Microcystis aeruginosa* (Ferrari et al., 2004; Liu et al., 2009 and Liu et al., 2012). This assessment demonstrates that the acceptable range of redox potentials within manure degradation assessments can drive differences within the identified risk.

Table 3.2: Environmental risk assessment risk quotients using the refined PEC values that were generated using the derived degradation data for sulfamethoxazole.

Veterinary medicine	Ecosystem	Redox potential	PEC refined (µg/kg	Target species	Ecotoxicological data	End point	PNEC (AF applied)	RQ	Reference

			or µg/L)						
	Terrestrial	-100 mV	32.06 µg/kg	Rice	Germination		10	**3.206**	
	Terrestrial	-250 mV	14.6 µg/kg	Rice	Germination	EC_{10} 100 µg/kg	10	**1.46**	Liu et al., (2009)
	Terrestrial	-400 mV	0.01 µg/kg	Rice	Germination		10	0.001	
	Aquatic	-100 mV	0.079 µg/L	*Synechococcus leopoliensis*	Growth		0.027	**2.92**	
	Aquatic	-250 mV	0.012 µg/L	*Synechococcus leopoliensis*	Growth	EC_{50} N/A	0.027	0.45	Ferrari et al., (2014)
SMX	Aquatic	-400 mV	0 µg/L	*Synechococcus leopoliensis*	Growth		0.027	0	
	Aquatic	-100 mV	0.079 µg/L	*Microcystis aeruginosa*	Antioxidant response		0.04	**1.975**	
	Aquatic	-250 mV	0.012 µg/L	*Microcystis aeruginosa*	Antioxidant response	EC_{50} 40 µg/L	0.04	0.31	Liu et al., (2012)
	Aquatic	-400 mV	0 µg/L	*Microcystis aeruginosa*	Antioxidant response		0.04	0	

Footnote: Bold = RQ > 1. As per the current regulatory guidance assessment factors were applied to the toxicological endpoints to derive the PNEC. An assessment factor of ten was applied to the terrestrial effect data (EC_{10}) and for aquatic a factor of one thousand was applied (EC_{50}) (VICH, 2003).

Due to seasonal variations within redox potentials and pig slurries, it is a possibility that degradation rates would differ between the summer and winter months (Park et al., 2006). For example, reduced methanogenic activity (i.e., elevated redox potential) during the winter months would inhibit FLO and SMX degradation; ultimately this would result in a greater environmental exposure and risk. Furthermore, the winter manure application timing is arguably the most important, increased volume of manures are often reported

due to the closed application period (October–January), increased housing, as well as increase precipitation for open lagoons, for example storage overflow contributed towards 24% of manure spills within Iowa (1992–2002) (Burkholder et al., 2007; and Armstrong et al., 2010). This phenomenon could result in excess environmental exposure during this application event (DEFRA, 2010). This is of course a concern regarding the reduced microbial activity within soils during these months, which would result in greater persistence and environmental concern (Srinivasan and Sarmah, 2014; and Bansal, 2012).

3.4 Conclusion

The presented study demonstrates a cost-effective means of controlling redox potentials within laboratory scale experiments. Improved accessibility for redox control could result in further research and understanding regarding the influence of redox potentials on contaminant fate. The derived data regarding the fate of veterinary medicines under anaerobic redox potentials was significant and compound specific. Given what we now know regarding anaerobic redox potentials and veterinary medicine fate it is clear that, in order to have uniform assessments, tighter redox potential control is required. Under the currently available manure degradation guidance and OECD documents the acceptable range of anaerobic conditions is − 230 to − 400 mV and >-100 mV respectively, here we demonstrate such a range can drive differences within the outlined risks towards aquatic and terrestrial organisms. Therefore, it is critical to harmonise manure degradation protocols at an EU or OECD level, during the development of such guidance we would suggest the usage of a range of slurries and redox potentials. Not doing so will continue to contribute to inaccurate predictions of environmental exposure. Furthermore, until now the influence of aerating pig slurries on the degradation of a broad range of veterinary medicines was unknown. This work highlights that such manure processing techniques could in fact reduce the degradation of veterinary medicines which would in return increase the exposure of the environment.

References

Akın, B.S., Ugurlu, A., 2005. Monitoring and control of biological nutrient removal in a sequencing batch reactor. *Process Biochemistry*. 40 (8), 2873–2878.

Ali, M., Wang, J.J., DeLaune, R.D., Seo, D.C., Dodla, S.K., Hernandez, A.B., 2013. Effect of redox potential and pH status on degradation and adsorption behavior of tylosin in dairy lagoon sediment suspension. *Chemosphere.* 91 (11), 1583–1589.

Alvarino, T., Nastold, P., Suarez, S., Omil, F., Corvini, P.F.X., Bouju, H., 2016. Role of biotransformation, sorption and mineralization of 14C-labelled sulfamethoxazole

under different redox conditions. *Science of the Total Environment*. 542, 706–715.

Armstrong, S.D., Smith, D.R., Owens, P.R., Joern, B., Williams, C., 2010. Manure spills and remediation methods to improve water quality. In: Genetic Engineering,

Biofertilisation, *Soil Quality and Organic Farming*. Springer, Dordrecht, pp. 201–215.

Bachmann, A.P., Walet, P., Wijnen, W., de Bruin, J.L.M., Huntjens, W., Zehnder, R.A., 1988. Biodegradation ofα- and β-hexachlorocyclohexane in a soil slurry under

different redox conditions. Applied Environmental Microbiology. 54 (1), 143–149.

Balzer, F., Zühlke, S., Hannappel, S., 2016. Antibiotics in groundwater under locations with high livestock density in Germany. Water Science and Technology. Water Supply 16 (5), 1361–1369.

Bansal, O.P., 2012. A laboratory study on degradation studies of tetracycline and chlortetracycline in soils of Aligarh district as influenced by temperature, water content, concentration of farm yield manure, nitrogen and tetracyclines. *Proceedings of the National Academy of Sciences, India Section B*. 82 (4), 503–509.

Béline, F., Daumer, M.L. and Guiziou, F., 2004. Biological aerobic treatment of pig slurry in France: nutrients removal efficiency and separation performances. *Transactions of the ASAE*, 47(3), p.857.

Berendsen, B.J.A., Lahr, J., Nibbeling, C., Jansen, L.J.M., Bongers, I.E.A., Wipfler, E.L., Van de Schans, M.G.M., 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. *Chemosphere* 204, 267–276.

Blackwell, P.A., Boxall, A.B., Kay, P., Noble, H., 2005. Evaluation of a lower tier exposure assessment model for veterinary medicines. *Journal of Agricultural Food and Chemistry*. 53 (6), 2192–2201.

Bogaard, A., Fraser, R., Heaton, T.H., Wallace, M., Vaiglova, P., Charles, M., Jones, G., Evershed, R.P., Styring, A.K., Andersen, N.H., Arbogast, R.M., 2013. Crop manuring and intensive land management by Europe's first farmers. *Proceedings of the National Academy of Sciences of the United States of America* 110 (31), 12589–12594.

Burkholder, J., Libra, B., Weyer, P., Heathcote, S., Kolpin, D., Thorne, P.S., Wichman, M., 2007. Impacts of waste from concentrated animal feeding operations on water quality. *Environmental Health Perspective*. 115 (2), 308–312.

Burton, C.H., 1992. A review of the strategies in the aerobic treatment of pig slurry: purpose, theory and method. Journal of Agricultural Engineering. Res. 53, 249–272.

Calvet, S., Hunt, J. and Misselbrook, T.H., 2017. Low frequency aeration of pig slurry affects slurry characteristics and emissions of greenhouse gases and ammonia. *Biosystems Engineering*, 159, pp.121-132.

Canh, T.T., Aarnink, A.J.A., Verstegen, M.W.A., Schrama, J.W., 1998a. Influence of dietary factors on the pH and ammonia emission of slurry from growing-finishing pigs. *Journal of Animal Science*. 76 (4), 1123–1130.

Canh, T.T., Sutton, A.L., Aarnink, A.J.A., Verstegen, M.W.A., Schrama, J.W., Bakker, G.C. M., 1998b. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. *Journal of Animal Science*. 76 (7), 1887–1895.

Carbonell-Barrachina, A.A., Jugsujinda, A., Burlo, F., Delaune, R.D., Patrick Jr., W.H., 2000. Arsenic chemistry in municipal sewage sludge as affected by redox potential and pH. *Water Research*. 34 (1), 216–224.

Charpentier, J., Florentz, M., David, G., 1987. Oxidation-reduction potential (ORP) regulation: a way to optimize pollution removal and energy savings in the low load activated sludge process. *Water Science Technology*. 19 (3–4), 645–655.

Charpentier, J., Martin, G., Wacheux, H., Gilles, P., 1998. ORP regulation and activated sludge: 15 years of experience. *Water Science Technology*. 38 (3), 197–208.

Chuan, M.C., Shu, G.Y., Liu, J.C., 1996. Solubility of heavy metals in a contaminated soil: effects of redox potential and pH. *Water Air Soil Pollution*. 90 (3), 543–556.

Cook, K.L., Whitehead, T.R., Spence, C., Cotta, M.A., 2008. Evaluation of the sulfate reducing bacterial population associated with stored swine slurry. *Anaerobe* 14 (3),

172–180.

de Souza, Santos, Lucilaine, Val´eria, Teixeira, Danusa Campos, Jacob, Raquel Sampaio, Amaral, Míriam Cristina Santos do, Lange, Lis´ete Celina, 2014. Evaluation of the aerobic and anaerobic biodegradability of the antibiotic norfloxacin. *Water Science and Technology*. 70 (2), 265. https://doi.org/10.2166/wst.2014.214.

DEFRA, 2010. Fertiliser manual (RB209). eighth ed.. [online] Available at: http://file :///C:/Users/jnigh/AppData/Local/Temp/Defra%20Fertiliser%20manual%202011-1.pdf. (Accessed 21 July 2021).

Deng, L., Cai, C., Chen, Z., 2007. The treatment of pig slurry by a full-scale anaerobic adding raw wastewater-intermittent aeration process. *Biosystems Engineering*. 98 (3), 327–334.

Dorival-García, N., Zafra-Gómez, A., Navalón, A., González-López, J., Hontoria, E. and Vílchez, J.L., 2013. Removal and degradation characteristics of quinolone antibiotics in laboratory-scale activated sludge reactors under aerobic, nitrifying and anoxic conditions. *Journal of environmental management*, 120, pp.75-83.

Dusing, D.C., Bishop, P.L., Keener, T.C., 1992. Effect of redox potential on leaching from stabilized/solidified waste materials. *Journal of Air Waste Management Association*. 42 (1), 56–62.

EMA, 2011. "Guideline on Determining the Fate of Veterinary Medicinal Products in Manure" (EMEA/CVMP/ERA/430327/2009 (revised in 2011).

EMEA, 1997. Discussion Paper on Environmental Risk Assessment for Veterinary Medicinal Products Other than GMO-Containing and Immunological Products. EMEA, London, UK.

EMA, 2016. Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 and GL38. EMA/CVMP/ERA/418282/2005-Rev.1-Corr.1.

Fan, C.H., Yang, C.W., Chang, B.V., 2019. Anaerobic degradation of sulfamethoxazole by mixed cultures from swine and sewage sludge. *Environmental Technolology* 40 (2), 210–218.

Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Pax´eus, N., Lo Giudice, R., Pollio, A., Garric, J., 2004. Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? *Environmental Toxicology and Chemistry*. 23 (5), 1344–1354.

FDA, 2013. Environmental Assessment for Aquaflor (Florfenicol). Aquaflor for Freshwater-Reared Finfish in Recirculating Aquaculture Systems [online]. EPA. Available at: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/113. (Accessed 7 May 2020).

FOCUS, 2014. Generic Guidance for Tier 1 FOCUS Ground Water Assessments. https://esdac.jrc.ec.europa.eu/public_path/projects_data/focus/gw/NewDocs/GenericGuidance2_2.pdf.

Gambrell, R.P., Patrick, W.H., 1988. In: The Influence of Redox Potential on the Environmental Chemistry of Contaminants in Soils and Sediments. *The Ecology and Management of Wetlands*, pp. 319–333.

Ghernaout, D., Elboughdiri, N., 2020. Advanced oxidation processes for wastewater treatment: facts and future trends. *Open Access Library Journal* 7 (2), 1–15.

Gilbertson, T.J., Hornish, R.E., Jaglan, P.S., Koshy, K.T., Nappier, J.L., Stahl, G.L., Cazers, A.R., Nappier, J.M., Kubicek, M.F., 1990. Environmental fate of ceftiofur sodium, a cephalosporin antibiotic. Role of animal excreta in its decomposition. *Journal of Agricultural Food and Chemistry*. 38 (3), 890–894.

Goncharuk, V.V., Bagrii, V.A., Mel'nik, L.A., Chebotareva, R.D., Bashtan, S.Y., 2010. The use of redox potential in water treatment processes. *Journal of Water Chemistry Technology*. 32 (1), 1–9.

Halling-Sørensen, B., Jensen, J., Tjørnelund, J., Montforts, M.H.M.M., 2001. Worst-case estimations of predicted environmental soil concentrations (PEC) of selected veterinary antibiotics and residues used in Danish agriculture. In: Kümmerer, K. (Ed.), *Pharmaceuticals in the Environment*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-04634-0_13.

Hanajima, D., Fukumoto, Y., Yasuda, T., Suzuki, K., Maeda, K., Morioka, R., 2011. Bacterial community dynamics in aerated cow manure slurry at different aeration intensities. *Journal of Applied Microbiology*. 111 (6), 1416–1425.

Hjorth, M., Pedersen, C.Ø., Feilberg, A., 2012. Redox potential as a means to control the treatment of slurry to lower H2S emissions. *Sensors* 12 (5), 5349–5362.

Hollis, J.M., 1991. Mapping the vulnerability of aquifers and surface waters to pesticide contamination at the national/regional scale. *Pesticides in Soils and Water*, BCPC Monograph 47, 165–174.

Jang, Y.N., Hwang, O., Jung, M.W., Ahn, B.K., Kim, H., Jo, G., Yun, Y.M., 2021. Comprehensive analysis of microbial dynamics linked with the reduction of odorous compounds in a full-scale swine manure pit recharge system with recirculation of aerobically treated liquid fertilizer. *Science of the Total Environment*. 777, 146122.

Joy, S.R., Li, X., Snow, D.D., Gilley, J.E., Woodbury, B., Bartelt-Hunt, S.L., 2014. Fate of antimicrobials and antimicrobial resistance genes in simulated swine manure storage. *Science of the Total Environment*. 481, 69–74.

Jia, Yanyan, Khanal, Samir Kumar, Zhang, Huiqun, Chen, Guang-Hao, Lu, Hui, 2017. Sulfamethoxazole degradation in anaerobic sulfate-reducing bacteria sludge system. *Water Research.* 119, 12–20. https://doi.org/10.1016/j.watres.2017.04.040.

Junker, T., Atorf, C., Berkner, S., Düring, R.A., Hennecke, D., Herrchen, M., Konradi, S., Merrettig-Bruns, U., Rombke, ¨ J., Wagner, J., Weinfurtner, K., 2020. Development of a test method for transformation of veterinary pharmaceuticals and biocides in anaerobic liquid manure. *Environmental Sciences Europe* 32 (1), 1–21.

Junker, T., Hennecke, D., Herrchen, M., Düring, R., Thiele-Bruhn, S., Meinerling, M., Fiebig, S., Topp, E., Volkel, ¨ W., 2016. Harmonization of Environmental Exposure Assessment for Veterinary Pharmaceuticals and Biocides: Ring Test for Validation of a Draft Test Protocol for Studies on Transformation in Manure [online]. Worlitzer: Umweltbundesamt. Available at: https://www.oecd.org/env/ehs/testing/validation-report-anaerobic-transformation-liquid-manure.pdf. (Accessed 23 November 2021).

Kang, A.J., Brown, A.K., Wong, C.S., Yuan, Q., 2018. Removal of antibiotic sulfamethoxazole by anoxic/anaerobic/oxic granular and suspended activated sludge processes. *Bioresource Technology*. 251, 151–157.

Karnachuk, O.V., Rusanov, I.I., Panova, I.A., Grigoriev, M.A., Zyusman, V.S., Latygolets, E.A., Kadyrbaev, M.K., Gruzdev, E.V., Beletsky, A.V., Mardanov, A.V., Pimenov, N.V., 2021. Microbial sulfate reduction by Desulfovibrio is an important source of hydrogen sulfide from a large swine finishing facility. *Scientific Reports* 11 (1),1–11.

Kolz, A.C., Moorman, T.B., Ong, S.K., Scoggin, K.D., Douglass, E.A., 2005. Degradation and metabolite production of tylosin in anaerobic and aerobic swine-manure lagoons. *Water Environmental Research*. 77 (1), 49–56.

Kim, K.R., Owens, G., Kwon, S.I., So, K.H., Lee, D.B., Ok, Y.S., 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water, Air, Soil Pollution.* 214 (1), 163–174.

Kreuzig, R., 2010. The reference manure concept for transformation tests of veterinary medicines and biocides in liquid manure. *CLEAN–Soil, Air, Water*, 38(8), pp.697-705.

Kuchta, S.L., Cessna, A.J., Elliott, J.A., Peru, K.M., Headley, J.V., 2009. Transport of lincomycin to surface and ground water from manure-amended cropland. *Journal of Environmental Quality*. 38 (4), 1719–1727.

Lamshoft, ¨ M., Sukul, P., Zühlke, S., Spiteller, M., 2010. Behaviour of 14C-sulfadiazine and 14C-difloxacin during manure storage. *Science of the Total Environment.* 408 (7), 1563–1568.

Lee, J., 2010. Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. *Scientia Horticulturae*. 124 (3), 299–305.

Lissner, J., Mendelssohn, I.A., Anastasiou, C.J., 2003. A method for cultivating plants under controlled redox intensities in hydroponics. *Aquatic Botany* 76 (2), 93–108.

Liu, Y, Guan, Y, Gao, B, Yue, Q, 2012. Antioxidant responses and degradation of two antibiotic contaminants in Microcystis aeruginosa. *Ecotoxicology and Environmental Safety.* 86, 23–30. https://doi.org/10.1016/j.ecoenv.2012.09.004.

Liu, F., Ying, G.G., Tao, R., Zhao, J.L., Yang, J.F., Zhao, L.F., 2009. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. *Environmental Pollution.* 157 (5), 1636–1642.

Liu, F., Ying, G.G., Yang, J.F., Zhou, L.J., Tao, R., Wang, L., Zhang, L.J., Peng, P.A., 2010. Dissipation of sulfamethoxazole, trimethoprim and tylosin in a soil under aerobic and anoxic conditions. *Environmental Chemistry*. 7 (4), 370–376.

Loke, M.L., Ingerslev, F., Halling-Sørensen, B., Tjørnelund, J., 2000. Stability of tylosin A in manure containing test systems determined by high performance liquid chromatography. *Chemosphere* 40 (7), 759–765.

Luo, A., Zhu, J., Ndegwa, P.M., 2002. Removal of carbon, nitrogen, and phosphorus in pig manure by continuous and intermittent aeration at low redox potentials. *Biosystem Engineering*. 82, 209–216.

Martínez-Carballo, E., González-Barreiro, C., Scharf, S. and Gans, O., 2007. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environmental Pollution*, 148(2), pp.570-579.

Nguyen Dang Giang, C., Sebesvari, Z., Renaud, F., Rosendahl, I., Hoang Minh, Q., Amelung, W., 2015. Occurrence and dissipation of the antibiotics sulfamethoxazole, sulfadiazine, trimethoprim, and enrofloxacin in the Mekong Delta, Vietnam. PLoS One 10 (7), e0131855.

OECD, n.d. Draft for an OECD Guideline for the Testing of Chemicals. Anaerobic Transformation in Liquid Manure. OECD [online]. Available at: < https://www.oecd.org/env/ehs/testing/draft-test-guideline-anaerobic-transformation-liquid manure.pdf > [Accessed 23 November 2021].

OECD, 2002a. Test No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems. OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing, Paris. https://doi.org/10.1787/9789264070523-en.

OECD, 2002b. Test No. 307: Aerobic and Anaerobic Transformation in Soil. OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing, Paris. https://doi.org/10.1787/9789264070509-en.

Ouyang, W.Y., Su, J.Q., Richnow, H.H., Adrian, L., 2019. Identification of dominant sulfamethoxazole-degraders in pig farm-impacted soil by DNA and protein stable isotope probing. *Environmental International* 126, 118–126.

Pain, B.F., Phillips, V.R., Clarkson, C.R., Misselbrook, T.H., Rees, Y.J., Farrent, J.W., 1990. Odour and ammonia emissions following the spreading of aerobically-treated pig slurry on grassland. *Biological Wastes* 34 (2), 149–160.

Park, K.-H., Thopson, A.G., Marinier, M., Clark, K., Wagner-Riddle, C., 2006. Greenhouse gas emissions from stored liquid swine manure in a cold climate. *Atmospheric Environment.* 40, 618–627.

Patel, S.J., Wellington, M., Shah, R.M., Ferreira, M.J., 2020. Antibiotic Stewardship in Food-Producing Animals: Challenges, Progress, and Opportunities. Clinical therapeutics.

Patrick Jr., W.H., Williams, B.G., Moraghan, J.T., 1973. A simple system for controlling redox potential and pH in soil suspensions. *Soil Science Society of America Proceedings*. 37, 331–332.

Pennington, Judith C., Patrick, William H., 1990. Adsorption and desorption of 2,4,6-trinitrotoluene by soils. *Journal of Environmental Quality*. 19 (3), 559. https://doi.org/10.2134/jeq1990.00472425001900030034.

Potter, P., Ramankutty, N., Bennett, E.M., Donner, S.D., 2010. Characterizing the spatial patterns of global fertilizer application and manure production. *Earth Interactions*. 14 (2), 1–22.

Poirier-Larabie, S., Segura, P.A., Gagnon, C., 2016. Degradation of the pharmaceuticals diclofenac and sulfamethoxazole and their transformation products under controlled environmental conditions. *Science of the Total Environment*. 557, 257–267.

Price, Cynthia B., Brannon, James M., Yost, Sally L., Hayes, Charolett A., 2001. Relationship between redox potential and pH on RDX transformation in soil-water slurries. *Journal of Environmental Engineering*. 127 (1), 26–31.https://doi.org/10.1061/(ASCE)0733-9372(2001)127:1.

Ribeiro, R., von Atzingen, G.V., Lima, F., Okamoto, V.H., Arce, A.I., Tomamso, G., da Costa, E.J., 2017. Real-time control system based on the values of derivative of the redox potential aiming nitrogen removal in a Sequencing Batch Reactor applied in treating dairy wastewater. *Water, Air, Soil Pollution*. 228 (7), 1–8.

Richter, E., Berkner, S., Ebert, I., Förster, B., Graf, N., Herrchen, M., Kühnen, U., Römbke, J. and Simon, M., 2016. Results of extended plant tests using more realistic exposure scenarios for improving environmental risk assessment of veterinary pharmaceuticals. *Environmental Sciences Europe*, 28(1), pp.1-17.

Seo, Y., Lim, S., Choi, S., Heo, S., Yoon, B., Park, Y., Hong, D., 2018. Aeration effect on degradation of veterinary antibiotics in swine slurry. *Korean Journal of Soil Science and Fertilizer* 51 (1), 8–15.

Singh, S.N., 2001. Exploring correlation between redox potential and other edaphic factors in field and laboratory conditions in relation to methane efflux. *Environmental International* 27 (4), 265–274.

Spaepen, K.R., van Leemput, L.J., Wislocki, P.G., Verschueren, C., 1997. A uniform procedure to estimate the predicted environmental concentration of the residues of veterinary medicines in soil. *Environmental Toxicology and Chemistry*. 16 (9).

Srinivasan, P., Sarmah, A.K., 2014. Dissipation of sulfamethoxazole in pasture soils as affected by soil and environmental factors. *Science of the Total Environment*. 479, 284–291.

Sukul, P., Lamshoft, ¨ M., Kusari, S., Zühlke, S., Spiteller, M., 2009. Metabolism and excretion kinetics of 14C-labeled and non-labeled difloxacin in pigs after oral administration, and antimicrobial activity of manure containing difloxacin and its metabolites. *Environmental Research.* 109 (3), 225–231.

Sun, J., Liang, P., Yan, X., Zuo, K., Xiao, K., Xia, J., Qiu, Y., Wu, Q., Wu, S., Huang, X., Qi, M., 2016. Reducing aeration energy consumption in a large-scale membrane bioreactor: process simulation and engineering application. *Water Research*. 93, 205–213.

Tessella, 2021. CAKE showcase computer assisted kinetic evaluation [online] Available at: https://www.tessella.com/showcase/computer-assisted-kinetic-evaluation.

Thiele-Bruhn, S., Beck, I.C., 2005. Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. *Chemosphere* 59 (4), 457–465.

VICH, 2000. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase I, 2000. CVMP/VICH, London. CVMP/VICH/592/98-final.

VICH, 2003. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase II Draft Guidance, vol. 38. CVMP/VICH GL, London. CVMP/VICH/790/03-Consultation.

Weinfurtner, K., 2011. Matrix Parameters and Storage Conditions of Manure.

Widyasari-Mehta, A., Suwito, H.R.K.A., Kreuzig, R., 2016. Laboratory testing on the removal of the veterinary antibiotic doxycycline during long-term liquid pig manure and digestate storage. *Chemosphere* 149, 154–160.

Willis, G.H., Wander, R.C., Southwick, L.M., 1974. Degradation of trifluralin in soil suspensions as related to redox potential. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America* 3 (3), 262–265.

Wohde, M., Berkner, S., Junker, T., Konradi, S., Schwarz, L., Düring, R.A., 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. *Environmental Science Europe* 28 (1), 1–25.

Yoon, S.H., Kim, H.S., Yeom, I.T., 2004. The optimum operational condition of membrane bioreactor (MBR): cost estimation of aeration and sludge treatment. *Water Research*. 38 (1), 37–46.

YSI, 2008. ORP Management in Wastewater as an Indicator of Process Efficiency. YSI, Yellow Springs, OH, 15.08. 13. http://www.ysi.com/media/pdfs/A567-ORP-Management-in-Wastewater-as-an-Indicator-of-Process-Efficiency.pdf.

Yu, K., Rinklebe, J., 2013. Soil redox potential and pH controllers. *Methods in Biogeochemistry of Wetlands* 10, 107–116.

Yu, T., Bishop, P.L., 2001. Stratification and oxidation–reduction potential change in an aerobic and sulfate-reducing biofilm studied using microelectrodes. *Water Environmental Research*. 73 (3), 368–373.

Chapter 4 :

Influence of manure application method on veterinary medicine losses to water

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Abstract

Veterinary medicines are routinely used within modern animal husbandry, which results in frequent detections within animal manures and slurries. The application of manures to land as a form of organic fertiliser presents a pathway by which these bioactive chemicals can enter the environment. However, to date, there is limited understanding regarding the influence of commonly used manure application methods on veterinary medicine fate in soil systems. To bridge this knowledge gap, a semi-field study was conducted to assess the influence of commonly used application methods such as, broadcast, chisel sweep, and incorporation on veterinary medicine losses to waters. A range of veterinary medicines were selected and applied as a mixture; these were enrofloxacin, florfenicol, lincomycin, meloxicam, oxytetracycline, sulfadiazine, trimethoprim and tylosin. All the assessed veterinary medicines were detected within surface runoff and leachates, and the concentrations generally decreased throughout the irrigation period. The surface runoff concentrations ranged from 0.49 to 183.47 µg/L and 2.26 to 236.83 µg/L for the bare soil and grass assessments respectively. The leachate concentrations ranged from 0.04 to 309.66 µg/L and 0.33 to 37.79 µg/L for the bare soil and grass assessments respectively. More advanced application methods (chisel sweep) were found to significantly reduce the mass loads of veterinary medicines transported to surface runoff and leachate by 13-56% and 49-88% over that of broadcast. Incorporating pig slurries reduced the losses further with surface runoff and leachate losses being 40-97% and 66-94% lower than broadcast. Our results show that manure application techniques have a significant effect on veterinary medicine fate in the environment and as such these effects should be considered in the decision-making processes for the management of manures as well as from a risk mitigation perspective for aquatic compartments.

4.0 Introduction

As a result of the intensification of animal husbandry veterinary medicines are routinely administered to improve and protect animal health, however, in some regions sub-therapeutic concentrations of antibiotics are used for growth promotion (Gaskins et al., 2002; Dibner and Richards. 2005; Sarmah et al., 2006 and Subbiah et al., 2011). Administered veterinary medicines are typically excreted in high concentrations which can result in their direct application to land; or following the use of animal manures as organic fertilizers, veterinary medicines can also become incorporated within the soil matrix (Thiele-Bruhn 2003; Carvalho and Santos, 2016; and Xu et al., 2020). These processes directly result in the exposure of veterinary medicines to the terrestrial environment (Kim et al., 2011). Moreover, there is the potential for veterinary medicines to be present in runoff, which has likely resulted in the contamination of surrounding surface waters (Kay et al., 2005a; Kreuzig et al., 2005; Pinheiro et al., 2013; and Milić et al., 2013). Concentrations of veterinary medicines in the ranges of ng/L to µg/L have previously been reported in surface waters surrounding agricultural fields following rainfall events (Kasprzyk-Hordern et al., 2008; and Boxall, 2004). The biological potency of veterinary medicines and their transformation products presents several risks including contributing to the development of antimicrobial resistance and endocrine disruption, as well as effects on non-target terrestrial and aquatic organisms (Shao et al., 2018; Heuer et al., 2011; Ingerslev et al., 2003; and Kemper., 2008). Joy et al., (2014) demonstrated the capability of antimicrobial resistant genes to form and persist during the storage of manures. Specifically, 10 mg/kg of tylosin was detected within pig slurries which was found to degrade rapidly (DT_{50}/half-life 9.7 d), but even when the concentration of tylosin dropped to 0.1 mg/kg the relative abundance of the antimicrobial resistance gene erm(B) remained at 50-60%.

The ability for veterinary medicines to be mobilised within runoff following manure applications has been demonstrated, for example early research conducted by Kay et al., (2005a) established that sulphachloropyridazine and oxytetracycline can be transported via runoff following the application of pig slurry, with concentrations detected in

overland flow at 703.2 µg/L and 71.7 µg/L respectively. Similar findings have been reported for a wide range of antibiotics from differing classes with varying physical-chemical properties. For example, field studies and semi-field trials have reported tylosin, chlortetracycline, oxytetracycline, sulphadiazine, sulphathiazole and sulphadimidine concentrations within runoff following the application of manure to land (soils and grasses) (Burkhardt et al., 2005; Blackwell et al., 2007; and Dolliver and Gupta, 2008). Moreover, a comprehensive runoff assessment conducted by Kreuzig et al., (2005) revealed 13-28% of the applied sulphonamides were present within runoff following 2 h of simulated rainfall at 50 mm/h.

Various processes govern the formation and rate of runoff generated following manure applications; some examples include tractor tramlines, manure properties/type, soil type, compaction, timing, and application method (Kay et al., 2005a; Smith et al., 2007; Rotz et al., 2011; and Le et al., 2018). The ability for advanced applicators to reduce nutrient loses via runoff or volatilization has been known for some time (Maguire et al., 2011). For example, Rotz et al., (2011) demonstrated a reduction of 48% and 70% for nitrogen and phosphorus respectively when shallow injection technologies were utilised over that of broadcast. Advanced manure application technologies are now being utilized to improve the retention of nutrients and crop yields (Webb et al., 2010). Based on these findings, it is likely that differences in manure application methods such as these will also influence veterinary medicine fate, and ultimately run-off (Bittman et al., 2005).

To date, the ability for advanced manure application methods to alter veterinary medicine exposure in surface waters has been demonstrated in a limited number of publications. For example, Joy et al., (2013), and Le et al., (2018) observed greater chlortetracycline runoff concentrations under broadcast application over that of injection/incorporation. Subsurface injections and the incorporation of manures to soils were reported to reduce runoff concentrations by 55 to 93%. Joy et al., (2013), also demonstrated that antimicrobial resistant genes present in manures can also be mobilised via runoff

following rainfall, this phenomenon presents a wider risk given the known societal risk of antimicrobial resistance (Zainab et al., 2020).

Previous publications such as Blackwell et al., (2009), have demonstrated that soil cultivation practices such as tilling can reduce sulphachloropyridazine leachate concentrations by 16.6%, with similar findings reported in a field study conducted by Dolliver and Gupta, (2008). The authors accredited this to an increase in soil surface area, which in return increased adsorption complexes between veterinary medicines and organic components. Despite current understanding of how soil cultivation can affect veterinary medicine concentrations in receiving waters, there is, to the best of our knowledge, no research that has considered the influence of injection technologies on the leaching of veterinary medicines. It is important this knowledge gap is addressed given previous concerns that injections can potentially promote the leaching of contaminants (Rotz et al., 2011; and Fangueiro et al., 2015). Moreover, tillage usage is declining in modern agriculture and shallow injection methods are becoming increasingly popular; their popularity stems from a reduction in farmers' time and money as no incorporation (ploughing) is required (CTIC. 2004; Maguire et al., 2011; and Busari et al., 2015; and Niles et al., 2019). Manure application method has been shown to influence the concentration of veterinary medicines in run off, however we currently lack the comprehensive understanding of surface runoff and leaching behaviours within soil and grass settings which is required in order to accurately and representatively understand risks associated with these exposure methods (Powell et al., 2011).

The aim of this study was to therefore assess the influence of a suite of manure application methods on the environmental exposure of veterinary medicines to receiving waters. Assessments were conducted on both bare soils and grasses to present a comprehensive evaluation of real-life exposures. A semi-field experiment with simulated irrigation was constructed, and the study encompassed a wide range of veterinary medicines, commonly reported in the environment, with a broad range of physical-chemical properties.

4.1 Methodology

4.1.1 Chemical and dosage concentrations

The veterinary medicines purchased were of the highest possible purity (94-99%). Florfenicol (FLO), and meloxicam (MLX) were purchased from Fischer Scientific (UK). Sulfadiazine (SDZ), tylosin (TYL) enrofloxacin (ENR), oxytetracycline (OTC), lincomycin (LNC), acetyl-salicylic acid (AS) and trimethoprim (TMP) were purchased from SLS (UK).

Pig slurry was dosed at the respective Predictive Environmental Concentration (PEC), this was calculated using the Spaepen et al., (1997) model and utilized the highest available administration dose within the Summary of Product Characteristics. Veterinary medicines were dosed into pig slurries using methanol as a carrier solvent, and concentrations were as follows: FLO 5.53 mg/kg, MLX 11.02 mg/kg, ENR 2.76 mg/kg, OTC 4.97 mg/kg, LNC 3.65 mg/kg, SDZ 6.9 mg/kg, TMP 1.38 mg/kg, SA 6.65 mg/kg and TYL 16.5 mg/kg (SI Table D1 for physical-chemical properties of the selected veterinary medicines).

4.1.2 Box plots and irrigation rates

Box plots were constructed using plastic Eurostack containers at a 3° incline (600 x 400 x 220 mm), there were three replicates per treatment for both grasses and bare soils. The boxes were amended to facilitate the collection of runoff and leachate (Figure 4.0), with PVC guttering attached at the top and bottom to enable sample collection. In order to achieve this, a 20 mm lip was removed from the front of the box and guttering was attached, for leachate 10 mm holes were drilled into the base. Pea shingle gravel (20 mm) was installed into each box to a depth of 5 cm and weed mesh used to promote leachate flow for collection. Dry soil (sieved to <4 mm) was uniformly packed into the box using a taper so that the bulk density of the soil was 1.3 g/cm^3. The soils were then wetted to saturation and left for 12 h after which additional soil was compacted into the box resulting in a total of 42 kg of soil being used in each box. For the grass treatments turf (*Lolium Perenne*) which was purchased from Inturf (UK) was laid onto the soil surface and irrigated for one month prior to the experiment to allow the grass' roots to establish.

The grass was uniformly cut to 7 cm using shears prior to the experiment. A corrugated plastic cover was installed (Figure 4.0) to prevent irrigation directly entering the runoff collector.

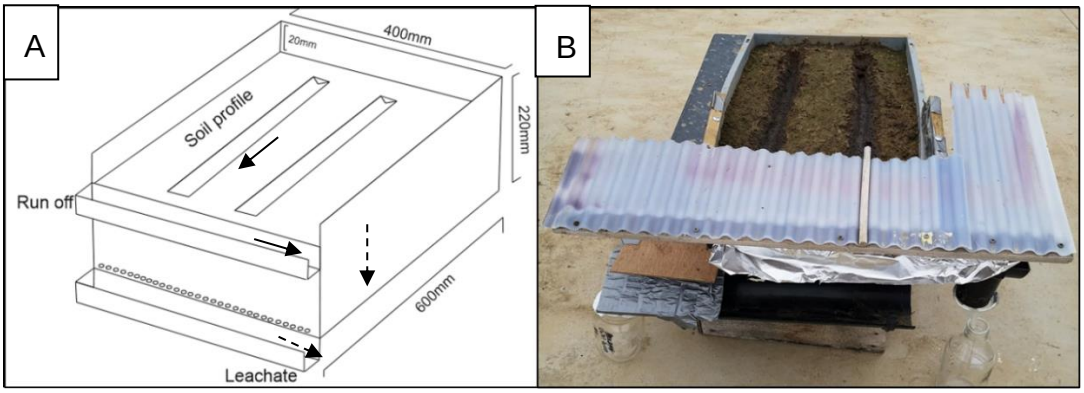


Figure 4.0: Schematic drawing of manure application box plots (chisel sweep) (A), and a picture demonstrating the design in real-life (B). These box plots were placed on wooden frames (100 x 50 and 35 cm). Runoff referred to the water that did not infiltrate the soil but became overland flow whilst leachate refers to the sample which infiltrated the soil profile and then was collected at the bottom of the box.

Key: ⟶ Run off, ⇢ Leachate

4.1.3 Manure sampling, properties and application rates

Pig manure was sampled from a farm in Welburn, York, UK (54°05'31.2"N, 0°53'03.0"W) and the manure was then stored a 3ºC for 1 week prior to the experiment. The pigs had not received any of the selected veterinary medicines that were used within the study. Manures were homogenised and the moisture was corrected to 5% dry weight (EMA, 2011). Please see SI section A1 for the manure characterization methodology and SI Table D2 for the manure properties. The soil utilized within this study was a clay loam from Surrey (UK) which had an organic carbon content of 4.3%; the soil's texture was 22% sand, 50% silt and 29% clay (parameters provided via the retailer Bourne Amenity Ltd).

Prior to dosing the boxes were irrigated with water for 3h to saturate the soils. Pig slurry containing the veterinary medicines was then applied to the boxes using a range of application methods. The rate of pig slurry that was applied was consistent across all treatments; specifically, manure was applied at 170 kg/N/ha which is typical of arable

farming practice within the UK (DEFRA, 2010). This corresponded to 0.72 kg of pig slurry per box; care was taken not to apply slurry close to the edge of the box plot to minimize edge effect (5cm from the width and 2 cm from the top/bottom) (Williams et al*.,* 2019). The manure application methods were broadcast, chisel sweep and immediate incorporation. Incorporation was not assessed within the grass treatments, this was sensible given this practice does not occur on grasses often. The broadcast treatment comprised of uniform application to the plots which was achieved using a watering can. Incorporation utilized a similar method to broadcast, only the slurry was immediately incorporated to 7cm depth using a trowel. To replicate the chisel sweep (shallow injection) application technique a gouge was drilled into the soil profile using a wooden stake; the dimensions of this were 3.8 cm deep and 3.4 cm wide. The drills were 14.3 cm apart and there were two in total per plot. Irrigation of the plots was initiated 24 h after slurry application (SI Figure 4.0D). There were 3 replicates per treatment and one contro.

4.1.4 Irrigation and sampling

The experiment was a semi-field assessment and was conducted within a polytunnel, this enabled the appropriate control that was required for the comparisons between treatments, including removal of external rainfall and irrigation drift. The box plots were irrigated at a rate of 5mm/h which is typical of heavy rainfall within the UK (DEFRA, 2002), this was achieved in cycles of irrigation (4 minutes off and 40 seconds on) and validated utilizing a rain gauge (large plastic rain gauge - Geopacks). A solenoid valve was attached to the water source and was controlled using a time dependent controller circuit. Copper tubing was used to construct the irrigators; these contained a nozzle outlet which created a fine mist. The irrigators were positioned in front of the boxes at height of 1.5 m. Water was distributed evenly between the irrigators using a manifold constructed using copper pipe fittings. The rates of irrigation were validated to ensure that each box received the same rate of rainfall as well as being evenly distributed over the soil profile, this was achieved utilizing 12 plastic cups to catch irrigation water over the course of 5 minutes, acceptable tolerances were set to 10% (SI Table D 3-4 and SI Figure D1). Runoff and leachate samples were collected in Schott bottles following three irrigation events (day 1, 2 and 3) and consisted of three sampling points per day post irrigation (30 mins, 75 mins and 135 mins). Samples were then filtered to 0.2 μm using

nylon syringe filters and fresh matrix matched standards were prepared. The standards encompassed a range of concentrations (0.004 ng/mL to 0.87 µg/mL), it was crucial to prepare these standards in the exact same matrix but also in parallel with the sample preparation. This provides a more accurate assessment of the concentration within the samples as it corrects for matrix suppression and the potential losses during storage. Samples and freshly prepared calibration standards were then stored at 3ºC prior to LC-MS/MS analyses via direct injection.

4.1.5 Analytical technique

Veterinary medicine analysis was achieved using LC-MS/MS (SCIEX Triple Quad 5500+ LC-MS/MS system). The method consisted of using a HST3 column at a set temperature of 40ºC and the mobile phases were 0.1% formic acid (aqueous) and 0.1% formic acid with 1 mM ammonium formate (methanol). The method utilized a 30 µl injection volume and the flow rate was set to 0.4 mL/min. The total chromatographic duration was 11 minutes, and the gradient was reversed phase. This consisted of the following organic percentages; 0mins (0%), 3mins (90%), 8mins (90%), 8.1mins (10%), and 11mins (0%) (see SI Table D5 for full analytical details). Analyst 1.6 was used for data processing and quantification.

4.1.6 Statistical analyses

Statistical comparisons were carried out using Minitab 18. An analysis of variance (two-way) (ANOVA) was used to statistically compare the application methods and concentration over time (mass=time*application technique). Tukey post hoc comparisons were utilized within the ANOVA to distinguish differences between the application methods. Statistical significance was reported at the 95% confidence level ($p \leq 0.05$).

4.2 Results

All of the veterinary medicines that were dosed into manures were detected within the surface runoff and leachate from both the soil and grass assessments (Figure 4.1). The

presented results in Figure 4.1 refer to the cumulative mass of veterinary medicines rather than concentrations, this interpretation of the dataset was conducted to provide a better comparison between the application methods. The absolute concentrations alone are unable to account for the large differences within the generated sample volumes. This was a result of the differences within soil hydrology's, which drove differences within the sample volumes between the application methods. For example, the incorporation method produced lower leachate and runoff volumes than that of broadcast and chisel sweep; this effect resulted in a high concentration being calculated. The concentrations were still reported as this data provides a better demonstration of the environmental risk but were not used within the statistical analyses, as it does not facilitate true comparisons between the application methods.

4.2.1 Arable soils vs grass assessment

The volumes of runoff and leachate were comparable between the same application methods conducted on arable soils and grass plots. The average total volume of runoff generated from broadcast and chisel sweep ranged from 1.0 to 2.1 L and 1.6 to 2.0 L for bare soils and grasses respectively (SI Figure D2-3 and SI Table D6-7). The average total leachate volumes were also comparable amongst replicates; under bare soils broadcast and chisel sweep methods the volume was 2.1 to 3.6 L and under the grass assessment this was 2.9 to 3.0 L (SI Figure D4-5 and Table D6-7). The mass loadings of veterinary medicines that were transported via runoff and leachate were slightly elevated under the bare soil assessment over that of the grasses (Figure 4.1). For example, the percentage of the nominal manure concentration that was transported in runoff and leachate ranged from 0.0003 to 0.46% for bare soils and 0.0003 to 0.1% for grasses under all the assessed application methods (Table 4.2).

4.2.2 Surface runoff vs leachate

Following the application of pig slurry and the sequence of irrigation events the volumes of leachate for both the bare soils and grasses was generally higher but more varied than that of surface runoff (SI Figure D2-5 and SI Table D6-7). Interestingly the percentage

of the nominal manure concentration that was transported in runoff was significantly greater than that of leachate for both bare soils and grasses ($p \leq 0.05$); MLX and TYL were exceptions of this where the concentrations were slightly elevated within leachate over that of the surface runoff via the broadcast method (Figure 4.1).

4.2.3 Veterinary medicine mass loads and concentrations under varying application methods

Following the application of pig slurries to bare soils under varying methods, the concentrations of veterinary medicines detected within surface runoff ranged from 0.49 to 183.46 µg/L (Table 4.0). The total percentage of the nominal veterinary medicine concentration in manure that was transported via runoff was compound specific and followed in the order of OTC<ENR<TMP<LNC<MLX<SDZ<TYL<FLO. FLO exhibited the greatest percentage loss via runoff from bare soils, this was found to be within a range of 0.08 to 0.21% for all assessed application methods (Fig.2 and Table.3). Within the grass assessment the concentrations of veterinary medicines detected within surface runoff ranged from 1.44 to 236.83 µg/L (Table 4.1). The susceptibility for veterinary medicines to form runoff under the grass plots was similar to that of bare soils, OTC<ENR<TYL<TMP<MLX<LNC<FLO. For both the soil and grass plots under all application methods peak runoff and leachate concentrations were detected within the first day of irrigation; generally, the concentration followed a decreasing trend over time (Figure 4.1).

Within the bare soil assessment, the assessed application treatments were observed to alter the soils hydrology and influence the total volumes of runoff that were generated, these were 1.5, 2.1 and 1.0 L for broadcast, chisel sweep, and incorporation respectively. Although the only significant difference was between broadcast and incorporation runoff volumes ($p \leq 0.05$). The manure application treatments not only influenced the soils hydrology but also the mass loadings of veterinary medicines that were detected within surface runoff. Within the bare soil assessment, the greatest veterinary mass loads were detected within surface runoff under the broadcast method, both chisel sweep and incorporation were observed to be significantly lower than that of broadcast ($p \leq 0.05$) (Figure 4.1 and Table 4.2). For ENR, FLO, LNC, and OTC, the cumulative masses within

the chisel sweep surface runoff were significantly reduced over that of broadcast ($p \leq 0.05$). For example, ENR and OTC mass loads were reduced by 55% (447.9 to 990.0 µg) and 83% (127.4 to 216.4 µg) respectively (Table D8-10). Conversely TYL runoff concentrations were higher under the chisel sweep technique; this is likely a result of the peak in the mass loads at 30 minutes (23.350 mg). The incorporation of manures substantially reduced the veterinary medicine mass loads within runoff over that of broadcast ($p \leq 0.05$); however, SDZ was observed to be an exception of this. For OTC, ENR and TMP, reductions of 97%, 92% and 89% were observed ($p \leq 0.05$). Incorporating manures resulted in a surface runoff and leachate lag phase, for example no sample was generated until the irrigation rate reached 6.25 mm/h (SI Table D6 and SI Figure D2-5).

Similarly, within the grass assessment the total surface runoff volume that was generated under the chisel sweep technique was slightly elevated in comparison to that of broadcast (1.6 to 2.0 L). However, no significant differences were observed between the veterinary mass loads that were detected within surface runoff from the differing application methods. There were however time specific differences observed between the application methods (Figure 4.1). Under the chisel sweep method, the cumulative masses for all assessed veterinary medicines (except for MLX) were elevated at 30 minutes when compared to broadcast ($p \leq 0.05$). For example, the LNC concentration range was 88.7 to 40 µg/L. Moreover, the broadcast method exhibited greater mass loads over that of chisel sweep when the irrigation rate reached 13.75 mm/h for MLX, 11.25 mm/h for TYL and ENR (Figure 4.1) ($p \leq 0.05$).

The application methods had little effect on the volumes of leachate that were collected from bare soils, volumes of 3.6, 3.4 and 2.9 L were recorded for broadcast, chisel sweep and incorporation respectively. The concentrations of all the veterinary medicines detected within the bare soil assessment for all application treatments ranged from 0.5 to 309.7 µg/L (Table 4.0). The ability for veterinary medicines to leach was also compound specific (Table 4.2 and Figure 4.1). TYL, MLX and LNC were readily leached following the application of manure to soil, the percentage of the nominal manure concentration

that was transported to leachate were, 0.25%, 0.096% and 0.08% respectively. Comparatively OTC and ENR mass loads were minimal within leachate, the percentages lost were 0.0014% and 0.005% respectively. Similar detections were made under the grass assessment, these ranged from 0.3 to 1255.9 µg/L for all of the assessed veterinary medicines (Table 4.1). The veterinary medicines most readily leached within the grass assessment were FLO, LNC and TYL, with the average total percentages lost at 0.027%, 0.011% and 0.01% respectively (Table 4.3). Interestingly, TMP, SDZ and OTC exhibited the lowest proportion to leachate under varying application methods to soils (Table 4.2 and Figure 4.1).

The veterinary medicine mass loads that were detected within bare soil leachate were found to be the highest under broadcast application over that of both chisel sweep and incorporation (Figure 4.1 and Table 4.3). Chisel sweep was found to reduce the leachate concentrations over that of broadcast, although not all the observed differences were significant. The mass loads of FLO, LNC and TYL within chisel sweep leachate were 60%, 63% and 88% lower than that of broadcast ($p \leq 0.05$). Incorporating manures resulted in a greater reduction in the veterinary medicine masses that were detected within leachate, ENR, LNC, and TMP were significantly reduced and their transfer to leachate was reduced by 94%, 91% and 88% over that of broadcast respectively ($p \leq 0.05$) (SI Table D10). No significant differences were observed between the leachate concentrations that were detected within the chisel sweep and incorporation technique, however by incorporating manures this reduced ENR and LNC concentrations by 83% and 76% over that of chisel sweep (Figure 4.1 and Table 4.2).

Within the grass assessment few differences were observed between the application methods and veterinary medicine mass loads within leachate; the total volume of leachate was similar between both broadcast and chisel sweep (2.9 L and 3.0 L). However, all the assessed veterinary medicines exhibited greater percentages lost under the broadcast treatment over that of chisel sweep, for OTC and TYL the differences in cumulative masses over time was significant ($p \leq 0.05$) (Figure 2). No difference between the

application methods was reported for FLO, although some degree of difference was noted ($p = 0.065$), this is likely attributed to the spiked in cumulative mass under broadcast application that was observed at an irrigation rate of 6.25 mm/h (360.3 µg).

Table 4.0: The total concentrations of veterinary medicines transported from manured soils into surface runoff and leachate.

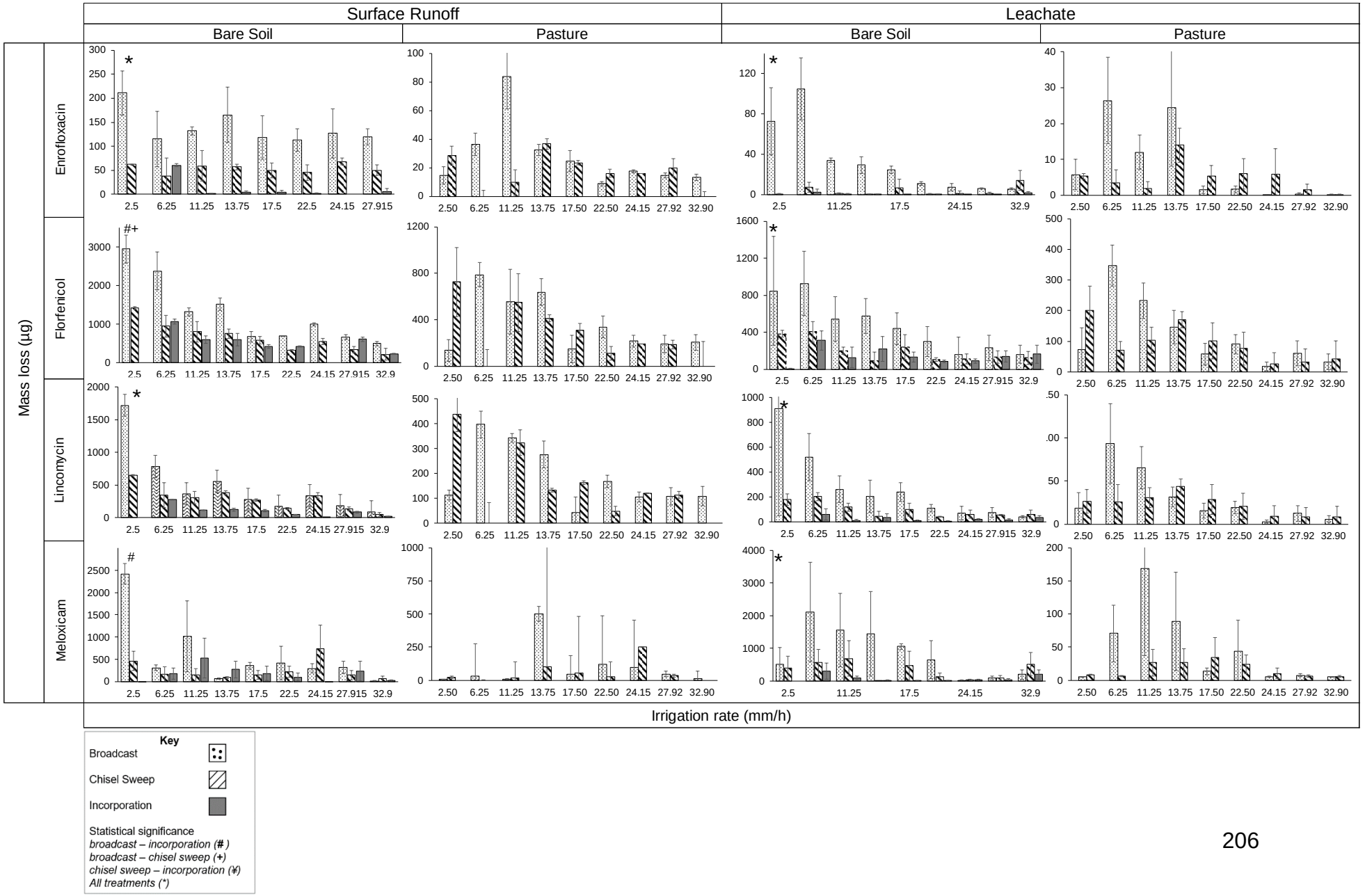
		Surface Runoff (µg/L)			Leachate (µg/L)		
Veterinary Medicine	Irrigation Rate (mm/hr)	BC	CS	IC	BC	CS	IC
ENR	2.5-11.25	2.34±0.34	0.54±0.13	1.48±0.33	0.47±0.14	0.04±0.007	0.02±0.009
	13.75-22.50	3.00±0.31	0.56±0.06	0.18±0.03	0.07±0.02	0.12±0.04	0.00±0.0
	24.15-32.90	1.72±0.39	0.50±0.12	0.06±0.03	0.05±0.02	0.07±0.02	0.02±0.004
FLO	2.5-11.25	31.77±3.06	13.64±2.93	28.54±6.73	15.34±4.41	9.50±3.79	2.56±0.95
	13.75-22.50	19.58±2.61	7.69±1.87	86.54±11.62	10.46±2.50	3.07±0.64	1.53±0.29
	24.15-32.90	14.39±2.54	7.09±2.19	22.55±7.69	3.19±0.37	3.85±0.91	6.74±2.42
LNC	2.5-11.25	20.73±5.25	6.18±1.35	6.20±1.52	9.69±3.38	4.15±1.59	0.47±0.21
	13.75-22.50	8.98±1.45	3.49±0.65	14.04±2.23	3.10±0.61	1.06±0.21	0.17±0.04
	24.15-32.90	4.23±0.90	3.48±1.15	2.41±2.56	1.00±0.16	1.64±0.40	1.01±0.37
MLX	2.5-11.25	11.69±1.89	4.06±1.15	21.05±7.98	21.12±4.56	17.85±6.9	3.10±1.35
	13.75-22.50	6.88±1.34	2.52±0.30	11.70±1.78	44.17±14.53	2.75±0.49	0.12±0.03
	24.15-32.90	4.61±1.18	6.92±2.59	3.72±1.08	0.90±0.12	2.90±0.64	4.85±1.61
OTC	2.5-11.25	0.55±0.09	0.23±0.04	0.49±0.18	0.34±0.13	0.10±0.04	0.03±0.015
	13.75-22.50	0.52±0.02	0.16±0.02	0.01±0.002	0.05±0.01	0.03±0.007	0.00±0.00
	24.15-32.90	0.48±0.07	0.18±0.05	0.00±0.0008	0.04±0.01	0.02±0.007	0.01±0.002
SDZ	2.5-11.25	13.95±3.85	6.48±1.01	7.57±1.90	6.19±2.12	3.08±1.01	0.83±0.21
	13.75-22.50	8.09±1.59	6.56±1.32	11.92±2.20	2.13±0.49	1.81±0.45	1.33±0.36
	24.15-32.90	3.35±0.93	8.67±3.25	2.22±0.40	0.77±0.10	1.66±0.40	1.00±0.30
TYL	2.5-11.25	11.97±1.54	158.57±64.02	37.71±11.10	283.24±122.3	25.38±9.96	25.55±11.3
	13.75-22.50	6.36±1.21	17.34±3.99	12.50±2.94	23.77±6.45	28.97±12.4	0.13±0.06
	24.15-32.90	2.50±0.80	7.56±2.79	0.00±0	2.65±0.73	1.47±0.40	0.21±0.1
TMP	2.5-11.25	3.54±0.33	0.89±0.13	1.61±0.39	1.48±0.42	0.19±0.04	0.11±0.05
	13.75-22.50	3.02±0.30	0.87±0.13	1.83±0.28	0.40±0.08	0.15±0.04	0.00±0.00
	24.15-32.90	1.35±0.31	1.12±0.38	0.19±0.08	0.12±0.02	0.12±0.04	0.02±0.005

Footnote: *Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC). Enrofloxacin (ENR), florfenicol (FLO), lincomycin (LNC), meloxicam (MLX), oxytetracycline (OTC), sulfadiazine (SDZ), tylosin (TYL), trimethoprim (TMP).*

Table 4.1: The total concentration of veterinary medicines transported from manured grass into surface runoff and leachate.

Veterinary Medicine	Irrigation Event	Surface Runoff (µg/L)		Leachate (µg/L)	
		BC	**CS**	**BC**	**CS**
ENR	2.5-11.25	0.43±0.03	2.18±0.89	0.41±0.10	0.10±0.027
	13.75-22.50	0.72±0.22	0.57±0.16	0.49±0.22	0.36±0.11
	24.15-32.90	0.29±0.06	0.32±0.07	0.00±0.00	0.26±0.12
FLO	2.5-11.25	5.63±0.64	50.16±20.79	6.11±1.41	6.34±2.53
	13.75-22.50	14.68±3.55	6.22±1.49	3.85±1.08	4.74±1.34
	24.15-32.90	4.69±0.93	2.91±0.37	0.66±0.49	0.48±0.02
LNC	2.5-11.25	4.24±0.51	36.80±15.23	1.64±0.35	1.80±0.69
	13.75-22.50	6.40±1.52	2.36±0.50	0.87±0.23	1.25±0.34
	24.15-32.90	2.03±0.30	1.84±0.04	0.11±0.01	0.14±0.01
MLX	2.5-11.25	1.12±0.41	4.55±1.40	1.12±0.23	0.48±0.14
	13.75-22.50	14.82±4.73	13.39±4.52	2.15±0.75	0.86±0.12
	24.15-32.90	8.20±3.57	0.69±0.09	0.11±0.01	0.39±0.15
OTC	2.5-11.25	0.97±0.25	7.40±3.18	1.11±0.34	0.38±0.086
	13.75-22.50	0.97±0.28	0.47±0.11	0.44±0.15	0.33±0.087
	24.15-32.90	0.32±0.06	0.28±0.05	0.01±0.001	0.03±0.004
SDZ	2.5-11.25	1.25±0.16	12.32±5.23	1.11±0.23	0.38±0.43
	13.75-22.50	1.37±0.42	0.39±0.10	0.44±0.06	0.33±0.11
	24.15-32.90	0.33±0.07	0.20±0.03	0.01±0.005	0.03±0.05
TYL	2.5-11.25	30.79±2.25	215.31±93.95	14.40±2.22	10.88±2.58
	13.75-22.50	34.22±8.41	13.52±2.52	15.18±3.24	17.38±4.23
	24.15-32.90	12.71±2.63	7.99±1.07	5.90±0.96	9.53±2.65
TMP	2.5-11.25	0.40±0.04	2.75±1.12	0.16±0.03	0.02±0.03
	13.75-22.50	1.07±0.33	0.82±0.21	0.20±0.08	0.23±0.07
	24.15-32.90	0.42±0.11	0.39±0.08	0.00±0.0003	0.09±0.04

Footnote: Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC). *Enrofloxacin (ENR), florfenicol (FLO), lincomycin (LNC), meloxicam (MLX), oxytetracycline (OTC), sulfadiazine (SDZ), tylosin (TYL), trimethoprim (TMP).*


Surface Runoff
Leachate
Bare Soil
Pasture
Bare Soil
Pasture
Mass loss (µg)
Enrofloxacin
Florfenicol
Lincomycin
Meloxicam
Irrigation rate (mm/h)
Key
Broadcast
Chisel Sweep
Incorporation
Statistical significance
broadcast – incorporation (#)
broadcast – chisel sweep (+)
chisel sweep – incorporation (¥)
All treatments (*)

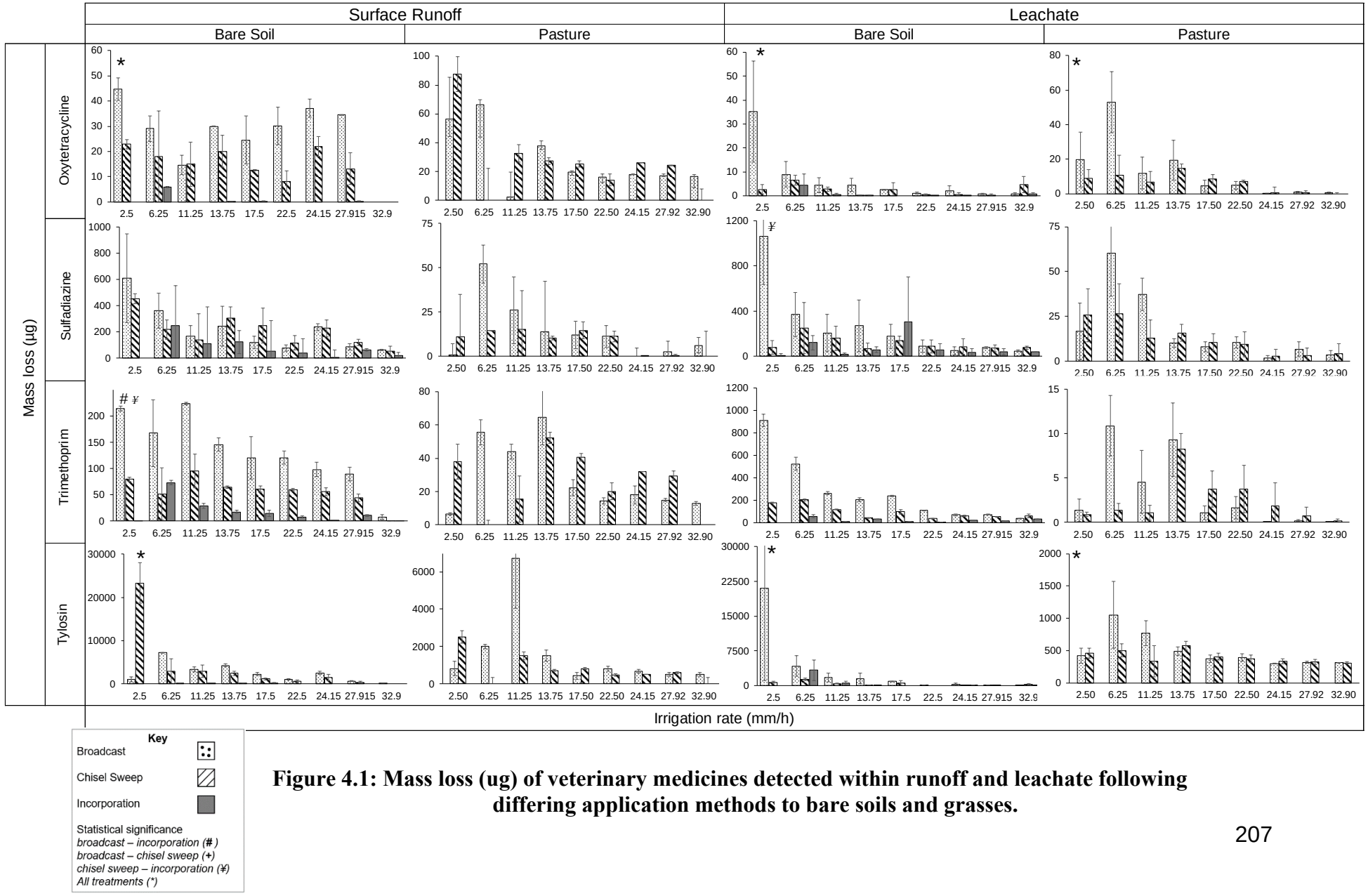


Figure 4.1: Mass loss (ug) of veterinary medicines detected within runoff and leachate following differing application methods to bare soils and grasses.

Table 4.2: Percentage of the veterinary medicine mass load transported via runoff and leachate under varying application methods on arable soils and grasses.

	Percentage Loss Arable Soils (%)								
	Runoff			Leachate			Total		
	BC	CS	IC	BC	CS	IC	BC	CS	IC
ENR	0.0498	0.0225	0.0039	0.0050	0.0017	0.0003	0.0549	0.0243	0.0042
FLO	0.2142	0.1111	0.0849	0.1051	0.0417	0.0318	0.3193	0.1528	0.1167
LNC	0.1499	0.0941	0.0262	0.0809	0.0303	0.0072	0.2308	0.1244	0.0334
MLX	0.0517	0.0304	0.0174	0.0962	0.0361	0.0086	0.1478	0.0665	0.0260
OTC	0.0060	0.0036	0.0002	0.0014	0.0006	0.0002	0.0074	0.0042	0.0004
SDZ	0.0486	0.0425	0.0293	0.0405	0.0207	0.0137	0.0891	0.0632	0.0430
TMP	0.1098	0.0482	0.0125	0.0298	0.0099	0.0024	0.1396	0.0582	0.0149
TYL	0.2108	0.2949	0.0273	0.2528	0.0291	0.0334	0.4635	0.3240	0.0606
	Percentage Loss Grasses (%)								
ENR	0.0096	0.0082		0.0036	0.0022		0.0132	0.0104	
FLO	0.0738	0.0723		0.0279	0.0204		0.1017	0.0927	
LNC	0.0603	0.0556		0.0114	0.0087		0.0717	0.0644	
MLX	0.0256	0.0343		0.0049	0.0019		0.0305	0.0362	
OTC	0.0070	0.0077		0.0035	0.0019		0.0104	0.0096	
SDZ	0.0074	0.0084		0.0035	0.0025		0.0108	0.0109	
TMP	0.0232	0.0269		0.0030	0.0022		0.0261	0.0291	
TYL	0.0133	0.0123		0.0106	0.0003		0.0239	0.0126	

Footnote: *Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC).*

4.3 Discussion

4.3.1 Veterinary medicine losses in surface runoff and leachate

Surface runoff concentrations generated in this study were found to vary to those presented within the literature; differences within experimental approach and design are suspected to be drivers of this. Dose may also play a role in the aforementioned differences, however given that PEC_{slurry} is calculated using the Spaepen, (1997) model which the risk assessments states to use the highest available SPC dosage concentration being used these comparisons are justifiable. Kay et al., (2005a) and Popova et al., (2013) both reported a greater percentage of OTC transported in runoff than that of this study (0.0064%), these values were 0.054% and 2.5% respectively. The differences here are most likely attributed to differences in angle of slope ($3 < 6^{o}$) (Kay et al., 2005a), irrigation duration and soil type (Popova et al., 2013). Similarly, Barrios et al., (2020) reported a total LNC concentration of 5.83 µg/L and a recovery of 122% following three

sets of 30-minute irrigations (70 mm/h) which were 24 h apart at 1 day, 1 week, 2 week and 3 weeks.

In the available literature the results for grassland plots are also varied. For example, Kreuzig et al*.,* (2005) and Knäbel et al*.*, (2016) reported greater SDZ loss via surface runoff, the reported values ranged from 0.56 to 28% whereas this study reported a loss of 0.048%. Both studies utilized much greater irrigation rates of 50-70 mm/h and angel of slope (5.1º). Until now direct comparisons between grasses and bare soils have not been achieved, Kreuzig et al., (2005) reported greater losses from grassland plots however their bare soil assessment contained incorporation to 15 cm meaning direct comparisons cannot be made. The presented dataset demonstrates that the risk of veterinary medicine exposure to surface waters is greatest following the broadcasting of slurries to bare soils. Similar findings were reported by Lin et al., (2010) who observed a 75% reduction in sulfamethazine runoff concentration when using tall grasses as a buffer zone over that of bare soils. Factors such as, inhibited water velocity, increased microbial degradation and adsorption within the trapped sediments are likely drivers of this (Krutz et al*.,* 2005; Liu et al*.,* 2008; Lin et al*.,* 2010 and Reichenberger et al*.,* 2007).

4.3.2 The influence of manure application methods on veterinary medicine fate

The replicated manure application methods altered the soil profiles which was hypothesised to affect the soils hydrology. This likely resulted in the observed differences between the timing and volume of surface runoff generated from the application methods. This was especially true for comparisons between broadcast and incorporation. The altered soil hydrology alone is unlikely to have driven differences in veterinary medicine concentrations. Other factors such as the increased exposure to soil particulates under the varying application methods most likely drove differences in the rates of adsorption, with similar findings previously reported for pesticides (Mickelson et al*.,* 2001; and Elias et al*.,* 2018). Both ENR and OTC have a high affinity for organic matter and carbon (Kim et al*.,* 2012; and Álvarez-Esmorís et al*.,* 2020) and as expected the differences observed

between the treatments was greater. Conversely, lower adsorption coefficients have previously been demonstrated to have an increased potential for surface runoff and leachate transport (Kay et al., 2005a; Dolliver and Gupta, 2008; Joy et al., 2013; Kim et al., 2010; and Popova et al., 2013), and manure application methods were observed to be comparatively less influential but still of great significance.

The presented dataset demonstrates that immediately incorporating slurries into soils prior to a rainfall event is best practice in terms of reducing veterinary medicine exposure to nearby water bodies. Injecting slurries is also an effective means in reducing the transport of veterinary medicines to waters although this was less effective than that of incorporation. Similar findings were reported via Joy et al., (2013), where a reduction in antibiotic runoff concentrations were observed when manures were incorporated or injected over that of broadcast. For example, the authors' reported 0.04% of TYL was transported via runoff under the broadcasting method, this was reduced to 0.028% and 0.011% via injection and incorporation methods. A similar significant effect was reported by Le et al., (2018), the authors reported a, 47, 50, 57, and 88% reduction in sulfamerazine, chlortetracycline, pirlimycin, and tylosin runoff concentrations through subsurface injection over that of broadcast.

Interestingly TYL was observed to deviate from this trend within the bare soil assessment; at 2.5 mm/h there was a spike in runoff concentration under the chisel sweep technique that far surpassed that of broadcast. Moreover, the dataset indicates that at this sampling point the broadcast method TYL was readily leachable which could be related to a reduction in TYL leaching under the chisel sweep application method. It has previously been demonstrated that TYL leaching can be promoted via facilitated transport associated with colloids from manure (Kolz et al., 2005). This is highly possible given that TYL's adsorption coefficient is greater to manure colloidal particulates than that of manure, and greater in manure over soil (Hu and Coats., 2009; and Kim et al., 2010). Therefore, colloidal facilitated transport would be greater under broadcast application,

and the chisel sweep technique could enhance runoff via channelization and desorption from manures (Hoese et al., 2009; and Amarakoon et al., 2014).

It is well known that veterinary medicines can leach following land application of animal manures (Kay et al., 2005b; Dolliver and Gupta., 2008; Popova et al., 2013; Spielmeyer et al., 2017; Pan and Chu., 2017; and Spielmeyer et al., 2020), however, research has seldom assessed the influence of manure application methods on veterinary medicine leaching. For the majority of the assessed veterinary medicines the mass loads detected within leachate were greatest under the broadcast application method over that of chisel weep and incorporation. This was expected when making comparisons to the incorporation technique but there has been concern regarding the influence of injecting slurries (chisel sweep) on leaching rates (Rotz et al., 2011; and Fangueiro et al., 2015). The presented dataset demonstrates that chisel sweep application methods do not enhance leaching rates, despite application into the soils subsurface. A possible explanation for this is capping of the silty clay soil as the drill is implemented into the soil profile. This is a likely explanation of why pig slurries were observed to pool within the injection slots. However, further research is required to assess this relationship for other soil types that may not cap (i.e., sandy or silty soils with lower clay contents).

4.3.3 Relevance to current agricultural practices and manure management

The presented study demonstrates the ability for veterinary medicines to be transported via runoff and leachate under a heavy rainfall event within the UK. Numerous farms within a catchment will apply manures within a similar timeframe, the joint contribution from several sources indicates that receiving waters within a catchment are potentially at a greater risk than this study anticipates. The derived fate data indicates the ability of advanced application technologies and immediate incorporation to reduce the risk of veterinary medicine exposure to surface and groundwaters. As we found to be true for both soils and grass plots, when possible, farmers should utilize shallow injection technologies (soils and grasslands) or immediately incorporate slurries into soils. Smith

et al., (2000) conducted a survey and reported that only 13-23% of farmers incorporate slurries on the day of application, hereby presenting a greater risk of runoff or leachate formation following a rainfall event. Moreover, the Nitrate Directive (91/676, EEC 2000/60/EC) states that slurries/manures should be applied from the 1st of September or 15th October (NVZ specific) to the 15th January or 31st December. Typically these application timings are within the wettest months indicating greater surface runoff and leaching risk (Defra, 2010). Moreover, it is debatable whether farmers adhere to current agricultural policies given problems in practicality and therefore environmental exposure maybe greater than anticipated or calculated under a typical environmental the risk assessment (Young and Mutchler, 1976; and Smith et al., 2000).

The results presented here suggest that the risks towards surface waters are lower when manure is applied to surrounding grasslands. Chisel sweep application methods were found to have very little influence on veterinary medicine runoff concentrations, however a reduction in leaching was observed suggesting this method to be a good measure to protect frequently contaminated groundwaters (Sui et al., 2015; Balzer et al., 2016; Kivits et al., 2018; and Boy-Roura et al., 2018). Slurry acidification is now being utilized within Holland and Denmark to better nutrient management and compliance to the Nitrate Directive, the Dutch authorities are also reducing the requirement to inject or incorporate slurries applied to land when utilizing this manure management technique (Hjorth et al., 2013; and Fangueiro et al., 2015). Recent research has demonstrated the capability of acidifying manures to reduce nitrate leaching when using broadcast application (do Rosário Cameira et al., 2019), however, very little is known regarding the influence of this on veterinary medicine fate and needs to be further investigated (degradation during storage, and mobility when applied to land) (Nightingale et al, 2022, Sassman et al., 2007; Ali et al, 2013; Joy et al, 2013 and Dolliver and Gupta, 2008).

4.4 Conclusion

The presented semi-field study provides a comprehensive evaluation of common manure application methods and their influence on veterinary medicine fate. The broadcasting of pig slurries with no incorporation was identified to present the greatest risk towards both surface and groundwaters. Comparatively, the incorporation of broadcasted pig slurries was observed to be of best practice in terms of the environmental exposure of veterinary medicines, however this practice requires more of the farmers valuable time and could result in greater soil compaction. Therefore, it is more likely that advanced manure application methods such as injection/chisel sweep will be utilized which were deemed appropriate to reduce the concentrations of veterinary medicines in surface runoff and leachate. The reduction in leachate concentrations using this method were surprising but beneficial given that this method is now widely adopted in modern farming. Moreover, the dataset demonstrates that the risk towards waterbodies is greatest surrounding bare soils over that of grasses, but chisel sweep methods are an effective means to reduce the veterinary medicine exposure to waters on both grasses and soils. The presented fate data is crucial to the management of manures and understanding veterinary medicine risk, however it indicates that further research is required to fully understand the influence of these application methods on variable soil types to be representative of the natural environment.

References

Ali, M., Wang, J.J., DeLaune, R.D., Seo, D.C., Dodla, S.K. and Hernandez, A.B., 2013. Effect of redox potential and pH status on degradation and adsorption behavior of tylosin in dairy lagoon sediment suspension. *Chemosphere*, *91*(11), pp.1583-1589.

Álvarez-Esmorís, C., Conde-Cid, M., Ferreira-Coelho, G., Fernández-Sanjurjo, M.J., Núñez-Delgado, A., Álvarez-Rodríguez, E. and Arias-Estévez, M., 2020. Adsorption/desorption of sulfamethoxypyridazine and enrofloxacin in agricultural soils. *Science of The Total Environment*, 706, p.136015.

Amarakoon, I.D., Zvomuya, F., Cessna, A.J., Degenhardt, D., Larney, F.J. and McAllister, T.A., 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure. *Journal of Environmental Quality*, 43(2), pp.549-557.

Balzer, F., Zühlke, S. and Hannappel, S., 2016. Antibiotics in groundwater under locations with high livestock density in Germany. *Water Science and Technology: Water Supply*, *16*(5), pp.1361-1369.

Barrios, R.E., Khuntia, H.K., Bartelt-Hunt, S.L., Gilley, J.E., Schmidt, A.M., Snow, D.D. and Li, X., 2020. Fate and transport of antibiotics and antibiotic resistance genes in runoff and soil as affected by the timing of swine manure slurry application. *Science of the total environment*, 712, p.136505.

Biosic, M. and Babic, S., 2020. Trimethoprim Elimination by Biotic and Abiotic Processes. *Feb Fresenius Environmental Bulletin*, p.7972.

Bittman, S., van Vliet, L.J., Kowalenko, C.G., McGinn, S., Hunt, D.E. and Bounaix, F., 2005. Surface-banding liquid manure over aeration slots. *Agronomy Journal*, 97(5), pp.1304-1313.

Blackwell, P.A., Kay, P. and Boxall, A.B., 2007. The dissipation and transport of veterinary antibiotics in a sandy loam soil. *Chemosphere*, 67(2), pp.292-299.

Blackwell, P.A., Kay, P., Ashauer, R. and Boxall, A.B., 2009. Effects of agricultural conditions on the leaching behaviour of veterinary antibiotics in soils. *Chemosphere*, 75(1), pp.13-19.

Boxall, A.B., 2004. The environmental side effects of medication: How are human and veterinary medicines in soils and water bodies affecting human and environmental health?. EMBO reports, 5(12), pp.1110-1116.

Boy-Roura, M., Mas-Pla, J., Petrovic, M., Gros, M., Soler, D., Brusi, D. and Menció, A., 2018. Towards the understanding of antibiotic occurrence and transport in groundwater: Findings from the Baix Fluvià alluvial aquifer (NE Catalonia, Spain). *Science of the Total Environment*, *612*, pp.1387-1406.

Burkhardt, M., Stamm, C., Waul, C., Singer, H. and Müller, S., 2005. Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. *Journal of Environmental Quality*, 34(4), pp.1363-1371.

Busari, M.A., Kukal, S.S., Kaur, A., Bhatt, R. and Dulazi, A.A., 2015. Conservation tillage impacts on soil, crop and the environment. *International soil and water conservation research*, 3(2), pp.119-129.

Carvalho, I.T. and Santos, L., 2016. Antibiotics in the aquatic environments: a review of the European scenario. *Environment International*, 94, pp.736-757.

Conde-Cid, M., Fernández-Calviño, D., Nóvoa-Muñoz, J.C., Arias-Estévez, M., Díaz-Raviña, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J. and Álvarez-Rodríguez, E., 2018. Degradation of sulfadiazine, sulfachloropyridazine and sulfamethazine in aqueous media. *Journal of Environmental Management*, 228, pp.239-248.

Conde-Cid, M., Ferreira-Coelho, G., Núñez-Delgado, A., Fernández-Calviño, D., Arias-Estévez, M., Álvarez-Rodríguez, E. and Fernández-Sanjurjo, M.J., 2019. Competitive adsorption of tetracycline, oxytetracycline and chlortetracycline on soils with different pH value and organic matter content. *Environmental Research*, 178, p.108669.

Czarniak, P., Boddy, M., Sunderland, B. and Hughes, J.D., 2016. Stability studies of lincomycin hydrochloride in aqueous solution and intravenous infusion fluids. *Drug Design, Development and Therapy,* 10, p.1029.

CTIC. 2004. National crop residue management survey. Available at http://www.ctic.purdue.edu/CRM/ (verified edition 26 Sept. 2010). *Conservation Technology*. Information Cent., West Lafayette, IN.

Williams, M.R., McAfee, S.J. and Kent, B.E., 2019. Dye Tracers Reveal Potential Edge-Flow Effects in Undisturbed Lysimeters Sealed with Petrolatum. Vadose Zone Journal, 18(1), pp.1-9.

DEFRA, 2002. Extreme Rainfall and Flood Event Recognition. R&D Technical Report FD2201. [online] Available at: <http://file:///C:/Users/jnigh/AppData/Local/Temp/FD2201_708_TRP.pdf> [Accessed 27 September 2021].

DEFRA, 2010. Fertiliser Manual (RB209). 8th Edition. [online] Available at: <http://file:///C:/Users/jnigh/AppData/Local/Temp/Defra%20Fertiliser%20manual%202011-1.pdf> [Accessed 21 July 2021].

Dibner, J.J. and Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. *Poultry Science*, 84(4), pp.634-643.

do Rosário Cameira, M., Valente, F., Li, R., Surgy, S., Abreu, F.G., Coutinho, J. and Fangueiro, D., 2019. Band application of acidified slurry as an alternative to slurry injection in Mediterranean winter conditions: Impact on nitrate leaching. Soil and Tillage Research, 187, pp.172-181.

Dolliver, H. and Gupta, S., 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *Journal of Environmental Quality*, *37*(3), pp.1227-1237.

Doretto, K.M. and Rath, S., 2013. Sorption of sulfadiazine on Brazilian soils. *Chemosphere*, 90(6), pp.2027-2034.

Elias, D., Wang, L. and Jacinthe, P.A., 2018. A meta-analysis of pesticide loss in runoff under conventional tillage and no-till management. *Environmental monitoring and assessment*, *190*(2), pp.1-17.

EMA, 2011. “Guideline on determining the fate of veterinary medicinal products in manure” (EMEA/CVMP/ERA/430327/2009 (revised in 2011).

Fangueiro, D., Hjorth, M. and Gioelli, F., 2015. Acidification of animal slurry–a review. *Journal of Environmental Management*, *149*, pp.46-56.

FDA, 2013. Environmental Assessment for Aquaflor (Florfenicol). Aquaflor for Freshwater-reared Finfish in Recirculating Aquaculture Systems. [online] EPA. Available at: <https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/113>.

Gaskins, H.R., Collier, C.T. and Anderson, D.B., 2002. Antibiotics as growth promotants: mode of action. *Animal Biotechnology*, 13(1), pp.29-42.

Hektoen, H., Berge, J.A., Hormazabal, V. and Yndestad, M., 1995. Persistence of antibacterial agents in marine sediments. *Aquaculture*, 133(3-4), pp.175-184.

Heuer, H., Kopmann, C., Zimmerling, U., Krögerrecklenfort, E., Kleineidamm, K., Schloter, M., Top, E.M. and Smalla, K., 2011. Effect of veterinary medicines introduced via manure into soil on the abundance and diversity of antibiotic resistance genes and their transferability. *Antimicrobial Resistance in the Environment*. Hoboken, NJ, USA: John Wiley & Sons, Inc, pp.453-63.

Hjorth, M., Cocolo, G., Jonassen, K. and Sommer, S.G., 2013. Acidifications effect on transformations in and composition of animal slurry. In *RAMIRAN 2013-15th International Conference*.

Hoese, A., Clay, S.A., Clay, D.E., Oswald, J., Trooien, T., Thaler, R. and Carlson, C.G., 2009. Chlortetracycline and tylosin runoff from soils treated with antimicrobial

containing manure. *Journal of Environmental Science and Health Part B*, 44(4), pp.371-378.

Hu, D. and Coats, J.R., 2009. Laboratory evaluation of mobility and sorption for the veterinary antibiotic, tylosin, in agricultural soils. *Journal of Environmental Monitoring*, 11(9), pp.1634-1638.

Ingerslev, F., Vaclavik, E. and Halling-Sørensen, B., 2003. Pharmaceuticals and personal care products-A source of endocrine disruption in the environment?. *Pure and Applied Chemistry*, 75(11-12), pp.1881-1893.

Joy, S.R., Bartelt-Hunt, S.L., Snow, D.D., Gilley, J.E., Woodbury, B.L., Parker, D.B., Marx, D.B. and Li, X., 2013. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. *Environmental Science & Technology*, *47*(21), pp.12081-12088.

Joy, S.R., Li, X., Snow, D.D., Gilley, J.E., Woodbury, B. and Bartelt-Hunt, S.L., 2014. Fate of antimicrobials and antimicrobial resistance genes in simulated swine manure storage. *Science of the Total Environment*, *481*, pp.69-74.

Kasprzyk-Hordern, B., Dinsdale, R.M. and Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Research*, 42(13), pp.3498-3518.

Kay, P., Blackwell, P.A. and Boxall, A.B., 2005a. Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. *Chemosphere*, 59(7), pp.951-959.

Kay, P., Blackwell, P.A. and Boxall, A.B., 2005b. Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land. *Chemosphere*, *60*(4), pp.497-507.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological indicators*, 8(1), pp.1-13.

Kim, K.R., Owens, G., Kwon, S.I., So, K.H., Lee, D.B. and Ok, Y.S., 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water, Air, & Soil Pollution*, *214*(1), pp.163-174.

Kim, S.C., Yang, J.E., Ok, Y.S. and Carlson, K., 2010. Dissolved and colloidal fraction transport of antibiotics in soil under biotic and abiotic conditions. *Water Quality Research Journal*, *45*(3), pp.275-285.

Kim, Y., Lim, S., Han, M. and Cho, J., 2012. Sorption characteristics of oxytetracycline, amoxicillin, and sulfathiazole in two different soil types. Geoderma, 185, pp.97-101.

Kivits, T., Broers, H.P., Beeltje, H., Van Vliet, M. and Griffioen, J., 2018. Presence and fate of veterinary antibiotics in age-dated groundwater in areas with intensive livestock farming. *Environmental Pollution*, *241*, pp.988-998.

Knäbel, A., Bundschuh, M., Kreuzig, R. and Schulz, R., 2016. Runoff of veterinary pharmaceuticals from arable and grassland—a comparison between predictions from model simulations and experimental studies. *Agriculture, Ecosystems & Environment*, *218*, pp.33-39.

Kolz, A.C., Ong, S.K. and Moorman, T.B., 2005. Sorption of tylosin onto swine manure. *Chemosphere*, *60*(2), pp.284-289.

Kreuzig, R., Höltge, S., Brunotte, J., Berenzen, N., Wogram, J. and Schulz, R., 2005. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. *Environmental Toxicology and Chemistry: An International Journal*, *24*(4), pp.777-781.

Krutz, L.J., Senseman, S.A., Zablotowicz, R.M. and Matocha, M.A., 2005. Reducing herbicide runoff from agricultural fields with vegetative filter strips: a review. *Weed Science*, 53(3), pp.353-367.

Le, H.T., Maguire, R.O. and Xia, K., 2018. Method of dairy manure application and time before rainfall affect antibiotics in surface runoff. *Journal of Environmental Quality*, 47(6), pp.1310-1317.

Lei, K.H. and Lai, H.T., 2019. Effects of sunlight, microbial activity, and temperature on the declines of antibiotic lincomycin in freshwater and saline aquaculture pond waters and sediments. *Environmental Science and Pollution Research*, *26*(33), pp.33988-33994.

Lin, C.H., Lerch, R.N., Goyne, K.W. and Garrett, H.E., 2011. Reducing herbicides and veterinary antibiotics losses from agroecosystems using vegetative buffers. *Journal of environmental quality*, *40*(3), pp.791-799.

Lin, J.S., Pan, H.Y., Liu, S.M. and Lai, H.T., 2010. Effects of light and microbial activity on the degradation of two fluoroquinolone antibiotics in pond water and sediment. *Journal of Environmental Science and Health Part B*, 45(5), pp.456-465.

Lin, K. and Gan, J., 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. *Chemosphere*, 83(3), pp.240-246.

Liu, X., Zhang, X. and Zhang, M., 2008. Major factors influencing the efficacy of vegetated buffers on sediment trapping: A review and analysis. *Journal of Environmental Quality*, 37(5), pp.1667-1674.

Loke, M.L., Tjørnelund, J. and Halling-Sørensen, B., 2002. Determination of the distribution coefficient (logKd) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. *Chemosphere*, *48*(3), pp.351-361.

Lukaszewicz, P., Kumirska, J., Bialk-Bielinska, A., Maszkowska, J., Mioduszewska, K., Puckowski, A. and Stepnowski, P., 2016. Application of high-performance liquid chromatography for hydrolytic stability assessment of selected antibiotics in aqueous environment. *Current Analytical Chemistry*, *12*(4), pp.324-329.

Maguire, R.O., Kleinman, P.J., Dell, C.J., Beegle, D.B., Brandt, R.C., McGrath, J.M. and Ketterings, Q.M., 2011. Manure application technology in reduced tillage and forage systems: A review. *Journal of Environmental Quality*, 40(2), pp.292-301.

Mickelson, S.K., Boyd, P., Baker, J.L. and Ahmed, S.I., 2001. Tillage and herbicide incorporation effects on residue cover, runoff, erosion, and herbicide loss. *Soil and Tillage Research*, *60*(1-2), pp.55-66.

Milić, N., Milanović, M., Letić, N.G., Sekulić, M.T., Radonić, J., Mihajlović, I. and Miloradov, M.V., 2013. Occurrence of antibiotics as emerging contaminant substances in aquatic environment. *International Journal of Environmental Health Research*, 23(4), pp.296-310.

Mitchell, S.M., Ullman, J.L., Teel, A.L. and Watts, R.J., 2015. Hydrolysis of amphenicol and macrolide antibiotics: chloramphenicol, florfenicol, spiramycin, and tylosin. *Chemosphere*, *134*, pp.504-511.

Niles, M.T., Horner, C., Chintala, R. and Tricarico, J., 2019. A review of determinants for dairy farmer decision making on manure management strategies in high-income countries. *Environmental Research Letters*, 14(5), p.053004.

Nightingale, J., Carter, L., Sinclair, C.J., Rooney, P., Dickinson, M., Tarbin, J. and Kay, P., 2022. Assessing the influence of pig slurry pH on the degradation of selected antibiotic compounds. *Chemosphere*, 290, p.133191.

Pan, M. and Chu, L.M., 2017. Leaching behavior of veterinary antibiotics in animal manure-applied soils. *Science of the Total Environment*, *579*, pp.466-473.

Pinheiro, A., Albano, R.M.R., Alves, T.C., Kaufmann, V. and da Silva, M.R., 2013. Veterinary antibiotics and hormones in water from application of pig slurry to soil. *Agricultural Water Management*, 129, pp.1-8.

Popova, I.E., Bair, D.A., Tate, K.W. and Parikh, S.J., 2013. Sorption, leaching, and surface runoff of beef cattle veterinary pharmaceuticals under simulated irrigated grass conditions. *Journal of Environmental Quality*, *42*(4), p.1167.

Powell, J.M., Jokela, W.E. and Misselbrook, T.H., 2011. Dairy slurry application method impacts ammonia emission and nitrate leaching in no-till corn silage. *Journal of Eenvironmental Quality*, *40*(2), pp.383-392.

Reichenberger, S., Bach, M., Skitschak, A. and Frede, H.G., 2007. Mitigation strategies to reduce pesticide inputs into ground-and surface water and their effectiveness; a review. *Science of the Total Environment*, 384(1-3), pp.1-35.

Rotz, C.A., Kleinman, P.J.A., Dell, C.J., Veith, T.L. and Beegle, D.B., 2011. Environmental and economic comparisons of manure application methods in farming systems. *Journal of Environmental Quality*, 40(2), pp.438-448.

Sarmah, A.K., Meyer, M.T. and Boxall, A.B., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*, 65(5), pp.725-759.

Sassman, S.A., Sarmah, A.K. and Lee, L.S., 2007. Sorption of tylosin A, D, and A-aldol and degradation of tylosin A in soils. Environmental Toxicology and Chemistry: *An International Journal*, 26(8), pp.1629-1635.

Shao, S., Hu, Y., Cheng, J. and Chen, Y., 2018. Research progress on distribution, migration, transformation of antibiotics and antibiotic resistance genes (ARGs) in aquatic environment. *Critical Reviews in Biotechnology*, 38(8), pp.1195-1208.

Smith, D.R., Owens, P.R., Leytem, A.B. and Warnemuende, E.A., 2007. Nutrient losses from manure and fertilizer applications as impacted by time to first runoff event. *Environmental Pollution*, 147(1), pp.131-137.

Smith, K.A., Brewer, A.J., Dauven, A. and Wilson, D.W., 2000. A survey of the production and use of animal manures in England and Wales. I. Pig manure. *Soil Use and Management*, *16*(2), pp.124-132.

Spaepen, K.R., van Leemput, L.J., Wislocki, P.G. and Verschueren, C., 1997. A uniform procedure to estimate the predicted environmental concentration of the residues of veterinary medicines in soil. *Environmental Toxicology and Chemistry: An International Journal*, 16(9), pp.1977-1982.

Spielmeyer, A., Höper, H. and Hamscher, G., 2017. Long-term monitoring of sulfonamide leaching from manure amended soil into groundwater. *Chemosphere*, *177*, pp.232-238.

Spielmeyer, A., Petri, M.S., Höper, H. and Hamscher, G., 2020. Long-term monitoring of sulfonamides and tetracyclines in manure amended soils and leachate samples-A follow-up study. *Heliyon*, *6*(8), p.e04656.

Subbiah, M., Mitchell, S.M., Ullman, J.L. and Call, D.R., 2011. β-Lactams and florfenicol antibiotics remain bioactive in soils while ciprofloxacin, neomycin, and tetracycline are neutralized. *Applied and environmental microbiology*, 77(20), pp.7255-7260.

Sui, Q., Cao, X., Lu, S., Zhao, W., Qiu, Z. and Yu, G., 2015. Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: a review. *Emerging Contaminants*, *1*(1), pp.14-24.

Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils–a review. *Journal of Plant Nutrition and Soil Science,* 166(2), pp.145-167.

Webb, J., Pain, B., Bittman, S. and Morgan, J., 2010. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—a review. *Agriculture, Ecosystems & Environment*, *137*(1-2), pp.39-46.

Xu, Y., Li, H., Shi, R., Lv, J., Li, B., Yang, F., Zheng, X. and Xu, J., 2020. Antibiotic resistance genes in different animal manures and their derived organic fertilizer. *Environmental Sciences Europe*, 32(1), pp.1-10.

Young, R.A. and Mutchler, C.K., 1976. Pollution Potential of Manure Spread on Frozen Ground 1. *Journal of Environmental Quality*, *5*(2), pp.174-179.

Zainab, S.M., Junaid, M., Xu, N. and Malik, R.N., 2020. Antibiotics and antibiotic resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water research*, p.116455.

Chapter 5

Validation of Current Groundwater Modelling Predictions within the Risk Assessment for Veterinary Medicines

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Abstract

The presented study investigated the environmental fate of veterinary medicines at field-scale in an attempt to validate the groundwater modelling suite FOCUS_PEARL. Veterinary antibiotics that were present on the available veterinary records were identified in the environment surrounding an area of intensive animal husbandry. The concentrations within pig slurries ranged from 0.004 µg/kg to 0.85 mg/kg (d/w) for oxytetracycline, lincomycin, sulfadiazine and trimethoprim. Following the application of this manure to land oxytetracycline, lincomycin, and sulfadiazine were detected in manured-soils to a depth of 10 cm. Immediate incorporation was revealed to substantially reduce the concentrations detected in the soils, which indicates a suitable means to reduce their environmental burden. Veterinary antibiotics were however, detected within groundwaters at depths ranging from 12.5 to 44 m with concentrations of lincomycin, sulfadiazine and trimethoprim ranging between 0.011 to 0.77 ng/L. These compounds were found to fluctuate in concentration within groundwater but be persistent throughout the duration of the study (13/12/20 to 07/07/21). The FOCUS_PEARL groundwater modelling predictions were found to surpass the concentrations detected in the environment by a factor 0.72 to 954 under the best suited default scenario (Châteaudun). The displayed results demonstrate that should good veterinary and farming practices be followed the risk of veterinary medicines in the environment can be reduced. However, the findings do demonstrate that future veterinary medicine effect assessments should be focused on the risks of lower and longer term antibiotic groundwater concentration (ng/L) as well as the problems faced with antimicrobial resistance.

5.0 Introduction

The usage of veterinary medicines within animal husbandry is not a new phenomenon, however, an ever-growing population coupled with the increased demand for a rich protein diet has resulted in its intensification and increased usage (Delgado, 2005; Thornton, 2010; and Wanapat et al., 2015). High excretion rates of veterinary medicines exist due to poor absorption within the gastrointestinal tract of the animal (Halling – Sørensen et al., 2001; Song and Guo 2014). Veterinary medicines are exposed to soils via the frequent application of animal manures, this is routine practice due to their excellent nutrient/organic matter contents, improved cycling via microbial populations as well as being a suitable means of waste disposal (Bogaard et al., 2013; Potter et al., 2010; and Lee, 2010). As a result, veterinary medicines are often detected within environmental compartments surrounding agriculture (Boxall et al., 2004; Hamscher et al., 2005; Li et al., 2018; and Ghirardini et al., 2020). The presence of veterinary antibiotics within the environment are of concern due to the formation of antibiotic resistant genes, as well as the toxicological effects towards terrestrial and aquatic organisms (Ahmad et al., 2014; Kotzerke et al., 2008, Cavalho and Santos, 2016, Klatte et al., 2017; Wallace et al., 2018; Koike et al., 2007; Allen et al., 2011; O'Neill, 2016; and Singer et al., 2016).

Veterinary medicines are well known to be transported via surface runoff and leachate into receiving waters, the extent of which is subject to their physiochemical properties and compound specific processes such as degradation, sorption-desorption, and hydrophilic/hydrophobic relationships (Thiele-Bruhn, 2003; Kreuzig and Höltge., 2005; Dolliver and Gupta; 2008; Lamshöft et al., 2010; and Srinivasn and Sarmah, 2014). Following the application of animal manures and a rainfall event veterinary medicines can leach into groundwater, this process has been demonstrated to be exacerbated via colloidal facilitated transport, macropores and high intensity rainfall events (Kay et al., 2004; Kay et al., 2005; Blackwell et al., 2009; Lee et al., 2009; Lee et al., 2014; Pan and Chu, 2017; and Mehrtens et al., 2020). For example, Kolz and Moorman, (2005), have demonstrated antibiotic leaching to be enhanced via colloidal facilitated transport. Moreover, longer term leaching has been demonstrated for sulphonamides for up to four years after the application of contaminated manures (Spielmeyer et al., 2017).

The concentrations of veterinary medicines that are detected within groundwater range from ng/L to µg/L, some examples of which include sulfamethazine, oxytetracycline, chlortetracycline, tylosin, and tiamuline (Boxall et al., 2004; Hamscher et al, 2005; Weis et al., 2008; Bartelt-Hunt et al., 2011; Kim et al., 2011; Boy-Roura et al., 2017; Yao et al., 2017; Klatte et al., 2017; and Li et al., 2018). There have also been reports of veterinary medicines that were previously considered immobile in the environment, an example of this is enrofloxacin which has a high K_{oc} value and has been detected at an average concentration of 44.5 µg/L in an area surrounding intensive animal agriculture within China (Li et al., 2018). The ability for veterinary antibiotics to persist in groundwaters has also been demonstrated, Kivits et al., (2018) reported sulphonamide detections in groundwaters at depths of 25 m which were characterised as being 40 years old. Their persistence is expected to be related to the reduction in hydrolytic degradation under denitrifying, cold and anoxic conditions (Ahmad et al., 2014).

Due to the environmental and societal risks of veterinary medicine contamination, the environmental risk assessment was implemented under Directive 2004/28/EC, this policy requires new veterinary medicines to be sold on the market to undergo a comprehensive assessment (VICH, 2000; and EMEA, 1997). Despite this, veterinary medicines are still exposed to the environment and our understanding of their fate is still developing. There are concerns regarding the risk assessments environmental representativeness. Some of these include, mixtures not being assessed, only one animal manure per animal type being used in degradation trials and the potential for non-extractable residues to desorb and leach (Gevao et al., 2000; EMA, 2011; Jechalke et al., 2014; Whode et al., 2016; Spielmeyer, 2018; Pan and Chu, 2017; and Kivits et al., 2018). The environmental quality standard for veterinary medicines within groundwater is set at 0.1 µg/L and should environmental modelling demonstrate that their usage will result in concentrations that are below this no further assessments are required (2014/80/AU). However, previous evidence suggests that the EQS may not be protective of all aquatic biota. For example, veterinary medicines have been previously identified to have lethal effects (LC_{50} or EC_{50}) below this value: ivermectin *Neomysis integer, Gammarus sp. and Daphnia magna* LC_{50}

from 25 – 70 ng/L, and cypermethrin arthropod EC_{50} 0.1 µg/L (Lumaret et al., 2012; Loetti and Bellocq, 2017; and EMA, 2018). Moreover, toxicological assessments are conducted at concentrations that typically exceed Measured Environmental Concentrations (MEC) and do not consider longer term chronic effects or the spread of antimicrobial resistance. For example, ciprofloxacin concentrations as low as 0.01 µg L^{-1} have been reported to result in selection of resistant genes for E. coli (*gyr*A) (Sandegren, 2014).

Groundwater prediction models are routinely used within the risk assessment (PEARL_FOCUS), the modelling suite was adopted from the pesticide risk assessment and there have been concerns regarding it's applicability (Montforts and Verschoor, 2003; FOCUS, 2014). The usage of these models within the risk assessment is a requirement under the Groundwater Directive 2014/80/AU (annex I) as well as Directive 2006/118/EC and the Drinking Water Directive (98/83/EC). These sophisticated modelling predictions are often found to surpass the MECs (Montforts and Verschoor, 2003; and Knapič, and Simončič, 2007). However, laboratory studies have suggested that this is not always the case indicating an underestimation. For example a column leaching study reported sulfachlorpuridizine concentrations of 0.66-8.5 µg/L under minor and extreme weather events and detailed that the PEARL modelled predictions were inadequate (Blackwell et al., 2009). In addition, Montforts and Verschoor, (2003) outlined that the sulphachloropyridazine modelling predictions were a factor of 50 below that of the concentrations detected in the leachate that was collected from the lysimeter.

These observations indicate that further research is required to assess the validity of the modelling suite in predicting groundwater. Therefore, the aim of this research was to contribute towards the understanding of the environmental occurrence and fate of veterinary medicines but also to assess the validity of modelling predictions at field scale.

5.1 Methods

5.1.2 Chemicals and reagents

All chemicals and solvents were of the highest available purity, methanol, orthophosphoric acid, NaOH 98 %, NaOH, Na_2EDTA, citric acid and di-sodium hydrogen orthophosphate were purchased from Fisher Scientific (UK). Antibiotics of analytical grade 94 - 98% were purchase from UK retailers. Sulfadiazine (SDZ), tylosin (TYL), enrofloxacin (ENR), oxytetracycline (OTC), lincomycin (LNC), trimethoprim (TMP) were purchased from SLS. Florfenicol (FLO), azaperone (AZA), altrenogest (ALT), ceftiofur (CFT), and meloxicam (MLX) were purchased from Fisher Scientific (UK). McIlvaine buffer (pH 4) was prepared by mixing 307.25 mL of 0.1M citric acid with 192.75 mL of 0.2M phosphate solution, pH 7 contained 7 329.4 mL of phosphate solution is mixed with 88.25 mL of citric acid. Irrespective of the pH, McIlvaine buffer was then mixed 50:50 with 0.1M Na_2EDTA. MeOH-McIlvaine buffer was made by mixing McIlvaine buffer pH 7 with methanol at a 50:50 ratio. SPE conditioning/washing buffer was prepared by diluting 15 mL of the soil extraction buffer to 400 ml total volume with distilled water and acidifying to pH 2.9 by adding 200 µL of H_3PO_4.

5.1.3 Experimental farm site details

The Leeds University research farm (Spen Farm) is located in North Yorkshire (53°51'45.6"N 1°20'03.9"W) (Figure 5.0). The farm is 765 acres in size and has an average total of 660 Hungarian White pigs, of which 230 pigs are housed inside and 230 pigs are located outside. Spen Farm's manure management practices depend on the housing, typically the housed pigs are kept on slats and the slurry is transported to an enclosed silo whilst the manure from the pigs reared outside is stored in manure compost heaps. The site is situated on a Nitrate Vulnerable Zone and therefore the application of manures is confined from the 31st November to the 1st of February. The soil type at the farm is generally sandy, although this differs between arable fields, it is reported that the soil depth is relatively shallow (1-3 m) and very well drained (ALC, 1994). The geology at the site is comprised of dolostone (limestone), associated with an aquifer below (Bgs, 2022). The bedrock is characterised by faults, fissures and cracks resulting in both

laminar flow and turbulent flows within groundwater (Medici *et al*., 2019). The field site contained 2 boreholes with 3 piezometers at differing depths which were sampled throughout the duration of this study. The depths of which were 47 m and 42.5 m at borehole 1 and 22 m, 17 m, and 12.5 m at borehole 2.

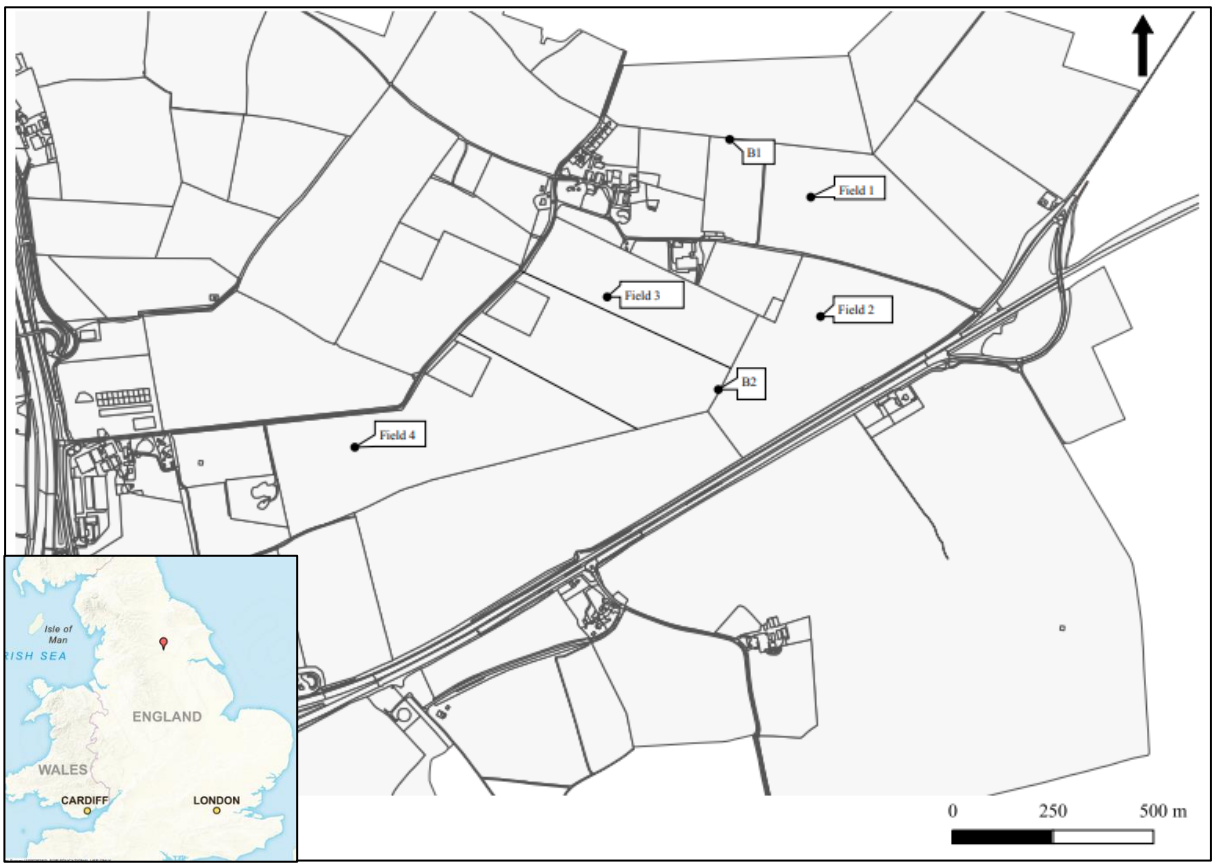


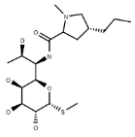
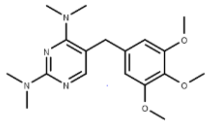
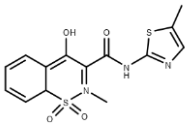
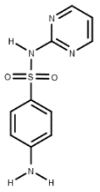
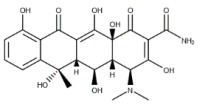
Figure 5.0: Map of Spen Farm (53°51'45.6"N 1°20'03.9"W) containing the sampling locations (fields and boreholes).

Footnote: *Borehole 1 (B1), Borehole 2 (B2).*

5.1.4 Properties of veterinary antibiotics detected

The physical-chemical property data for veterinary medicines that were detected at the farm are presented within Table 5.0.

Table 5.0: Physiochemical properties of veterinary medicines that were detected at Spen farm during the studies duration, the data presented here was utilized for the groundwater modelling predictions (PEARL_FOCUS).

Veterinary medicine	Chemical structure	Saturated water pressure at given temperature (Pa)	Solubility in water at 20°C (mg/L)	K_{oc} (L/kg)	K_{om}	Freundlich sorption coefficient 1/n	Soil DT_{50} (days) at PF 2
Lincomycin		178.65 a	927 a	69.76 b	40.09*	0.9 b	9 c
Trimethoprim		119.99 d (prediction ACD labs)	17.52 e	622 f	357.47*	1.06 f	4 g
Meloxicam		142.65 a	22000 a	318 h	182.75 h	N/A	6.99 h
Sulfadiazine		7.01e-7 a	77 i	15.6-17.6 j	10.11*	0.81-0.92 j	3 j
Oxytetracycline		1293.23 a	313 a	1032 k	80.99 k	0.5 k	56 l

K_f Freundlich sorption coefficient, N Freundlich exponent. *Worst case K_{om} instead of geomean (K_{om}= K_{oc}/1.74).

Footnote: *USEPA, (2010) a, Wang et al., (2012) b, Albero et al., (2018) c, Chemspider (2021) d, Yalkowsky and Jain (2016) e, Zhang et al., (2014) F, Wu et al., (2012) g, Koba et al.. (2017) h, Thiele-Bruhn et al., (2004) i, Sittig (2014) j, Kreuzig and Höltge. (2005) k, Jones et al., (2005), Wang and Yates (2008).*

Details of soil types used in cited studies: *geomean (Sandy clay loam, sandy loam and clay loam) b, Agricultural soils Spain (textures N/A) c, loamy sand f, clay soil g, sandy silt loam (river sediment) h, silty soil and clay soil j, geomean of six differing soils from varying regions k, sandy loam l.*

5.1.5 Sampling methodology

Pig slurry, soil and groundwater samples were collected bi-monthly for 8 months. Slurry samples were obtained from a collection pit that was used to transfer slurries from the rearing facilities into the silo. Grab samples were achieved using a ladle and

samples stored in clean plastic buckets with a tight seal. Soils were collected from four arable fields located around the farm which had a previous history of manure applications. Using a trowel soil was collected to a depth of 10 cm and then stored in ziplocked bags. There were four fields in total that were sampled throughout the course of the study (Field 1-4) (Figure 5.0). The soil samples were collected using the W transect method. Groundwater samples were obtained using a bailer device (point source bailer from Solinst UK) the dimensions of which were 84 cm in length and 1.47 inch in diameter. The bailer comprised of a ball lock system and the total volume was 1055 mL. A tagline was used to lower the bailer into the borehole to a specific depth. In-between sampling events the bailer was cleaned using methanol and deionized water to prevent cross contamination between the boreholes. The groundwater was stored in a 1 L duran bottle. All samples were then stored at 4^{o}C prior to extraction which never exceeded one week from the sampling date.

5.1.6 Sample characteristics

Pig slurry was characterised for the dissolved carbon and nutrient contents (Table 5.0). Slurries were centrifuged at 3,250 rpm for 2 h and the supernatant collected and sequentially filtered in this order; Whatman G/F membrane, Whatman 1PS, 20 µm syringe filter and 0.45 µm syringe nylon filter. The collected sample was then analysed using an Analytik Jena Multi NC2100 (carbon) and Autoanalyzer (nutrients). Both pH and redox potential were measured using a WTW xylem multi meter with the relevant probes. The soils were characterised in the laboratory for texture, pH (water and 1:5 0.01M $CaCl_2$) and carbon. The textures were derived using the sedimentation methodology, the settled sediment was measured, and the percentage sand, silt and clay recorded (FAO, n.d.). Carbon analysis was achieved using the loss on ignition method at a furnace temperature of 550^{o}C (see Table 5.2 for these properties).

Table 5.1: Dissolved pig slurry characteristics from the closed lagoon silo at Spen Farm.

Redox (mV)	pH	DOC, DIC (mg/ L)	NH_4^+, NO_2^-, Total N (mg/L)	PO_4^{3-} (mg/L)
- 434.1	7.81	1332.8±63.73, 589.8±25.88,	654.7±8.02, 0.3±0.084, 3.4±1.84	6.5±0.13

Table 5.2: Determined soil characteristics from the arable fields situated around the boreholes at Spen Farm.

Sample	Texture	pH (1:5 soil-water ratio)	pH (1:5 soil - 0.01 M $CaCl_2$ ratio)	SOM (%)
Field soil 1	Silty loam: 58% sand, 8.33% silt, 33.33% clay	7.35 ± 0.03	7.08 ± 0.02	5.15
Field soil 2	Silty loam: 48% sand, 29.6% silt, 22.22% sand	7.32 ± 0.005	7.12 ± 0.007	6.88
Field soil 3	Silty loam: 50% sand, 30.76% silt, 19.23% clay	7.21 ± 0.007	7.03 ± 0.016	7

5.1.7 Extractions and analytical details

In order to quantitate veterinary medicines within pig slurry, 12.5 ± 0.5 mL of sample was weighed and 25 mL of McIlvaine buffer (pH 4) was added. The sample was shaken at 250 rpm for 20 minutes using a rotary bed shaker and then centrifuged at 3,250 rpm for 20 minutes, the supernatant was decanted, and the extraction was repeated. The combined supernatant was then filtered to 0.2 µm using a nylon syringeless filter, samples were stored at 4°C prior to analysis. For the soils, 4 ± 0.2 g of soil was weighed, 5 mL of McIlvaine buffer (pH 7)-Methanol (50:50) was added and the sample was

vortexed for 30 s. The samples were then sonicated for ten minutes at 15ºC and shaken on the rotary bed shaker at 250 rpm for 20 minutes. The samples were then centrifuged at 2,500 rpm for ten minutes, the supernatant was collected, and the extraction was repeated 3 times. The soil extract was then filtered to 0.2 µm and stored at -20ºC until analysis.

The groundwater samples were filtered using a Whatman G/F filter paper, this filter paper was collected and extracted using McIlvaine buffer-methanol (50:50), however no veterinary medicines were detected during this process. Groundwater samples then underwent Solid Phase Extraction, and the methodology was closely related to the method devised via Blackwell et al., (2004). In order to do this 1 L of sample was accurately weighed, 50 mL of McIlvaine buffer and 20 mL of methanol were added. Oasis HLB cartridges (500 mg) were equilibrated using 5 mL of methanol and 5 mL of SPE conditioning buffer. The groundwater sample was then passed through the manifold at 5 mL/min, this was achieved using a viristep large sample loader. The cartridges were then washed sequentially with 5 mL of conditioning buffer, 2.5 mL 0.1M NaOAc and 5 mL of deionized water. Following the washes, the cartridges were dried under vacuum for ten minutes and eluted twice with 3 mL of methanol. The sample was then evaporated and reconstituted in 1.5 mL of 10% methanol.

Analysis of the samples for veterinary medicines was achieved using a Sciex triple quadrupole 5500+ Q-trap LC-MS/MS system. The analytical method utilized a HST3 column at a temperature of 40ºC, and the liquid chromatography conditions were as follows; 0.1% formic acid (aqueous purified water) and 0.1% formic acid, 1 mM ammonium formate (methanol and acetonitrile 50:50). The method was comprised of a 40 µl injection volume and the flow rate was set to 0.4 mL/min. The method's duration was 11 minutes in total and a reversed phased gradient was selected. The organic percentages were 0 mins (0%), 3 mins (90%), 8 mins (90%), 8.1 mins (10%), and 11 mins (0%). See SI Table E 1-2 for further analytical details and LODs. Analyst 1.6 was used for data processing and quantification.

5.1.8 Validation of extraction methods

The manure, soil, and groundwater veterinary medicine extraction techniques were validated to three concentration levels that were 100%, 10% and 1% of the Predicted Environmental Concentration (PEC). The PECs were derived using the Spaepen et al., (1997) model which utilized the highest possible administration dose from the available Summary of Product Characteristics (SPC) document. The percentage recoveries can be found in SI Tables E 1-3. The validation data produced a range of recoveries for the veterinary medicines used at the farm. The recoveries generated were compound specific but also dependent on the environmental matrix in which they were to be monitored in. The validation recoveries for the analytes detected ranged from, 59.37 to 95.85%, 13.24 to 102.24 %, and 71.98 to 110.17 % in slurry, soils and groundwaters respectively. This data demonstrates the appropriateness of the methods to extract veterinary medicines but also to be reproduceable facilitating concentration corrections to be made on field samples. For generation of the validation data, it was important to obtain a matrix that didn't contain any veterinary medicines. Therefore, for pig slurry farmyard manure was collected from pigs that had no history of veterinary medicine usage (Mount Pleasant Farm Welburn UK). The manure was then moisture corrected to replicate a slurry containing 95 % water (EMA, 2011). For soils standardised soils were purchased from Lufa-Speyer (Germany), and the textures were a sandy loam and clay loam (2.1 and 6s), these were maintained at a moisture content of PF 2.0 (OECD, 2002; and Lufa-speyer, 2022). For the validation of groundwater tap water was used as this provided a practical means of replicating groundwater properties.

5.1.9 Veterinary medicine modelling

For the tier 1 modelling assessment the PECs for pig slurry, soils, and groundwater were derived using the Spaepen et al., (1997) model. This was achieved by following the relevant guidance documents EMEA (1997) and EMA (2011). This is a pragmatic screening mechanism which is routinely utilized within the risk assessment to estimate

the concentrations of veterinary medicines in environmental matrices following the administration to livestock. The formula takes into account numerous arbitrary values such as administered dose (dose and duration), animal species, animal ages (weight), animal turnover (average), nitrogen produced each year (kg/N/year) and the housing factor. The parameters that are changed within this are the administration dosage as well as the duration, which can be found in the SPC documents, it was important to match the veterinary product to those used at the farm. The model then applies further default values such as fraction of herd treated, bulk density of soils and depth of soils to derive the PEC_{soil}. Using the PEC_{soil} at a plough depth of 20 cm the partition coefficient (K_d) is applied, and the groundwater PEC is derived. This can then be further refined using pre-existing manure degradation data (DT_{50}) to derive a $PEC_{soil\ refined}$ (Equations 1+2) (EMA, 2011; VICH, 2000; VICH, 2003). Please see SI Table E6 for the manure degradation data used to refine the PEC_{soil}.

$$\textbf{\textit{Equation 1:}}\ Mt = Mi \times e^{\left(\frac{(-\ln(2)\times\left(\frac{Tst}{2}\right))}{DT50}\right)}$$

$$\textbf{\textit{Equation 2:}}\ \text{PEC}_{\text{soil refined}} = \frac{Mt\times 170}{1500\times 10000\times 0.05\times Ns}$$

Where: Mt = mass of active in manure/slurry after the mean storage time (mg), Mi = Mass of active in manure/slurry (mg), Tst = length of time manure is stored (days), DT50 = half-life of active in manure (days), 170 = EU nitrogen spreading limit, 1500 = bulk density of dry soil (kg/m^3), 10,000 = area of 1 hectare (m2/ha), Ns = nitrogen produced during storage time (kg/N), Fa = fraction of the dose considered to be active.

The PEARL groundwater model was then employed to produce a more accurate prediction of concentrations within groundwater. This suite of models (PELMO, PRZM and MACRO) incorporates site conditions for standardised scenarios (i.e., soil types and climatic conditions), as well as chemical property data such as molar mass (g/mol), Freundlich isotherms (K_f), dissociation constant K_{om}, solubility, soil DT_{50}, and saturated vapour pressure (Pa) (FCOUS, 2014). Using the SPCs of the veterinary medicines used at Spen Farm, the $PEC_{soil\ refined}$ was utilized to calculate the application rate that was inputted into the model (FOCUS, 2014). The default scenarios that were best suited the climate and soil types at Spen Farm were Okehampton, Hamburg and Châteaudun, and therefore these were selected for the assessment (FOCUS, 2014). The

Okenhampton scenario is often utilized within the UK risk assessment for veterinary medicines, this is considered the worst-case prediction (DEFRA, 2013). For this assessment winter cereals were selected, and the application of animal manures included incorporation to 0.2 m depth.

The soil at Spen farm (Table 5.2) was found to be a silty loam with an average organic matter content of 6.34% and the annual average rainfall within this region is 644 mm (Met Office, 2021). Based of the available climate and soil data the Châteaudun scenario is best suited to the field site, with an annual rainfall average of 648 mm, and soil comprised of a silty clay loam with an organic matter content of 2.4%. The Hamburg scenario was generated although not used in comparisons (FOCUS, 2014).

5.2 Results

5.2.1 The occurrence of veterinary antibiotics

In comparison to soils and groundwaters the concentrations of veterinary medicines were elevated within the slurry samples. Of which four of the eleven veterinary medicines that were monitored were detected, the concentrations were found to be within the range of µg/kg to mg/kg. The antibiotic compounds that were detected within pig slurries were lincomycin, oxytetracycline, sulfadiazine and trimethoprim. At no point during the sampling regime were there no detections of antibiotics in pig slurries. Trimethoprim was detected at the highest concentration of 0.85 mg/kg but also the lowest at 80 µg/kg (Figure 5.1). As expected very little trends were observed within the concentrations detected, in that they fluctuated but too many variables existed to extrapolate a clear trend.

Only one of the four fields that were monitored for veterinary medicines had detections above the LOQ, the concentrations of which were in the range of µg/kg. Fields 1 and 2 received no manure applications since before 2019 and therefore no veterinary medicine detections were made. However field 3 had received 24.8 m^3/ha and 23.7 m^3/ha in 2019 (February and September respectively), similarly to that of fields 1 and 2 no detections

of veterinary medicines were made throughout the duration of this study. Veterinary antibiotics were identified in field 4 soils upon the timing of application and these were, oxytetracycline, lincomycin and sulfadiazine. Differences in concentration were observed between the sampling areas (i.e., ploughed and un-ploughed); at the timing of sampling pig slurries were being incorporated into the soil profile. The average concentrations that were detected within the un-ploughed regions were 1.1, 0.7, and 0.14 µg/kg for oxytetracycline, lincomycin, and sulfadiazine respectively, these concentrations were identified to decrease to 0.02, 0, and 0.08 µg/kg following incorporation (ploughed) (Table 5.3). Interestingly just one month after application (13/04/21) no veterinary medicines were detected. Although on 07/07/21 sulfadiazine was detected but these detections were below the LOQ (SI Table.2).

The groundwater surrounding the area of intensive animal husbandry was found to be contaminated with lincomycin, sulfadiazine, and trimethoprim. The concentrations that these antibiotic compounds were detected at were between 0.01 and 1 ng/L (Figure 5.2). The antibiotics were shown to persist throughout the sampling period 18/12/20 to 07/07/21. Small fluctuations were observed within the concentrations detected. For example, trimethoprim and sulfadiazine exhibited a seven-fold and eleven-fold increase in concentration respectively, from the 18/12/20 to 07/07/21 the concentration increased from 0.10 - 0.77 ng/L and 0 – 1.25 ng/L. Although, there was little compelling evidence to relate the increase in groundwater concentrations to the leaching of antibiotics detected within soils.

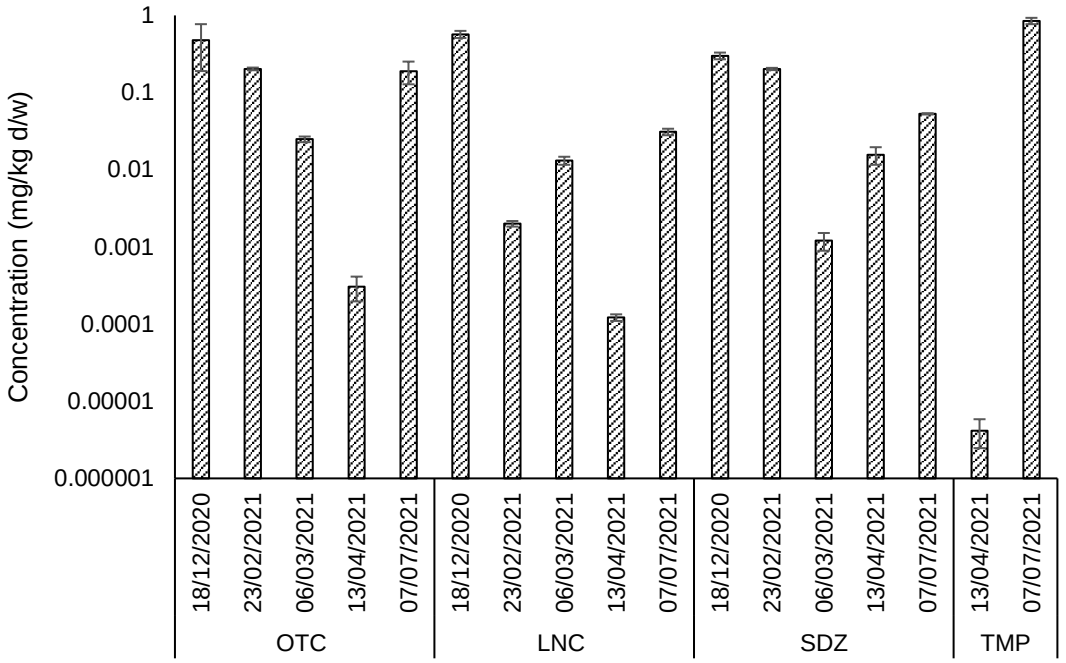


Figure 5.1: Concentrations of veterinary antibiotics detected within pig slurry.

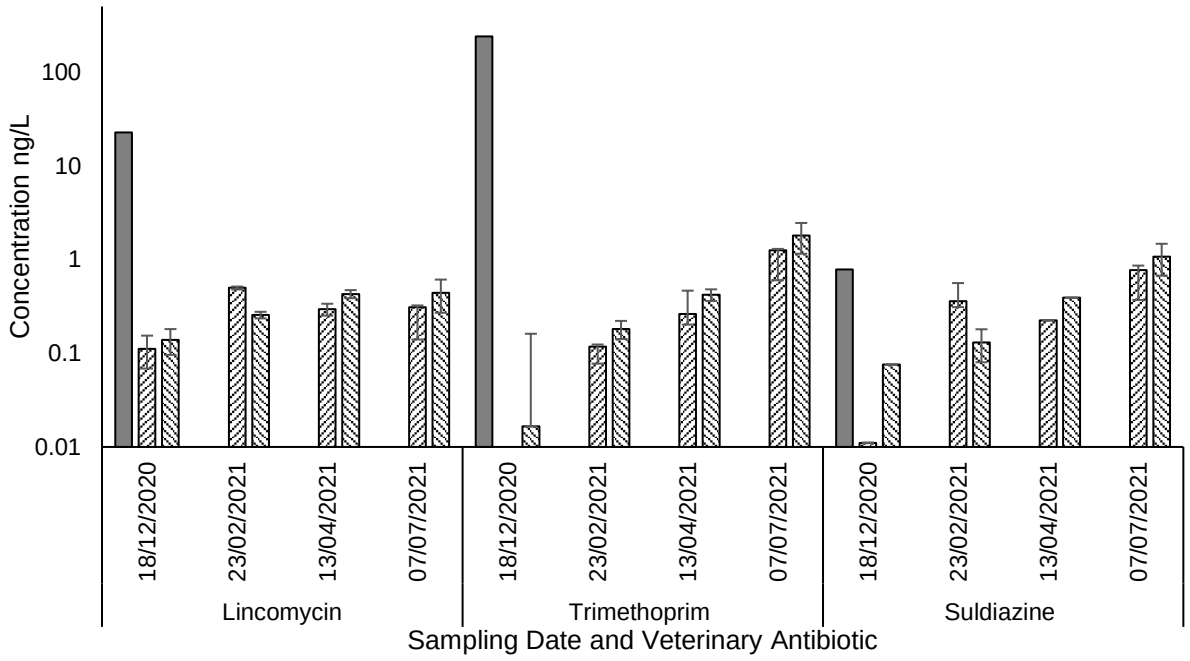


Figure 5.2: Concentrations of veterinary antibiotics detected within groundwater and FOCUS_PEARL predictions.

Key ■ Prediction (Pearl) ▨ Borehole 1 ▧ Borehole 2

Footnote: S*cenario: Châteaudun*

Table 5.3: Concentrations of veterinary antibiotics present in soils (10 cm) at field 4.

		Concentration (µg/kg d/w)		
Sampling area	Date	OTC	LNC	SDZ
Un-ploughed	06/03/21	1.1	0.7	0.14
Ploughed	06/03/21	0.02	0	0.08
	13/04/21	0	0	0

Footnote: No detections occurred in fields 1, 2, and 3 throughout the course of the study.

5.2.2 Veterinary medicine modelling predictions

5.2.3 Tier 1

The lower tier modelled predictions are presented within Table 5.4. Generally, the PEC values range from 1.38 to 6.9 mg/kg in pig slurries, 3.7 to 194.5 µg/kg in soils, and 12.58 to 100.30 µg/L in groundwater. As expected, the PEC_{manure} for all of the assessed analytes were over predicted by a factor of 25 to 69. This was expected given that the model utilizes a 'total residue approach'. The modelled predictions for groundwater were also found to surpass the MEC. Using the available manure degradation data, the soil PECs were refined. However, the $PEC_{soil\ refined}$ were also overestimated even with the available environmental fate data incorporated. Unfortunately, there is currently very little literature regarding the degradation of trimethoprim in slurries and therefore to conduct this assessment manured-soil degradation data was used (Wu et al., 2012).

5.2.4 Tier 2 assessment (FOCUS_PEARL)

The PEARL_FOCUS modelled predictions are presented within Table 5.5. The $PEC_{initial}$ values were found to surpass that of the MEC. Within the groundwater $PEC_{refined}$ assessment the Châteaudun was found to be the best suited default scenario in comparison to the detections made at Spen Farm although the predictions were found to overestimate by a factor of 0.72 to 954 (Table 5.5 and Figure 5.2). Interestingly the sulfadiazine prediction was found to be in-line for the Châteaudun scenario with the measured

environmental concentration being 0.37 ng/L on average and the prediction being 0.78 ng/L.

Table 5.4: Manure soil and groundwater predictions using the lower tier assessment models.

	Manure PEC (mg/kg)	**PECsoil (µg/kg soil) (5 cm mixing depth)**	**Soil PECsoil initial (µg/kg soil) (20 cm mixing depth)**	**Groundwater PEC (µg/L)**	**$PEC_{soil\ refined}$ (5 cm mixing depth)**	**$PEC_{soil\ refined}$ (20 cm mixing depth)**
Lincomycin	3.64	194.5	48.6	91.8	186.64	46.7
Oxytetracycline	4.97	117.9	29.5	100.3	108.4	27.1
Sulfadiazine	6.9	14.7	3.7	12.58	4.9	1.2
Trimethoprim	1.38	N/A	N/A	29.51	N/A	N/A

Footnote: Where appropriate fate data was not available the refined PECs are indicated as N/A.

Table 5.5: FOCUS groundwater modelled predictions for the selected EU scenarios using the refined soil PECs and initial PECs.

	Groundwater $PEC_{initial}$ (ng/L)			**Groundwater $PEC_{refined}$ (ng/L)**		
Scenario	Lincomycin	Sulfadiazine	Trimethoprim	Lincomycin	Sulfadiazine	Trimethoprim
Okenhampton	3700.67	7281.26	2415.29	217.48	183.81	1192.71
Hamburg	2002.36	1007.63	1283.84	129.6	147.33	615.26
Châteaudun	505.10	286.05	4931.99	22.65	0.78	238.82

5.3 Discussion

The concentrations of veterinary antibiotics detected at Spen farm were generally lower in comparison to the available literature. Previous publications have reported the concentrations for oxytetracycline, lincomycin, and trimethoprim within pig slurries to be between 0.21 – 354 mg/kg, 0.17 – 29 mg/kg, and 1.1 – 3.3 mg/kg respectively (Haller et al., 2002; Martínez-Carballo et al., 2007; Rasschaert et al., 2020; and Wang et al., 2017). Similarly, the reported concentrations of antibiotic compounds in soils are variable and typically higher than that of this study despite the majority of the detections being made on manured-soils; for example, oxytetracycline 0.9 – 322 µg/kg, 0.97 µg/kg, 3.2 µg/kg have been reported for oxytetracycline, lincomycin, and sulfadiazine respectively (Boxall et al., 2004; Hamscher et al., 2004; Sattelberger et al., 2005; Li et al., 2013; Hou et al., 2015). An exception of this was lincomycin for which very little literature exists and the concentration was below that of this study (Li et al., 2013). Differences within the reported values are expected to be related to, regional differences in veterinary practice, timing of sampling, climatic conditions and soil types (Campagnolo et al., 2002; Ungemach et al., 2006; Berendsen et al., 2015; Zainab et al., 2020; and Quaik et al., 2020).

Generally, the reported concentrations of antibiotics in groundwater are within the ng/L range (Sui et al., 2015). For example, previous publications have reported concentrations of, 1.5 - 12 ng/L, 8.5 ng/L, 90 – 211.8 ng/L for sulfadiazine, sulfamethoxazole and ciprofloxacin respectively (Balzer et al., 2016; Boy-Roura et al., 2018; and Kivits et al., 2018). However, there are reports of greater concentrations to those detected within this study, for example sulfadimidine and sulfachloropyridazine have been detected at concentrations of 680 µg/L and 3 µg/L respectively (Pei et al., 2006; Weiss et al., 2007; and Díaz-Cruz et al., 2008).

5.3.1 Environmental fate and modelled predictions

Out of the monitored veterinary antibiotics their presence is likely attributed to their usage, physical-chemical properties and environmental fate characteristics. For example,

lincomycin and oxytetracycline are persistent within pig slurries (DT_{50} values > 75 d) (Blackwell et al., 2009; Kuchta and Cessna, 2009; Hollis, 1991), and therefore their presence was expected during storage. The veterinary medicines present within slurries were transferred to soils via the application of contaminated slurries, interestingly these analytes were found to dissipate quickly within the soils. Perhaps this could be attributed to the sampling depth which was only 10 cm whilst incorporation of the slurries was carried out to 20 cm. Moreover, it could also be related to the increased formation of non-extractable residues via longer term adsorption which would not have been assessed within the extraction validation (Wehrhan et al., 2010). Moreover, no detections of the monitored veterinary medicines were achieved during the duration of the study, whilst fields, 1, 2, and 3 had a history of manure applications no slurry has been applied since 2019. Therefore, their lack of presence is likely attributed to a combination of the following, sampling depth, degradation, long-term adsorption, leaching, or even plant uptake.

The presence of antibiotics within groundwater was generally found to relate to the physio-chemical properties of the analytes, for example lincomycin and sulfadiazine have K_{oc} values of 69.76 and 16.6-17.6 L/kg and have been previously demonstrated to readily leachable (Kreuzig and Höltge. 2005; Kuchta et al., 2009; Albero et al., 2018). Interestingly trimethoprim has a high affinity for sorption and would not be expected to contaminate groundwaters (Wu et al., 2012). In column leaching assessments conducted via Burke et al., (2016) trimethoprim produced a K_{oc} value of 725 L/kg, despite this the authors still detected a concentration of 5 - 12 ng/L in groundwater samples. Moreover, Hirsch et al., (1999) conducted a monitoring study and reported no detections of trimethoprim and Fram and Belitz, (2011) only reported one detection out of 1231 groundwater samples which were suggested to be related to human pharmacology. Trimethoprim's presence in groundwater could be related to its desorption rate (8 – 12.4 %), for which has been demonstrated to be enhanced in the presence of nutrients (Sukul et al., 2008; and Li and Zhang., 2015).

Throughout the literature authors accredit the presence of veterinary antibiotics to long term leaching and persistence within the environment (Kivits et al., 2018), for example Spielmeyer et al., (2017) reported sulphonamide leaching for up to years after the application of manure. Hereby presenting that veterinary antibiotic detected within groundwater could be related to previous manure applications and their persistence within groundwater (up to 40 years) (Kivits et al., 2018). Therefore, it is currently unknown whether veterinary medicine fluctuations are related to, previous manure applications, nearby farms, or fluctuations within groundwater flow (Kuchta et al., 2009; Medici et al., 2019).

The modelled predictions under the Châteaudun scenario for sulfadiazine were in-line the detections that were reported at Spen Farm, although this was not the case for all of the antibiotics detected. The appropriateness of the sulfadiazine predictions could be related to comparable soil types being used in degradation experiments as well as he DT_{50} value originating from a radiolabelled study (Kreuzig and Höltge, 2005). Previous assessments that have compared FOCUS_PEARL modelled predictions to the MECs have reported similar overestimations (Montforts, 2003; Labite et al., 2013). In some cases, predictions have been outlined to be underestimated, but typically these involved controlled laboratory experiments (Blackwell et al., 2009). Therefore, in this case it can be concluded that the FOCUS_PEARL models are inadequate at predicting the fate of veterinary medicines within the environment. The overestimation of the modelled predictions could be attributed to the fact that the model does not consider the dilution effect within aquifers (Montforts, 2003). This aspect should be considered when conducting assessments of risk in varying regions. The low concentrations of veterinary medicines detected at the farm is a precursor of their good veterinary and husbandry practices. Such practices include, good housing standards, administering veterinary medicines on an individual basis, and immediately incorporating slurries into soils. This field study demonstrates how best practices can be adopted to reduce the exposure of veterinary medicines to the environment whilst meeting the farmers production requirements.

5.3.2 Environmental significance and future recommendations

The presence of antibiotics within groundwaters is of both an environmental and societal concern. However, to date there is a lack of understanding regarding the risk of lower antibiotic concentrations and chronic exposure in the environment. It is a possibility that even lower concentrations of antibiotics may exert a selective pressure on microbial populations and enhance the formation of resistance (Zainab et al., 2020). Regardless of whether the resistance forms within groundwaters at these concentrations there is also a risk of direct transfer of antimicrobial resistant genes to aquatic compartments (Sapkota et al., 2007). Moreover, there is compelling evidence suggesting antibiotics in groundwater can alter microbial diversity and promote pathogens (*Acinetobacter*), which in return could have negative effects towards humans' gastrointestinal microbial populations (Wang et al., 2019; Gao et al., 2020). There is also a growing concern that the altered microbial dynamics could decrease denitrification which would in return exacerbate the problems faced with nitrification of groundwaters, for example Zou et al., (2021) stated lomefloxacin concentrations of ng/L to reduce denitrification by 8.7%. The presented study demonstrates that the FOCUS_PEARL modelling software's predictions are poor and that current regulatory assessments are likely to overestimate the concentrations in the environment (UK). On one hand this could be seen as beneficial due to the requirement for further ecotoxicological assessments (VICH, 2003), however, it could be debated that such assessments are conducted at unrealistic concentrations and timeframes which indicates a lack of understanding regarding their effects in the environment. Further research is required to confirm these points, this could be achieved via more in-depth monitoring work and site-specific modelling approaches. A consequence of not doing so is to continuously conduct fate/effect assessments at environmentally unrealistic concentrations and overlook the issues faced with antimicrobial resistance (O'Dwyer et al., 2017).

5.4 Conclusion

The presented study demonstrates the fate of veterinary medicines at field-scale. The selected study site utilizes good veterinary and husbandry practices and therefore demonstrates the capability for intensive agriculture to be conducted in a manner that doesn't result in high environmental risks. Factors such as administering veterinary medicines on a case by case basis via injection, immediately incorporating manured-soils, and better housing conditions are suspected to be a movement in the right direction. However, groundwaters were still identified to be present and persist within groundwaters to a depth of 44 m, which highlights that their accumulation in groundwaters is of concern. The modelled predictions of these were found to surpass the measured concentrations, which highlights the requirement to conduct environmental effect assessments at lower concentrations for longer durations. Currently very little literature exists regarding this and there is of course still the complications with antimicrobial resistance.

References

Ahmad, M., Vithanage, M., Kim, K., Cho, J.S., Lee, Y.H., Joo, Y.K., Lee, S.S. and Ok, Y.S., 2014. Inhibitory effect of veterinary antibiotics on denitrification in groundwater: a microcosm approach. *The Scientific World Journal*, *2014*.

Albero, B., Tadeo, J.L., Escario, M., Miguel, E. and Pérez, R.A., 2018. Persistence and availability of veterinary antibiotics in soil and soil-manure systems. *Science of the Total Environment*, *643*, pp.1562-1570.

ALC, 1994. Agricultural land classification A1 (M) Bramham to Wertherby North Yorkshire proposed road widening. Leeds statutory group.

Allen, S.E., Boerlin, P., Janecko, N., Lumsden, J.S., Barker, I.K., Pearl, D.L., Reid-Smith, R.J. and Jardine, C., 2011. Antimicrobial resistance in generic Escherichia coli isolates from wild

small mammals living in swine farm, residential, landfill, and natural environments in southern Ontario, Canada. *Applied and Environmental Microbiology*, 77(3), pp.882-888.

Balzer, F., Zühlke, S. and Hannappel, S., 2016. Antibiotics in groundwater under locations with high livestock density in Germany. *Water Science and Technology: Water Supply*, *16*(5), pp.1361-1369.

Bartelt-Hunt, S., Snow, D.D., Damon-Powell, T. and Miesbach, D., 2011. Occurrence of steroid hormones and antibiotics in shallow groundwater impacted by livestock waste control facilities. *Journal of Contaminant Hydrology*, 123(3-4), pp.94-103.

Berendsen, B.J., Wegh, R.S., Memelink, J., Zuidema, T. and Stolker, L.A., 2015. The analysis of animal faeces as a tool to monitor antibiotic usage. *Talanta*, 132, pp.258-268.

Blackwell, P.A., Boxall, A.B., Kay, P. and Noble, H., 2005. Evaluation of a lower tier exposure assessment model for veterinary medicines. *Journal of Agricultural and Food Chemistry*, *53*(6), pp.2192-2201.

Blackwell, P.A., Kay, P., Ashauer, R. and Boxall, A.B., 2009. Effects of agricultural conditions on the leaching behaviour of veterinary antibiotics in soils. *Chemosphere*, *75*(1), pp.13-19.

Blackwell, P.A., Lützhøft, H.C.H., Ma, H.P., Halling-Sørensen, B., Boxall, A.B. and Kay, P., 2004. Fast and robust simultaneous determination of three veterinary antibiotics in groundwater and surface water using a tandem solid-phase extraction with high-performance liquid chromatography–UV detection. *Journal of Chromatography A*, 1045(1-2), pp.111-117.

Bogaard, A., Fraser, R., Heaton, T.H., Wallace, M., Vaiglova, P., Charles, M., Jones, G., Evershed, R.P., Styring, A.K., Andersen, N.H. and Arbogast, R.M., 2013. Crop manuring and intensive land management by Europe's first farmers. *Proceedings of the National Academy of Sciences*, 110(31), pp.12589-12594.

Boxall, A.B., Fogg, L.A., Blackwell, P.A., Blackwell, P., Kay, P., Pemberton, E.J. and Croxford, A., 2004. Veterinary medicines in the environment. *Reviews of Environmental Contamination and Toxicology*, pp.1-91.

Boy-Roura, M., Mas-Pla, J., Petrovic, M., Gros, M., Soler, D., Brusi, D. and Menció, A., 2018. Towards the understanding of antibiotic occurrence and transport in groundwater: Findings from the Baix Fluvià alluvial aquifer (NE Catalonia, Spain). *Science of the Total Environment*, 612, pp.1387-1406.

British Geological Survey. 2022. BGS maps portal. [online] Available at: <https://www.bgs.ac.uk/information-hub/bgs-maps-portal/> [Accessed 22 February 2022].

Burke, V., Richter, D., Greskowiak, J., Mehrtens, A., Schulz, L. and Massmann, G., 2016. Occurrence of antibiotics in surface and groundwater of a drinking water catchment area in Germany. *Water Environment Research*, 88(7), pp.652-659.

Campagnolo, E.R., Johnson, K.R., Karpati, A., Rubin, C.S., Kolpin, D.W., Meyer, M.T., Esteban, J.E., Currier, R.W., Smith, K., Thu, K.M. and McGeehin, M., 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. *Science of the Total Environment*, *299*(1-3), pp.89-95.

Chemspider. 2021. Trimethoprim | C14H18N4O3 |. [online] Available at: <http://www.chemspider.com/Chemical-Structure.5376.html> [Accessed 23 July 2021].

Chen, L., Lang, H., Liu, F., Jin, S. and Yan, T., 2018. Presence of antibiotics in shallow groundwater in the northern and southwestern regions of China. *Groundwater*, *56*(3), pp.451-457.

Chen, Y., Zhang, H., Luo, Y. and Song, J., 2012. Occurrence and assessment of veterinary antibiotics in swine manures: a case study in East China. *Chinese Science Bulletin*, *57*(6), pp.606-614.

DEFRA, 2013. Comparison of National Approaches for Pesticide Exposure Assessment in the EU Central Zone. EVID4 Evidence Project Final Report. [online] Available at: <http://file:///C:/Users/jnigh/AppData/Local/Temp/11954_PS2253NationalScenariosevid4FinalReport.pdf> [Accessed 22 September 2021].

Delgado, C., 2005. Rising demand for meat and milk in developing countries: implications for grasslands-based livestock production. *Grassland: a global resource*, pp.29-39.

Díaz-Cruz, M.S., García-Galán, M.J. and Barceló, D., 2008. Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography–quadrupole linear ion trap–mass spectrometry. *Journal of Chromatography A*, 1193(1-2), pp.50-59.

Dolliver, H. and Gupta, S., 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *Journal of Environmental Quality*, 37(3), pp.1227-1237.

Du, L. and Liu, W., 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agronomy for sustainable development*, *32*(2), pp.309-327.

EMA (2011) “Guideline on determining the fate of veterinary medicinal products in manure” (EMEA/CVMP/ERA/430327/2009 (revised in 2011).

EMA, 2018. *Guideline on assessing the environmental and human health risks of veterinary medicinal products in groundwater*. EMA/CVMP/ERA/103555/2015.

EMEA, 1997. Discussion Paper on Environmental Risk Assessment for Veterinary Medicinal Products Other than GMO-Containing and Immunological Products. EMEA, London, UK.

FAO, n.d. 6. Soil Texture. [online] Fao.org. Available at: <https://www.fao.org/fishery/docs/CDrom/FAO_Training/FAO_Training/General/x6706e/x6706e06.htm> [Accessed 19 October 2021].

FOCUS. (2014), “groundwater scenarios in the EU review of active substances" - The report of the work of the Groundwater Scenarios Workgroup of FOCUS (Forum for the Co-ordination of pesticide fate models and their USe), Version 1 of November 2000. EC Document Reference Sanco/321/2000 rev.2, 202pp

Fram, M. S.; Belitz, K. (2011) Occurrence and Concentrations of Pharmaceutical Compounds in Groundwater Used for Public Drinking-Water Supply in California. *Science of the Total Environment*., 409, 3409-3417

Gao, F.Z., Zou, H.Y., Wu, D.L., Chen, S., He, L.Y., Zhang, M., Bai, H. and Ying, G.G., 2020. Swine farming elevated the proliferation of Acinetobacter with the prevalence of antibiotic resistance genes in the groundwater. *Environment International*, *136*, p.105484.

Gevao, B., Semple, K.T. and Jones, K.C., 2000. Bound pesticide residues in soils: a review. *Environmental Pollution*, 108(1), pp.3-14.

Ghirardini, A., Grillini, V. and Verlicchi, P., 2020. A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure–environmental risk due to antibiotics after application to soil. *Science of the Total Environment*, 707, p.136118.

Haller, M.Y., Müller, S.R., McArdell, C.S., Alder, A.C. and Suter, M.J.F., 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography–mass spectrometry. *Journal of Chromatography* A, 952(1-2), pp.111-120.

Halling-Sørensen, B., Jensen, J., Tjørnelund, J. and Montforts, M.H.M.M., 2001. Worst-case estimations of predicted environmental soil concentrations (PEC) of selected veterinary antibiotics and residues used in Danish agriculture. *In Pharmaceuticals in the Environment* (pp. 143-157). Springer, Berlin, Heidelberg.

Hamscher, G., Pawelzick, H.T., Höper, H. and Nau, H., 2004. Antibiotics in soil: routes of entry, environmental concentrations, fate and possible effects. *In Pharmaceuticals in the Environment* (pp. 139-147). Springer, Berlin, Heidelberg.

Hamscher, G., Pawelzick, H.T., Höper, H. and Nau, H., 2005. Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. *Environmental Toxicology and Chemistry: An International Journal*, *24*(4), pp.861-868.

Hirsch, R.; Ternes, T.; Haberer, K.; Kratz, K. L. (1999) Occurrence of Antibiotics in the Aquatic Environment. Sci. Total Environ., 225, 109-118.

Hollis, J.M., 1991. Mapping the vulnerability of aquifers and surface waters to pesticide contamination at the national/regional scale. Pesticides in Soils and Water, BCPC Monograph, 47, pp.165-174.

Hou, J., Wan, W., Mao, D., Wang, C., Mu, Q., Qin, S. and Luo, Y., 2015. Occurrence and distribution of sulfonamides, tetracyclines, quinolones, macrolides, and nitrofurans in livestock manure and amended soils of Northern China. *Environmental Science and Pollution Research*, 22(6), pp.4545-4554.

Jechalke, S., Heuer, H., Siemens, J., Amelung, W. and Smalla, K., 2014. Fate and effects of veterinary antibiotics in soil. *Trends in Microbiology*, 22(9), pp.536-545.

Jiménez, J.J., Muñoz, B.E., Sánchez, M.I. and Pardo, R., 2018. Forced and long-term degradation assays of tenoxicam, piroxicam and meloxicam in river water. Degradation products and adsorption to sediment. *Chemosphere*, 191, pp.903-910.

Jones, A.D., Bruland, G.L., Agrawal, S.G. and Vasudevan, D., 2005. Factors influencing the sorption of oxytetracycline to soils. *Environmental Toxicology and Chemistry: An International Journal*, *24*(4), pp.761-770.

Kay, P., Blackwell, P.A. and Boxall, A.B., 2004. Fate of veterinary antibiotics in a macroporous tile drained clay soil. *Environmental Toxicology and Chemistry: An International Journal*, *23*(5), pp.1136-1144.

Kay, P., Blackwell, P.A. and Boxall, A.B., 2005. Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. *Chemosphere*, *59*(7), pp.951-959.

Kim, K.R., Owens, G., Kwon, S.I., So, K.H., Lee, D.B. and Ok, Y.S., 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water, Air, & Soil Pollution*, 214(1), pp.163-174.

Kivits, T., Broers, H.P., Beeltje, H., Van Vliet, M. and Griffioen, J., 2018. Presence and fate of veterinary antibiotics in age-dated groundwater in areas with intensive livestock farming. *Environmental Pollution*, *241*, pp.988-998.

Klatte, S., Schaefer, H.C. and Hempel, M., 2017. Pharmaceuticals in the environment–a short review on options to minimize the exposure of humans, animals and ecosystems. *Sustainable Chemistry and Pharmacy*, 5, pp.61-66.

Knapič, M. and Simončič, A., 2007. Comparison of leaching prediction for selected pesticides using PELMO and PEARL FOCUS models on medium depth eutric cambisol in the Savinja valley. Hmeljarski Bilten, 14, pp.55-61.

Koba, O., Golovko, O., Kodešová, R., Fér, M. and Grabic, R., 2017. Antibiotics degradation in soil: A case of clindamycin, trimethoprim, sulfamethoxazole and their transformation products. *Environmental Pollution*, *220*, pp.1251-1263.

Koike, S., Krapac, I.G., Oliver, H.D., Yannarell, A.C., Chee-Sanford, J.C., Aminov, R.I. and Mackie, R.I., 2007. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Applied and Environmental Microbiology*, 73(15), pp.4813-4823.

Kolz, A.C., Ong, S.K. and Moorman, T.B., 2005. Sorption of tylosin onto swine manure. *Chemosphere*, *60*(2), pp.284-289.

Kotzerke, A., Sharma, S., Schauss, K., Heuer, H., Thiele-Bruhn, S., Smalla, K., Wilke, B.M. and Schloter, M., 2008. Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure. *Environmental Pollution*, 153(2), pp.315-322.

Kreuzig, R. and Höltge, S., 2005. Investigations on the fate of sulfadiazine in manured soil: laboratory experiments and test plot studies. *Environmental Toxicology and Chemistry: An International Journal*, *24*(4), pp.771-776.

Kuchta, S.L. and Cessna, A.J., 2009. Lincomycin and spectinomycin concentrations in liquid swine manure and their persistence during simulated manure storage. *Archives of Environmental Contamination and Toxicology*, *57*(1), pp.1-10.

Kuchta, S.L., Cessna, A.J., Elliott, J.A., Peru, K.M. and Headley, J.V., 2009. Transport of lincomycin to surface and ground water from manure-amended cropland. *Journal of Environmental Quality*, *38*(4), pp.1719-1727.

Labite, H., Holden, N.M., Richards, K.G., Kramers, G., Premrov, A., Coxon, C.E. and Cummins, E., 2013. Comparison of pesticide leaching potential to groundwater under EU FOCUS and site specific conditions. *Science of the Total Environment*, *463*, pp.432-441.

Lam, M.W., Young, C.J., Brain, R.A., Johnson, D.J., Hanson, M.A., Wilson, C.J., Richards, S.M., Solomon, K.R. and Mabury, S.A., 2004. Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environmental Toxicology and Chemistry: An International Journal*, *23*(6), pp.1431-1440.

Lamshöft, M., Sukul, P., Zühlke, S. and Spiteller, M., 2010. Behaviour of 14C-sulfadiazine and 14C-difloxacin during manure storage. *Science of the Total Environment*, 408(7), pp.1563-1568.

Lee, H.Y., Lim, J.E., Kim, S.C., Kim, K.R., Kwon, O.K., Yang, J.E. and Ok, Y.S., 2009. Transport of selected veterinary antibiotics (tetracyclines and sulfonamides) in a sandy loam soil: laboratory-scale soil column experiments. *Journal of Korean Society of Environmental Engineers*, 31(12), pp.1105-1112.

Lee, J., 2010. Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. *Scientia Horticulturae*, 124(3), pp.299-305.

Lee, J., Seo, Y. and Essington, M.E., 2014. Sorption and transport of veterinary pharmaceuticals in soil—a laboratory study. *Soil Science Society of America Journal*, 78(5), pp.1531-1543.

Li, J. and Zhang, H., 2017. Factors influencing adsorption and desorption of trimethoprim on marine sediments: mechanisms and kinetics. *Environmental Science and Pollution Research*, *24*(27), pp.21929-21937.

Li, L., Sun, J., Liu, B., Zhao, D., Ma, J., Deng, H., Li, X., Hu, F., Liao, X. and Liu, Y., 2013. Quantification of lincomycin resistance genes associated with lincomycin residues in waters and soils adjacent to representative swine farms in China. *Frontiers in microbiology*, 4, p.364.

Li, X., Liu, C., Chen, Y., Huang, H. and Ren, T., 2018. Antibiotic residues in liquid manure from swine feedlot and their effects on nearby groundwater in regions of North China. *Environmental Science and Pollution Research*, 25(12), pp.11565-11575.

Loetti, V. and Bellocq, I., 2017. Effects of the insecticides methoxyfenozide and cypermethrin on non-target arthropods: a field experiment. *Austral Entomology*, 56(3), pp.255-260.

Lufa-speyer. 2022. Standard Soils. [online] Available at: <https://www.lufa-speyer.de/index.php/dienstleistungen/standardboeden/8-dienstleistungen/artikel/57-standard-soils> [Accessed 21 February 2022].

Lumaret, J.P., Errouissi, F., Floate, K., Rombke, J. and Wardhaugh, K., 2012. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. *Current Pharmaceutical Biotechnology*, *13*(6), pp.1004-1060.

Martínez Salgado, M.M., Ortega Blu, R., Janssens, M., Fincheira, P., 2019. Grape pomace compost as a source of organic matter: evolution of quality parameters to evaluate maturity and stability. *Journal of Clean Production* 216, 56e63. https://doi.org/10.1016/j.jclepro.2019.01.156.

Martínez-Carballo, E., González-Barreiro, C., Scharf, S. and Gans, O., 2007. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environmental Pollution*, *148*(2), pp.570-579.

Medici, G., West, L.J., Chapman, P.J. and Banwart, S.A., 2019. Prediction of contaminant transport in fractured carbonate aquifer types: A case study of the Permian Magnesian Limestone Group (NE England, UK). *Environmental Science and Pollution Research*, *26*(24), pp.24863-24884.

Mehrtens, A., Licha, T., Broers, H.P. and Burke, V., 2020. Tracing veterinary antibiotics in the subsurface–A long-term field experiment with spiked manure. *Environmental Pollution*, 265, p.114930.

Met Office. 2021. Topcliffe (North Yorkshire) UK climate averages. [online] Available at: <https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gcxh27ec4> [Accessed 22 September 2021].

Mohring, S.A., Strzysch, I., Fernandes, M.R., Kiffmeyer, T.K., Tuerk, J. and Hamscher, G., 2009. Degradation and elimination of various sulfonamides during anaerobic fermentation: a promising step on the way to sustainable pharmacy?. *Environmental Science & Technology*, *43*(7), pp.2569-2574.

Montforts, M.H.M.M. and Verschoor, A.J., 2003. Environmental Risk Assessment for Veterinary Medicinal Products Part 3. Validation of environmental exposure models.

O'Dwyer, J., Hynds, P., Pot, M., Adley, C.C. and Ryan, M.P., 2017. Evaluation of levels of antibiotic resistance in groundwater-derived E. coli isolates in the Midwest of Ireland and elucidation of potential predictors of resistance. *Hydrogeology Journal*, *25*(4), pp.939-951.

OECD 2002, Test No. 307: Aerobic and Anaerobic Transformation in Soil, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070509-en.

OECD, 2000. Adsorption -- Desorption Using a Batch Equilibrium Method. OECD 106 - Guidelines for the Testing of Chemicals. [online] Available at: <https://read.oecd-ilibrary.org/environment/test-no-106-adsorption-desorption-using-a-batch-equilibrium-method_9789264069602-en#page1> [Accessed 20 October 2021].

O'Neill, J., 2016. Review on antimicrobial resistance: tackling drug-resistant infections globally: final report and recommendations. Review on antimicrobial resistance: tackling drug-resistant infections globally: final report and recommendations.

Pan, M. and Chu, L.M., 2017. Leaching behavior of veterinary antibiotics in animal manure-applied soils. *Science of the Total Environment*, 579, pp.466-473.

Pei, R., Kim, S.C., Carlson, K.H. and Pruden, A., 2006. Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research*, 40(12), pp.2427-2435.

Potter, P., Ramankutty, N., Bennett, E.M. and Donner, S.D., 2010. Characterizing the spatial patterns of global fertilizer application and manure production. *Earth Interactions*, 14(2), pp.1-22.

Quaik, S., Embrandiri, A., Ravindran, B., Hossain, K., Al-Dhabi, N.A., Arasu, M.V., Ignacimuthu, S. and Ismail, N., 2020. Veterinary antibiotics in animal manure and manure laden soil: scenario and challenges in Asian countries. *Journal of King Saud University-Science*, *32*(2), pp.1300-1305.

Rasschaert, G., Van Elst, D., Colson, L., Herman, L., de Carvalho Ferreira, H.C., Dewulf, J., Decrop, J., Meirlaen, J., Heyndrickx, M. and Daeseleire, E., 2020. Antibiotic residues and antibiotic-resistant bacteria in pig slurry used to fertilize agricultural fields. *Antibiotics*, *9*(1), p.34.

Sandegren, L., 2014. Selection of antibiotic resistance at very low antibiotic concentrations. *Upsala journal of medical sciences*, 119(2), pp.103-107.

Sapkota, A.R., Curriero, F.C., Gibson, K.E. and Schwab, K.J., 2007. Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. *Environmental Health Perspectives*, *115*(7), pp.1040-1045.

Sattelberger, R., Gans, O. and Martínez, E., 2005. Veterinärantibiotika in Wirtschaftsdünger und Boden. Umweltbundesamt.

Singer, A.C., Shaw, H., Rhodes, V. and Hart, A., 2016. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in microbiology*, 7, p.1728.

Sittig, S., 2014. *Sorption, Transformation and Transport of Sulfadiazine in a loess and a sandy Soil* (Vol. 225). Forschungszentrum Jülich.

Song, W. and Guo, M., 2014. Residual veterinary pharmaceuticals in animal manures and their environmental behaviors in soils. In *Applied manure and nutrient chemistry for sustainable agriculture and environment* (pp. 23-52). Springer, Dordrecht.

Spaepen, K.R., van Leemput, L.J., Wislocki, P.G. and Verschueren, C., 1997. A uniform procedure to estimate the predicted environmental concentration of the residues of veterinary medicines in soil. *Environmental Toxicology and Chemistry*: *An International Journal,* 16(9), pp.1977-1982.

Spielmeyer, A., 2018. Occurrence and fate of antibiotics in manure during manure treatments: a short review. *Sustainable Chemistry and Pharmacy*, 9, pp.76-86.

Spielmeyer, A., Höper, H. and Hamscher, G., 2017. Long-term monitoring of sulfonamide leaching from manure amended soil into groundwater. *Chemosphere*, *177*, pp.232-238.

Srinivasan, P. and Sarmah, A.K., 2014. Assessing the sorption and leaching behaviour of three sulfonamides in pasture soils through batch and column studies. Science of the Total Environment, 493, pp.535-543.

Sui, Q., Cao, X., Lu, S., Zhao, W., Qiu, Z. and Yu, G., 2015. Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: a review. *Emerging Contaminants*, *1*(1), pp.14-24.

Sukul, P., Lamshöft, M., Zühlke, S. and Spiteller, M., 2008. Sorption and desorption of sulfadiazine in soil and soil-manure systems. *Chemosphere*, *73*(8), pp.1344-1350.

Thiele-Bruhn S, Seibicke T, Schulten H-R, Leinweber P (2004) Sorption of sulfonamide pharmaceutical antibiotics on whole soils and particle-size fractions. *Journal of Environmental Quality* 33:1331–1342.

Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils–a review. *Journal of Plant Nutrition and Soil Science*, 166(2), pp.145-167.

Thornton, P.K., 2010. Livestock production: recent trends, future prospects. Philosophical Transactions of the Royal Society B: *Biological Sciences*, 365(1554), pp.2853-2867.

Ungemach, F.R., Müller-Bahrdt, D. and Abraham, G., 2006. Guidelines for prudent use of antimicrobials and their implications on antibiotic usage in veterinary medicine. *International Journal of Medical Microbiology*, *296*, pp.33-38.

US EPA. (2010). Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11 or insert version used]. United States Environmental Protection Agency, Washington, DC, USA.

VICH. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase I. London: CVMP/VICH, 2000. CVMP/VICH/592/98-final.

VICH. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase II Draft Guidance. London: CVMP/VICH, 2003. CVMP/VICH/790/03-Consultation.

Wallace, J.S., Garner, E., Pruden, A. and Aga, D.S., 2018. Occurrence and transformation of veterinary antibiotics and antibiotic resistance genes in dairy manure treated by advanced anaerobic digestion and conventional treatment methods. *Environmental Pollution*, 236, pp.764-772.

Wanapat, M., Cherdthong, A., Phesatcha, K. and Kang, S., 2015. Dietary sources and their effects on animal production and environmental sustainability. *Animal Nutrition*, 1(3), pp.96-103.

Wang, C., Teppen, B.J., Boyd, S.A. and Li, H., 2012. Sorption of lincomycin at low concentrations from water by soils. *Soil Science Society of America Journal*, *76*(4), pp.1222-1228.

Wang, H., Chu, Y. and Fang, C., 2017. Occurrence of veterinary antibiotics in swine manure from large-scale feedlots in Zhejiang Province, China. *Bulletin of environmental contamination and toxicology*, *98*(4), pp.472-477.

Wang, M., Xiong, W., Zou, Y., Lin, M., Zhou, Q., Xie, X. and Sun, Y., 2019. Evaluating the net effect of sulfadimidine on nitrogen removal in an aquatic microcosm environment. *Environmental Pollution*, *248*, pp.1010-1019.

Wang, Q. and Yates, S.R., 2008. Laboratory study of oxytetracycline degradation kinetics in animal manure and soil. *Journal of Agricultural and Food Chemistry*, 56(5), pp.1683-1688.

Wehrhan, A., Streck, T., Groeneweg, J., Vereecken, H. and Kasteel, R., 2010. Long-term sorption and desorption of sulfadiazine in soil: Experiments and modeling. *Journal of Environmental Quality*, 39(2), pp.654-666.

Weiss, C., Conte, A., Milandri, C., Scortichini, G., Semprini, P., Usberti, R. and Migliorati, G., 2007. Veterinary drugs residue monitoring in Italian poultry: *Current strategies and possible developments. Food control*, 18(9), pp.1068-1076.

Weiss, K., Schüssler, W. and Porzelt, M., 2008. Sulfamethazine and flubendazole in seepage water after the sprinkling of manured areas. *Chemosphere*, 72(9), pp.1292-1297.

Wohde, M., Berkner, S., Junker, T., Konradi, S., Schwarz, L. and Düring, R.A., 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. *Environmental Sciences Europe*, 28(1), pp.1-25.

Wu, Y., Williams, M., Smith, L., Chen, D., & Kookana, R. (2012). Dissipation of sulfamethoxazole and trimethoprim antibiotics from manure-amended soils. *Journal of*

Environmental Science and Health, Part B, 47(4), 240–249. doi:10.1080/03601234.2012.636580.

Yalkowsky and Jain, (2016). Handbook of aqueous solubility data. CRC press.

Yao, L., Wang, Y., Tong, L., Deng, Y., Li, Y., Gan, Y., Guo, W., Dong, C., Duan, Y. and Zhao, K., 2017. Occurrence and risk assessment of antibiotics in surface water and groundwater from different depths of aquifers: a case study at Jianghan Plain, central China. *Ecotoxicology and Environmental Safety*, *135*, pp.236-242.

Zainab, S.M., Junaid, M., Xu, N. and Malik, R.N., 2020. Antibiotics and antibiotic resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water Research*, p.116455.

Zhang, Y.L., Lin, S.S., Dai, C.M., Shi, L. and Zhou, X.F., 2014. Sorption–desorption and transport of trimethoprim and sulfonamide antibiotics in agricultural soil: effect of soil type, dissolved organic matter, and pH. *Environmental Science and Pollution Research*, *21*(9), pp.5827-5835.

Zou, H., He, J., Guan, X., Zhang, Y., Deng, L., Li, Y. and Liu, F., 2021. Microbial responses underlying the denitrification kinetic shifting exposed to ng/L-and μg/L-level lomefloxacin in groundwater. *Journal of Hazardous Materials*, *417*, p.126093.

Chapter 6

Insights towards a representative assessment of veterinary medicine risk in the environment - Synthesis and Discussion

Recently there has been a wealth of research investigating the fate of veterinary medicines within the environment. Research of this particular focus entails both commercial regulatory and academic research regarding the environmental fate and risk of veterinary medicines within the environment (EMEA, 1997; Halling-Sørensen et al., 1998; VICH, 2000; VICH 2003; Wohde et al., 2016 and EMA, 2016). Whilst this field is now well established, there is still a lack of understanding of how environmentally realistic processes can influence the fate of veterinary medicines within the environment. Such an understanding is critical to achieve an accurate assessment of the risk of veterinary medicine. Within the published literature it is apparent that there is variation within environmental assessments of veterinary medicine fate, this was found to be especially true within manure degradation assessments as well as studies investigating the transport of veterinary medicines to surface waters (Loke et al., 2000; Kreuzig et al., 2005; Knäbel et al., 2016; and Berendsen et al., 2018). Therefore, the main focus of this thesis was to investigate this variation and whether the environmental representativeness of laboratory assessments is contributing towards inaccurate assessments.

6.0 Research synthesis

The presented research chapters demonstrate that environmentally realistic scenarios and processes can influence the environmental fate of veterinary medicines (see section 6.2 to 6.3.5). For example, commonly reported manure properties have been outlined to be key parameters determining the fate of veterinary medicine degradation (via both biotic and abiotic processes) (Chapters 2 and 3). Moreover, commonly used manure application methods have also been identified to influence transport of veterinary medicines to surface waters and groundwaters via surface runoff and leaching (Chapter 4). The following sections (6.1.1 to 6.1.2) provide a summary and synthesis to the findings that are presented in these research Chapters.

6.1.1 Investigations into the influence of manure properties on veterinary medicine degradation rates - a potential contributor towards variability

Commonly reported pig slurry pHs were observed to significantly influence the degradation rate for the majority of the selected antibiotics (Chapter 2). Within the scientific literature a wide range of pH values are documented (pH 5 to 9), this is a result of differences in animal diet, water, climate, storage conditions as well as the processing of manures (Weinfurtner. 2011; Canh et al., 1998a; Canh et al., 1998b; and Joubin, 2018). Currently manure degradation assessments are only required to utilize one manure per animal type, whereas it could be argued that this is not representative of reality given the heterogenic nature of manures (Weinfurtner, 2011). Therefore, Chapter 2 employed novel experimental techniques to investigate the influence of pig slurry pH on veterinary medicine degradation rates. The experimental aspects of importance were, a suitable means of pH adjustment, maintaining sterility, and anaerobic conditions. These were achieved via unique vessels which were designed with permanently installed pH/redox probes, a detachable lid to facilitate constant pH adjustment and inlets/outlets to flush nitrogen and remove any oxygen. Sterility of the slurries were achieved via autoclaving and using formaldehyde as a sterilant. The adopted techniques were appropriate with pH values being within a tolerance of 0.5, sterility being maintained, and redox potentials remaining below -100 mV (EMA, nd).

The presented fate data in Chapter 2 demonstrates a compound specific effect where, ceftiofur, florfenicol and tylosin's degradation was enhanced within alkaline and inhibited under acidic slurries. Comparatively, sulfamethoxazole was observed to degrade faster in acidic conditions over that of alkaline. Both biotic and abiotic processes associated with pH were identified to drive differences within the degradation rates. For example, the differences within the degradation rates for sulfamethoxazole and tylosin were speculated to be driven via differences in rates of adsorption. This is likely a result of their acid dissociation constant (pKa) and their ionized state at specific pHs which most likely drove differing rates of adsorption via caring mechanisms (hydrophobic interactions, cation exchange, and electrostatic forces) (Srinivasan et al., 2013; Jia et al., 2017; Hu et al., 2019; and Boguta and Sokołowska, 2020). Interestingly the differences

in florfenicol degradation under varying pHs was speculated to be related to microbial mineralization. At alkaline and neutral pHs monochloroflorfenicol was identified to rapidly form, whereas at acidic pH very little of this metabolite formed indicating differences within the rates of microbial mineralization. Moreover, during the abiotic – biotic assessments of pH it was revealed that hydrolysis played a small contribution towards differences in degradation rates. Although significant differences were observed for ceftiofur, tylosin, and sulfamethoxazole, the timeframe at which these differences occurred was much greater than that of the non-sterile assessment.

High resolution mass spectrometry analysis was employed to investigate whether the observed differences within degradation rates were a product of increased rate of formation or alternative degradation routes. Here transformation products which are unreported in pig slurries were identified for the first time. For example, florfenicol metabolite 338 m/Z and Florfenicol metabolite 271 m/Z have recently been identified within soils but not pig slurries (Qiu et al., 2021), and unknown sulfamethoxazole metabolites were detected (ie., SMX_M_259 m/Z and SMX_M_239 m/Z).

The results of Chapter 2 demonstrate: *1. pH is an important parameter in determining the fate of veterinary antibiotics in pig slurries. 2. pH of pig slurries could be driving differences seen between manure degradation assessments. 3. Both abiotic and biotic factors were affected. 4. Future research should conduct such assessments on numerous manures with varying realistic pH's. 5. Future research should be concerned about transformation products and their fate or effects in the environment.*

Similarly Chapter 3 investigated whether manure properties were contributing towards variability seen within manure degradation assessments. Redox potential is a parameter which is often used to determine oxygen content of environmental matrices. The following factors are known to influence the redox potential of slurries, pH, storage conditions, temperature, age, microbial activity and handling (Canhn et al., 1998a; Canhn

et al., 19988b; Weinfurtner et al., 2011). The current manure degradation guideline EMA, (nd) indicates that anaerobic conditions are achieved at ≤ -100 mV for liquid slurries and pig slurries are suggested to range from -250 to -400 mV. Moreover, a wide array of redox potentials have been documented within the literature, for example pig slurry redox potentials have been found to range from +50 to -410 mV (Kolz et al., 2005; Kreuzig. 2010; Lamshöft et al., 2010; Widyasari-Mehta et al., 2016; Richter et al., 2016; and Junker et al., 2020). It was therefore hypothesized whether the differing degrees of oxygen and therefore redox potential was contributing to the variability and uncertainty seen between manure degradation assessments.

In order to investigate this, an experimental system that is capable of controlling the redox potentials of pig slurries (moisture content 95%) was devised. Current systems are either, outdated, expensive, or require sufficient knowledge in electric wiring (Patrick et al., 1973; Hjorth et al., 2012; and Ali et al., 2013). Therefore a novel, cheap and cost-effective experimental system was developed. Briefly this method included a biodegradation reactor that was constructed from a 2L Erlenmeyer flask. This vessel was connected to a redox potential controlling unit and an air pump. When the redox potential fell below the critical value the pump would initiate to oxygenate the system, for anaerobic conditions nitrogen was used to purge the system. The experimental system worked well and the redox potentials of the pig slurries were maintained within a tolerance of ± 50 mV for one month. Until now there have been no reports of a system capable of controlling the redox potentials of pig slurries in a laboratory setting. The development of this system should provide a grounding that will make future investigations into environmental fate assessments and redox feasible.

The presented results of Chapter 3 demonstrate the significant effect of anaerobic redox potentials on veterinary medicine degradation. A compound specific effect was observed where ceftiofur, florfenicol, and sulfamethoxazole's degradation was inhibited under reduced conditions (-100 mV) over that of very anaerobic (-400 mV), the DT_{50} values were 0.7–1.84 h, 1.35–3.61 h, 22.2–49.8 h, 131–211 h and 35.4–94 h respectively. Comparatively the degradation of tylosin was enhanced under reduced (-100 mV) over

that of very anaerobic conditions (DT_{50} 6.88–19.4 h). The results from this study demonstrate that the acceptable range of redox potentials that are considered anaerobic for pig slurries could be contributing towards variability. Through evaluating the literature the cause of these differences were speculated to be a result of differences in microbial population dynamics (FDA, 2013; Alvarino et al., 2016). For example, recent research demonstrate that sulfamethoxazole's degradation is enhanced under anaerobic sludge conditions, the authors accredited this towards the presence of sulphate-reducing-bacteria (*Desulfovibrio Vulgari* and *Clostiridum* sp) (Jia et al., 2017; Ouyang et al., 2019). Interestingly there has been some evidence to suggest abiotic adsorption may influence the degradation of veterinary medicines at different redox potentials, however no hard conclusions have been made (Kolz et al., 2005; Alvarino et al., 2016).

The results of Chapter 3 demonstrate: *1. The redox potentials of pig slurries are an important parameter in determining the fate of veterinary antibiotics. 2. Current accepted anaerobic redox potential values could be contributing towards the differences seen between manure degradation assessments. 3. Future research should conduct such assessments on numerous manures with varying redox potentials.*

6.1.2 Transfer of Veterinary Medicines to Waters

Chapters 3 and 4 specifically were interested in the fate of veterinary medicines post manure storage. Here the potential for veterinary medicines to be transported to waters was observed. The application of animal manures to land as a form of organic fertilizer is commonplace within agriculture and presents a source of veterinary medicines into the environment (Thiele-Bruhn 2003; Kreuzig et al., 2005; Carvalho and Santos, 2016; Spielmeyer et al., 2017). There are now numerous methods in which farmers can apply animal manures to land, these application methods are known to influence the retention of nutrients within soils. It was therefore seen as fitting to assess whether advanced application methods could be used as a mitigative measure to reduce veterinary medicine exposure (Webb et al., 2010; Rotz et al., 2011).

In order to do so a semi-field experiment was designed to investigate the influence of manure application methods on veterinary medicine losses to waters. The application methods that were assessed were conventional broadcast, chisel sweep (injection), and incorporation. A realistic heavy rainfall event (5 mm/h) was simulated using an irrigation system that consisted of a series of copper pipes with spray nozzles (Defra, 2002). The water flow was controlled using a time depending circuit that powered a solenoid valve, and the irrigation system was validated so that the irrigators distributed the rain evenly over the plots but also be within tolerance between the replicates (validated to a difference of 10%). Moreover, boxplots were designed which facilitated the collection of runoff and leachate from both bare soils and grasses. Both arable soils and grass assessments were conducted in an attempt to produce a more rounding and representative conclusion of the risk surrounding typical intensive agriculture.

All of the investigated veterinary medicines (8 in total) were detected within the surface runoff and leachate samples. The concentration of which was compound specific and differed over time. This was expected given what is currently known regarding the influence of organic compounds physiochemical properties on their mobility in soils (Kay et al., 2005; Dolliver and Gupta, 2008; Kim et al., 2010; Joy et al., 2013; and Popova et al., 2013). Parameters such as solubility, octanol-water coefficient, and their affinity for organic matter/carbon (K_{oc}), are understood to influence a compounds mobility in soils (Kim et al., 2012; Álvarez-Esmorís et al., 2020). Generally the concentrations detected in surface runoff were from 0.49 to 183.47 µg/L and 2.26 to 236.83 µg/L for the bare soil and grass assessments. Whilst leachate concentrations were found to be in the range of 0.04 to 309.66 µg/L and 0.33 to 37.79 µg/L for the bare soil and pasture assessments respectively. Of the assessed application methods broadcasting slurries was identified to result in the highest percentage of veterinary medicines lost to surface runoff and leachate (0.006 – 0.21 %, and 0.005 – 0.25 %). Advanced application methods such as chisel sweep were observed to reduce the transport of veterinary medicines via runoff and leaching by 13-56 % and 49-88 % over that of broadcast. Whilst incorporating the slurry was reported to be the most effective measure of reducing the transport of veterinary

medicines, surface runoff and leachate mass loads were reduced by 40 - 97 % and 66 - 94 % over that of broadcast.

This work demonstrated that conventional broadcasting application methods result in the greatest losses to runoff and leachate following a rainfall event. Advanced application methods were found to reduce veterinary medicine losses to runoff and leaching and therefore limit exposure towards receiving waters surrounding bare fields and pastures. Therefore using advanced methods presents a mitigative measure that farmers can use to reduce the exposure of veterinary medicines to aquatic compartments. Interestingly incorporating slurries was found to be the most appropriate means to reduce the exposure of veterinary medicines. However, advanced injection methods are more favourable for farmers as they reduce the requirement for pig slurries to be incorporated into soils which saves valuable time, resources and cost (Busari et al., 2015; Niles et al., 2019). Whilst this maybe a pragmatic approach to applying slurries it was identified that incorporation techniques (ploughing) is the best suited practice in reducing the exposure of veterinary medicines towards both surface waters and groundwaters. Protecting water bodies from veterinary medicine exposure is essential to protect aquatic organisms such as, cyanobacteria, algae, freshwater plants and aquatic invertebrates such as daphnids and fish (Ingerslev et al., 2003; and Kemper., 2008; Heuer et al., 2011; and Shao et al., 2018).

The results of Chapter 4 demonstrate: *1. Advanced manure application methods can be used to mitigate the exposure of veterinary medicines to waters 2. Conventional broadcasting of slurries prior to a rainfall event presents the largest environmental risk in terms of aquatic compartments. 3. Some veterinary medicines are more influenced by application method thank others. 4. Future considerations in manure management and the risk assessment for veterinary medicines should entail the use of injection technologies.*

The ability for veterinary antibiotics to leach into groundwater surrounding intensive animal husbandry was demonstrated within Chapter 5. The chapter also investigated the appropriateness of the pesticide leaching model FOCUS_PEARL in predicting veterinary medicine concentrations in groundwater. In order to meet these aims, the study consisted of a yearlong field monitoring exercise which comprised of bi monthly sampling of slurry, soil and groundwater at the University of Leeds research farm (Spen Farm, 53°51'45.6"N, 1°20'03.9"W). Extraction methods and solid phase extraction techniques were developed for the veterinary medicines that had recent records of administration (2018 – 2020). A singular analytical method capable of detecting nine veterinary medicines was devised using LC-MS/MS, the limits of detections ranged from 1 ng/L to 0.05 µg/L were devised (matrix and compound specific).

Five of the nine monitored veterinary medicines were detected at the farm in various matrices. The highest concentrations reported were within pig slurry and ranged from 0.004 µg/kg to 0.85 mg/kg (d/w). The antibiotics present within slurries were identified to be transferred to land following the application of manure to land, sulfadiazine, trimethoprim, and lincomycin were detected in soils to a depth of 10 cm. The concentrations in soils were observed to quickly dissipate below the LOQ following incorporation to a depth of 20 cm. Perhaps this is attributed to longer term sorption that the validation would have overlooked or alternatively the difference in sample depth and incorporation (10 < 20 cm) (Wehrhan et al., 2010). The three antibiotics that were detected within the groundwater were lincomycin, sulfadiazine and trimethoprim. The concentrations were in the range of ng/L and they were found to persist for up to nearly a year. Currently there is very little literature available regarding the persistence of antibiotics within groundwaters, however it has been a concern amongst researchers for some time now (Bilal et al., 2020). Of the published literature Ahmad et al., (2014) demonstrated chlortetracycline to remain stable for 12 d in synthetic groundwater and Frey et al., (2015) reported tylosin to persist for 211 d in groundwaters. Persistence within groundwaters presents a concern that veterinary medicines may accumulate within groundwater following the repeat application of contaminated animal manures (Kampouris et al., 2022).

The aim of this chapter was to investigate whether FOCUS_PEARL modelled predictions were in-line with the concentrations monitored in groundwater at Spen Farm. The default Châteaudun scenario predictions were the best suited to the measured environmental concentrations, this was expected given that the soil and climatic conditions were similar. The majority of the assessed antibiotic compounds the predictions were found to surpass the detections made in groundwater. An exception of this was for sulfadiazine under the Châteaudun scenario. The concentrations presented were comparatively minor in comparison to the detections made within groundwaters at the Farm. These results were generally in-line with other studies that have investigated this (Montforts, 2003; Labite et al., 2013). This study demonstrates that the risks of veterinary medicines in the environment can be alleviated should good husbandry and veterinary practices be followed. Good practices may include, improved housing for animals such as sanitary conditions with adequate space and light, as well as the administration of veterinary medicines on a case-by-case basis via injection (Ricker et al., 2020).

The results of Chapter 5 demonstrate: *1. Intensive animal husbandry can contaminate groundwaters 2. Antibiotic compounds can persist within groundwaters to a depth of 44 m for up to a year. 3. Current veterinary medicine leaching modelling predictions surpass those of measured environmental concentrations.*

6.2 Implications of Findings Towards the Risk Assessment for Veterinary Medicines and Future Research

The presented data in Chapters 1 and 2 demonstrates that environmentally realistic manure properties such as pH and redox can significantly alter the degradation rates of veterinary medicines. Therefore, it was only fitting to assess whether these differences would result in variation for the assessed risk within the environment. In order to do so the following section used the derived fate data (DT_{50}) to calculate the $PEC_{soil\ refined}$ for each experimental treatment, this data was then compared to terrestrial and aquatic effect

data to distinguish whether altered degradation rates drove differences in identified risk (VICH 2000; VICH 2003). For the purpose of this exercise fattening pigs (62 to 125 kg) were selected.

In order to quantify the differences in risk an environmental risk assessment was conducted which encompassed both phases I and II. The analytes selected for this were florfenicol, tylosin, and sulfamethoxazole, the veterinary products of which are Amphen, Pharmacin, and T.sol respectively. These antibiotic compounds were selected for this evaluation due to, their significant differences within degradation rates, as well as the availability of reliable ecotoxicological effect data. The products were selected for this assessment as they contained the highest available administration dose within the available Summary of Product Characteristics documents (VMD, 2021). This was considered an essential aspect in order to investigate the variation on a worse case basis; this is also routine practice within the environmental risk assessment. The physiochemical properties of the selected antibiotic compounds that were used in the model are presented in Table 6.0.

Table 6.0: Summary of florfenicol, tylosin and sulfamethoxazole physical-chemical properties.

Substance	**Molecular mass (g/mol)**	**Water solubility (mg/L)**	**Dissociation constant range K_f or K_{oc} (geomean)**	**Vapour pressure (Pa)**	**Octanol-water partition coefficient (Log K_{ow})**
Florfenicol	358.21	1320 b	Range of four soil types K_{oc} 10 -27 (18.38) b	18.26 c	0.37 b
Sulfamethoxazole	253.279	610 c	219 d	$9.2x10^{-6}$ c	0.89 e
Tylosin	916.1	5000 a	Range of four soil types K_{oc} 553 – 7988 (2095.73), K_f 2.3 – 7 (3.97) f	13.33 c	-3.41 g

Footnote: *Yalkowsky and He (2003) a, FDA, (2013) b, Chemspider, (2022) (estimated) c, Nguyen Dang Gjang et al., (2015) d , Chen et al., (2017) e, Rabølle and Spliid, (2000) f, Loke et al., (2000) g.*

6.2.1 Phase I

Using the Spaepen, (1997) model and the aforementioned SPC documents the PEC $_{soil\ initial}$ was calculated. The simple model and scenarios used are also defined within the guidance document EMA, (2016). The model encompasses a total residue approach and is designed to consider a 'worst-case' scenario. This assumes that the total administered dose is excreted, and no dissipation occurs during manure storage or following the application of slurries to land. The model also utilizes a soil incorporation depth of 5 cm following the application of manures to land. Other default values utilized are, bulk density, nitrogen produced in one year per place, animal turnover, and the fraction of the herd being treated (Table 6.1). The only values that are altered within this assessment are the administration dose and the duration of administration.

As can be seen in Table 6.2 all of the assessed products resulted in PEC $_{soil\ initial}$ values that far exceeded the action limit of 100 µg/kg (VICH, 200; and EMA, 2016). Therefore, a Phase II tier A risk assessment would be required to fully assess products the risk towards terrestrial and aquatic organisms.

Table 6.1: Phase I tier A assessment for Amphen, Pharmacin, and T.sol containing the husbandry scenarios and treatment data that was used to calculate the PEC soil initial (spaepen et al., 1997).

Active	Animal	P (Animal place/year)	BW (Kg)	Ny (Kg/N/ place/year)	H (-)	D (mg/kg$_{bw}$ /d)	Ad (d)
Florfenicol						5	5
	Fattening pigs	3	25-125	7.5	1		
Tylosin						20	10
Sulfamethoxazole						20	4

Table 6.2: Phase I tier A PEC initial derived using the Spaepen, (1997) model.

Active	PEC $_{soil\ initial}$ (µg/kg)	PEC groundwater (µg/L)	PEC surface water (µg/L)
Florfenicol	147.3	83.22	27.74
Sulfamethoxazole	1178 .7	29.61	9.87
Tylosin	471.5	7.94	2.65

Footnote: PEC $_{soil\ initial}$ = Predicted environmental concentration in soil (µg/kg). D = Daily dose of the active ingredient (mg/kg$_{bw}$/day), Ad = Number of days of treatment (days), BW = Animal body weight (kg$_{bw}$), P = Animal turnover rate per place per year (place/year), 170 = EU nitrogen spreading limit (Kg/N/ha), Fh = Fraction of herd treated (0 - 1), 1500 = Bulk density of dry soil (kg/m^3), 10000 = Area of 1 hectare (m^2/ha), 0.05 = Depth of incorporation into soil (m), Ny = Nitrogen produced in one year per place (kg/N/place/y), H = Housing factor (0.5 - 1)

6.3 Phase II Tier A

6.3.1 Risks to soil microbes

At concentrations of 0.5 to 2.5 mg/kg (d/w) florfenicol was identified to result in a transient effect on nitrogen transformation in soils. The authors reported a <25% reduction in nitrogen transformation in comparison to the controls (FDA, 2013). In a similar assessment Tylosin reduced nitrogen transformation by 66.3 % over a 28 day exposure assessment (Anses, 2018). Sulfamethoxazole was identified to be the least toxic towards microorganisms responsible for nitrogen transformation with a 10% reduction observed (Zheng et al., 2021).

The antibiotics effects towards soil fauna (*E.fetida*) are summarised in Table 6.3, where florfenicol was observed to have a NOEC of 1.56, sulfamethoxazole had a LC_{50} of > 4000 mg/kg, and tylosin's NOEC was 149 mg/kg. Sulfamethoxazole was reported to be non-toxic towards *E.fetida* with a LC_{50} value of >4000 mg/kg being reported, therefore there were no available NOEC values within the literature (Anses, 2018; FDA, 2013; and Pino et al., 2015).

The presented terrestrial effect data (Table.6.3) was compared to the PEC $_{soil\ initial}$ to generate a risk quotient (RQ) (Table.6.4). In order to do so an assessment factor of 10 was applied to the NOEC to generate the Predicted No Effect Concentration, the PEC soil initial (µg/kg) was then compared to the PNEC (µg/kg) to derive the RQ (0-1). From this assessment it is apparent that at the PEC $_{soil\ initial}$ florfenicol would present a risk towards *E.fetida* whilst for tylosin and sulfamethoxazole no risk was identified (Table 6.4).

Table 6.3: Summarized terrestrial ecotoxicological effect data from the available literature.

	Micro-organisms		**Terrestrial organisms**	
Substance	**Nitrogen transformation (28 days)**	**Toxicity Endpoint (mg/kg $_{d/w}$)**	**Earthworm reproduction (NOEC)**	**Toxicity Endpoint (mg/kg $_{d/w}$)**
Florfenicol	<25% a	0.5 to 2.5 a	*E.fetida*	1.56 a
Sulfamethoxazole	10% b	0.1 b	*E.fetida*	LC_{50} > 4000 c
Tylosin	66.3% d	N/A	*E.fetida*	149 d

Footnote: FDA, (2013) a, Zheng et al., 2021 b, Pino et al., 2015 c, Anses, (2018) d.

Guideline: ***a*** *– Nitrogen transformation OECD 216 (OECD, 2004), earthworm reproduction OECD 222 (OECD, 2000);* ***b*** *nitrogen transformation in accordance with OECD 216 (OECD, 2004), earthworm reproduction did not follow any OECD compliance.*

Table 6.4: Risk quotient for florfenicol and tylosin using the collated terrestrial effect data.

Substance	**PEC $_{soil\ initial}$ (µg/kg)**	**NOEC (µg/kg)**	**PNEC (µg/kg) (NOEC/10)**	**Risk quotient**
Florfenicol	294.67	1560	156	1.88
Sulfamethoxazole	471.35	N/A	N/A	N/A
Tylosin	1186.67	149000	14900	0.079

Footnote: *A assessment factor of 10 was applied to the NOEC to generate a PNEC.*

6.3.2 Risks to Plants

Presented below is the obtained ecotoxicological effect data of florfenicol, sulfamethoxazole, and tylosin towards plants (Table 6.5) The most sensitive species was selected for the calculation of the RQ, for this assessment the effect concentration that caused a 50% effect towards the population (EC_{50}) was utilized. The most sensitive species were *L.sativum*, *A.cepa*, and *O.sativa* for florfenicol, tylosin and sulfamethoxazole respectively (FDA, 2013; Richter et al., 2016; Liu et al., 2009). It is apparent that florfenicol is more toxic towards a range of plants than tylosin and sulfamethoxazole however all RQ assessments resulted in extreme risk when comparisons were made to the $PEC_{soil\ initial}$ (Table 6.6). This indicates that should 100% of the administered active ingredients be excreted and applied to land a negative affect towards plant species is to be expected.

Table 6.5: Florfenicol and tylosin ecotoxicological endpoints for terrestrial plants.

Substance	Species	Endpoint	EC_{50} (mg/kg d/w)	Reference
Florfenicol	*L.Sativum* (Cress)	Biomass	0.5	FDA, (2013)
	B.nigra (Mustard)		1.7	FDA, (2013)
	Triticum (Wheat)		6.7	FDA, (2013)
	B.oleracea (Cabbage)		0.85	FDA, (2013)
Sulfamethoxazole	*A.Sativa* (Oat)	Germination	69	Liu et al., (2009)
	Oryza sativa (rice)	Root length	13	Liu et al., (2009)
	Cucumis sativus (cucumber)	Root length	>300	Liu et al., (2009)
Tylosin	*A.cepa* (Onion)	Biomass	41.3	Richter et al., (2016)
	A.Sativa (Oat)		603	Richter et al., (2016)
	B.napus (Rapeseed)		61.9	Richter et al., (2016)

S. lycopersicum (Tomato)	74	Richter et al., (2016)
P.Vulgaris (Bean)	107	Richter et al., (2016)

Footnote: FDA, (2013) plant tests were in accordance with OECD 208 (OECD, 2006). Liu et al., (2009) plant tests followed OECD 208 (OECD, 1984).

Table 6.6: Risk quotient for florfenicol and tylosin using the collated terrestrial effect data.

Substance	**PEC $_{soil\ initial}$ (µg/kg)**	**EC_{50} (µg/kg)**	**PNEC (µg/kg) (EC50/100)**	**Risk quotient**
Florfenicol	294.67	500	5	**58.93**
Sulfamethoxazole	471.5	13000	130	**3.62**
Tylosin	1186.67	4130	41.3	**28.73**

Footnote: *A assessment factor of 10 was applied to the NOEC to generate a PNEC. Values in **bold** indicate a risk.*

6.2.2 Aquatic

6.2.3 Primary producers

Presented below is the available literature regarding the ecotoxicological effect of the assessed antibiotics towards aquatic organisms (Table 6.7). Within aquatic effect assessments cyanobacteria are considered the most sensitive taxa, which is not surprising given their bioactive mode of action (EMA, 2018). Therefore, to consider their 'worst-case' effect comparisons are made towards cyanobacteria. As can be seen within Table 6.8 *Anabaena flos-aquae*, *R.subcapitata* and *Microcystis aeruginosa* were the most sensitive towards florfenicol, sulfamethoxazole and tylosin respectively. Table 6.9 demonstrates that should the assessed antibiotics be present within surface waters at the PEC $_{surface\ water\ initial}$ then a substantial effect towards cyanobacteria will occur.

6.2.4 Aquatic invertebrates

Routine practice is to then assess the risk towards *Daphnia magna* as these are typically the most sensitive aquatic invertebrate and their presence within the environment is

critical as they play a fundamental role within the aquatic food chain (Tkaczyk et al., 2021). The risk quotient for florfenicol demonstrated a risk towards *Daphnia magna,* whereas for sulfamethoxazole and tylosin no risk was identified (Table 6.10). There was no available literature regarding the toxicological effect of tylosin or sulfamethoxazole to fish, whereas for florfenicol there was (FDA, 2013). The calculated risk quotient demonstrates that florfenicol would present a high risk towards *Oncorhynchus mykiss* following exposure to the aquatic environment (Table.11).

Table 6.7: Collated aquatic ecotoxicological data for florfenicol and tylosin.

Substance	**Species**	**Toxicity Value EC_{50} (mg/L)**	**Reference**
Florfenicol	*Oncorhynchus mykiss*	<780	FDA, (2013)
	Lepomis marochirus	>830	FDA, (2013)
	Daphnia magna (reproduction)	7.6	Martins et al., (2013)
	Navicula pelliculosa	61	FDA, (2013)
	Pseudokirchneriella subcapitata	1	FDA, (2013)
	Lemina gibba	0.76	FDA, (2013)
	Anabaena flos-aquae	0.23	FDA, (2013)
Sulfamethoxazole	*R. subcapitata*	0.49	Zhang et al., (2021)
	V.fischeri	>100	Osorio et al., (2016)
	Daphnia magna	75.3	Osorio et al., (2016)
	Lemna gibba	0.81	Grenni et al., (2019)
Tylosin	*Daphnia magna* (reproduction)	680	Wollenberger et al., (2000)
	Lemina gibba (growth)	7.25	Brain et al., (2005)
	Microcystis aeruginosa (growth)	0.034	Halling-Sørensen (2000)

Footnote: All studies used in risk quotient derivation followed the appropriate OECD guidelines such as ISO, (1989) protocol and OECD 211 (OECD, 2012).

Table 6.8: Risk quotient for florfenicol and tylosin towards primary producers (cyanobacteria).

Substance	PEC $_{surface\ water\ initial}$ (µg/L)	EC_{50} (µg/L)	PNEC (µg/L) (EC_{50}/100)	Risk quotient
Florfenicol	55.5	230	2.3	**24.13**
Sulfamethoxazole	9.87	490	4.9	**2.01**
Tylosin	2.65	34	0.34	**7.79**

Footnote: *A assessment factor of 100 was applied to the EC_{50} to generate a PNEC.*

Table 6.9: Risk quotient for florfenicol and tylosin towards *Daphnia magna*.

Substance	PEC $_{surface\ water\ initial}$ (µg/L)	EC_{50} (µg/L)	PNEC (µg/L) (EC_{50}/1000)	Risk quotient
Florfenicol	55.5	7,600	7.6	**7.30**
Sulfamethoxazole	9.87	75,300	75.3	0.13
Tylosin	2.65	680,000	680	0.003

Footnote: *A assessment factor of 100 was applied to the EC_{50} to generate a PNEC.*

Table 6.10: Risk quotient calculation for florfenicol to Oncorhynchus mykiss.

Substance	PEC $_{surface\ water\ initial}$ (µg/L)	EC_{50} (µg/L)	PNEC (µg/L) (EC_{50}/1000)	Risk quotient
Florfenicol	55.5	780,000	780	0.071

Footnote: *A assessment factor of 1000 was applied to the EC_{50} to generate a PNEC.*

6.2.3 Groundwater

In order to assess the risks of florfenicol and tylosin towards groundwater organisms an assessment of risk was conducted which first investigated the PEC $_{groundwater\ initial}$ (Table 6.11). This involved using the calculated PNEC for cyanobacteria; for which a further AF of 10 was applied. For groundwater initial (Step 1) the generated RQ's were elevated for all antibiotics indicating a risk towards groundwaters.

Table 6.11: Groundwater step 1 assessment towards cyanobacteria the most sensitive species for the selected substances.

Substance	PEC $_{groundwater\ initial}$ (µg/L)	PNEC (µg/L)	RQ
Florfenicol	166.65	0.23	**7.24×10^5**
Sulfamethoxazole	29.61	0.49	**60.42**
Tylosin	7.94	0.034	**2.3×10^5**

Footnote: *PNEC generated from cyanobacteria PNEC $_{surface\ water}$ /10.*

6.2.4 Phase II Tier B

6.2.4 Terrestrial

Should a risk be identified then a tier B study is routinely conducted which considers environmentally relevant processes such as, excretion, dissipation, adsorption, manure degradation, and soil degradation. For the purpose of this exercise the data derived in chapters (1 and 2) were utilized to refine the PEC $_{soil\ initial}$. The purpose of doing this was to assess whether the differing environmentally relevant manure treatments (pH's and redox potentials) would outline differences in risk following the exposure to land.

For the purpose of this equation's 1 and 2 was used to refine the PEC $_{soil\ initial}$, this involved using the calculated DT_{50} values (Table 6.12). As can be seen from the dataset the differing rates of degradation in varying treatments resulted in varying rates of risks towards the most sensitive terrestrial plant . As a result of reduced degradation florfenicol and tylosin were identified to be a risk towards *B.napus* and *T.pratense* when pig slurries are acidic (pH 5.5). Comparatively sulfamethoxazole was identified to a risk towards *Oryza sativa* when pig slurries have a pH of 8.5, this was a result of the inhibited degradation under alkaline conditions.

Equation 1: $$Mt = Mi \times e\left(\frac{(-\ln(2)\times\left(\frac{Tst}{2}\right))}{DT50}\right)$$

Equation 2: $$\text{PEC}_{\text{soil refined}} = \frac{Mt\times 170}{1500\times 10000\times 0.05\times Ns}$$

Where: Mt = mass of active in manure/slurry after the mean storage time (mg), Mi = Mass of active in manure/slurry (mg) (PEC manure initial), Tst = length of time manure is stored (days), DT_{50} = half-life of active in manure (days), 170 = EU nitrogen spreading limit, 1500 = bulk density of dry soil (kg/m^3), 10,000 = area of 1 hectare (m^2/ha), Ns = nitrogen produced during storage time (kg/N).

Table 6.12: Terrestrial risk quotient derivation using manure degradation data derived in Chapters 2 and 3 for florfenicol, sulfamethoxazole tylosin at differing pH's and redox conditions.

Substance	pH/redox potential	DT_{50}	PEC $_{\text{soil refined}}$ (µg/kg)	Most sensitive plant species	EC_{10} µg/kg	PNEC (µg/kg) (EC_{50}/100)	Risk quotient
Florfenicol	8.5	0.08d	2.18 x 10^{-93}	*B.napus* (biomass)	5000	50	2.135x10^{-95}
	7	0.08d	2.18 x 10^{-93}				2.135x10^{-95}
	5.5	8.7d	141.11				**2.82**
	-100 mV	2.07d	0.005				0.0001
	-250 mV	1.23d	1.2 x 10^{-5}				2.39 x10^{-7}
	-400 mV	0.92d	8.5 x 10^{-8}				1.7 x 10^{-5}
	8.5	0.14d	1.89 x 10^{-50}				1.41x10^{-26}
	7	1.47d	0.017				0.022
	5.5	9.75d	707.16				**918.38**

Tylosin	-100 mV	0.28d	1.09 x 10^{-26}	*T.pratense* (biomass)	7700	77	8.98 x 10^{-27}
	-250 mV	0.39d	1.45 x 10^{-18}				6.26 x 10^{-19}
	-400 mV	0.81d	1.9 x 10^{-8}				8.17 x 10^{-9}
Sulfamethoxazole	8.5	5.11	51.36	*Oryza sativa* (root length)			**2.57**
	7	1.31	0.0016				0.00008
	5.5	0.85	9.46 x 10^{-7}				4.73 x 10^{-8}
	-100 mV	1.48	17.10		2000	20	0.85
	-250 mV	3.92	7.78				0.39
	-400 mV	3.35	0.0073				0.000365

Footnote: *A assessment factor of 100 was applied to the EC_{50} to generate a PNEC. Ecotox data derived from Richter et al., (2016).*

6.3.5 Aquatic

The $PEC_{groundwater\ refined}$ was calculated using the calculated $PEC_{soil\ refined}$ to generate the application rate (kg/ha) which was then used as an input into the modelling software PEARL. This provides a more accurate assessment of the concentrations likely to be detected within groundwaters following the application of manures to land. Such an assessment includes additional parameters not utilized within step 1, these values include the manure degradation data that was derived under differing pH and redox conditions (DT_{50} values generated during Chapter's 1 and 2), soil degradation (DT_{50}) and adsorption coefficients (K_{oc} and K_f). In order to conduct this assessment, the DT_{50} values were applied to equation's 1 and 2 to derive the $PEC_{soil\ refined}$, this value was then converted into application rate (kg/ha) using equation 3. The application rate was then used as the input value within FOCUS_PEARL in order to generate the $PEC_{groundwater\ refined}$.

Equation 3: Application rate (kg/ha) = $(PEC_{soil\,refined}\ (\mu g/kg) \times soil\ depth\ (m) \times bulk\ density\ (kg/m^3))\ /10000$

Within step 2 tylosin was not estimated to be leachable into groundwater and therefore no comparisons could be made. For florfenicol a risk was identified when using the acidic manure DT_{50}, a RQ of 144.3 was calculated indicating a considerable risk (Table 6.13). Conversely sulfamethoxazole was identified to be a risk towards cyanobacteria when manures are stored under alkaline conditions. In addition, anaerobic to reduced redox conditions (-100 to -250 mV) were identified to have a groundwater risk.

Table 6.13: Groundwater step 2 a refined assessment of the effect of florfenicol towards cyanobacteria utilizing the modelling suite FOCUS_PEARL to provide a more accurate estimation of the concentrations likely to be leached to groundwater following the degradation in pig slurries at differing pH's and redox potentials.

Substance	Manure degradation treatment	PEC $_{groundwater\ refined}$ (µg/L)	PNEC (µg/L)	RQ
Florfenicol	pH 8.5	0	0.23	0
	pH 7	0		0
	pH 5.5	33.19		**144.3**
	-100 mV	0.001851		0.008
	-250 mV	0.000005		2.17×10^{-5}
	-400 mv	0		0
Sulfamethoxazole	pH 8.5	6.05	0.49	**12.34**
	pH 7	0.000192		3.92×10^{-4}
	pH 5.5	0		0
	-100 mV	2.02		**4.12**
	-250 mV	0.918		**1.87**
	-400 mv	0.00085		0.0017

Footnote: *PNEC generated from cyanobacteria PNEC $_{surface\ water}$ /10.* **Bold = risk (RQ <1).**

6.3 Discussion

As expected various risks were identified within Phase I of the risk assessment. This screening exercise investigated the risks with a 'total residue' approach assuming the worst case scenario in that 100% of the administered dose was excreted and applied to land (Spaepen, 1997; VICH, 2000; VICH, 2002). In conducting this assessment it was clear that florfenicol was the most toxic of the assessed analytes. Risks were identified in both the terrestrial and aquatic compartments, for example *E.fetida, L.satvium, Daphnia magna,* and cyanobacteria species had RQ values > 1 for the Amphen product (FDA, 2013). Sulfamethoxazole was also identified to have risks in both the aquatic compartment and the terrestrial with effects being noted towards cyanobacteria and *Oryza sativa* (Liu et al., 2009; Zhang et al., 2021). Similarly risks were observed for cyanobacteria and the most sensitive terrestrial plant for tylosin (*B.napus*) (Halling-Sørensen, 2000; Richter et al., 2016).

The presented risk assessment demonstrates all of the veterinary medicines used in this exercise will have moved into Phase II of the risk assessment (VICH, 2000). The derived manure degradation data that was used in Chapters 2 and 3 was used to calculate a $PEC_{soil\ refined}$ which was then used for further assessments of risk (sections 6.2.4 to 6.3.5). Here it is clear that manure properties such as pH and redox potential could ultimately drive differing outcomes within the risk assessment. For example, florfenicol and tylosin were reported to be a risk towards *B.napus* and *T.pratense* when slurries are stored under acidic conditions (pH 5.5) (FDA, 2013; Richter et al., 2016). Conversely sulfamethoxazole was revealed to present a risk towards *Oryza sativa* when pig slurries are alkaline (pH 8.5). This is an interesting outcome in that manure processing such as acidification would ultimately increase the risk of florfenicol and tylosin in the environment but not sulfamethoxazole. Sulfamethoxazole was identified to be a risk towards rice under alkaline conditions, which is a commonly reported pH within the scientific literature (Weinfurtner. 2011; Joubin 2018). This indicates that differences in identified risk can occur at pH values routinely reported in the scientific literature. Interestingly no differences in risk towards terrestrial plants was observed under differing anaerobic redox potentials.

As a result of varied degradation rates in differing manure properties differences in the groundwater leaching assessment were observed. Typically, florfenicol's presence in groundwater would not be expected, this is a result of the compounds instability (Chapters 2 and 3) (FDA, 2013). However, as a result of inhibited degradation in acidic conditions the groundwater concentration was estimated to be 33.9 µg/L and a risk towards the most sensitive taxa, *Anabaena flos-aquae* (cyanobacteria) was identified. Although a significant difference was observed between the degradation rates of florfenicol at varying anaerobic redox potentials no difference in risk was identified within the risk assessment. For tylosin no difference in risk was identified towards groundwater, this was a result of its high dissociation constant which indicates that it would be immobile.

Based on the presented risk assessment for florfenicol, sulfamethoxazole, and tylosin it is clear that manure degradation assessments should be conducted on numerous manures with differing properties. Otherwise the risk assessment could result in the overestimation of concentrations applied to land and inaccurately assess their risk towards non-target terrestrial and aquatic organisms (Ingerslav et al., 2003; Kemper et al., 2008; Heuer et al., 2011; and Shao et al., 2018).

Agricultural techniques are constantly evolving which creates a knowledge gap regarding our understanding of how these processes effect the environmental fate of veterinary medicines. Chapter 4 attempted to bridge this knowledge gap in terms of how manure application method can effect veterinary medicine losses to water. The study clearly demonstrates that conventional slurry broadcasting prior to an irrigation event results in the greatest losses of veterinary medicines to water and therefore creates the greatest risk to aquatic species. Typically farmers will incorporate slurries after broadcasting which was identified to be in the best practice in terms of reducing veterinary medicine mass loads in water, although timely incoropration does not always occur. For example, Smith et al., (2016) completed a survey of UK farms and

demonstrated that 80% of pig farms broadcasted their slurries and only 30% were incorporated within 1-7 days and 20% within > 1 week. Moreover the author outlined 20% of slurries were not incorporated, although it was not clear whether this was related to the reduced requirement to do so when using advanced injection technologies (CTIC. 2004; Maguire et al., 2011; and Niles et al., 2019). It is therefore evident that there is currently a risk of greater expossure to receiving waters surrounding areas of intensive agriculture using broadcast alone. Should we wish to achieve good surface water quality and protect aquatic species considerations towards this phenomenon is needed (European Commission 2000/60/EC).

Advanced injection methods have been identified to reduce the transport of veterinary medicines to receiving waters over that of broadcasting. Although this practice was not found to be as efficient as incorporating slurries. However, the benefits of injecting slurries for soil health potentially outweigh the efficiency of incorporating for retaining veterinary medicines in soils. For example, regenerative farming approaches have identified that routinely incorporating slurries via ploughing can impair soil health and functioning via compaction below 20 cm depth which impairs root functioing and the soils hydrology as well as disrpution to terrstrial organisms (Hetesi, 2019). De Oliveira et al., (2012) demonstrated that ploughing impaired earthworm populations (*A. caliginosa* and *A. rosea*) by up to 80% which was also found to persist throughout the studies duration (2 years).

Manure application techniques evidently have a significant effect on the transport of veterinary medicines to waters. Therefore these techniques need to be considered within the risk assessment for veterinary medicines, specifically within the modelling approaches such as SWASH and FOCUS_PEARL (VICH, 2000). Currently these models assume slurries to be immediately incorporated into soils, which often is not the case. Moreover, no consideration is given towards advanced applications in these models, consequently these modelling approaches could be resulting in a underestimation of the concentrations that are transported to water bodies. Interestingly this study outlined injecting slurries did not increase leaching over that of broadcast,

however more assessments are required in order to make validate this (Rotz et al., 2011; and Fangueiro et al., 2015). The soil type used in this study was a silty clay loam which was found to cap when replicating injection methodologies, this may not be the case for other soil types such as sandy soils and therefore this risk is not outlined.

Chapter 5 demonstrated the ability of modern animal husbandry with good veterinary practices to reduce the exposure of the environment to veterinary medicines. Spen farm is a smart farm which is adjusting husbandry practices to improve the well being of the pigs but also reduce the requirement of veterinary medicines whilst maintaining a high production rate. Antibiotic compounds were detected within the pig slurries that was sampled from the closed storage silo. The concentrations of these antibiotics were observed to fluctuate (increase and decrease), although no clear relationship could be achieved between concentration and degradation. This is a result of potential dilution from dirty water used to wash the rearing facilities or even antibiotic free excrement as well as differences in sampling and the homogenerity of the slurry. Interestingly very little veterinary medicines used at the farm were detected within the soils surrounding the farm. Field 4 was identified to have oxytetracycline, sulfadiazine, and lincomycin, however these were found to dissipate quickly (1 month). This could be related to the shallow sampling method that was used (10 cm) where soils are incorporated to 20 cm or alternatively due to fate processes such as degradation, long-term adsorption or to a lesser extent leaching/plant uptake.

Interestingly sulfadiazine, lincomycin, and trimethoprim antibiotics were detected within groundwater below the farm itself. These antibiotics have a history of use at the farm as well as being detected within the pig slurry during the studies duration. Whilst there is potential these detections arose from the farms usage, there was no clear relationship seen within the soil sampling. Therefore, their origin is currently unknown given that there are numerous farms within the area which also could have contributed. These detections were made at depths of 40 m, which indicates their input is dated. It was interesting to see that these antibiotics persisted within groundwater for the duration of the study. It is clear from the presented research that ecotoxicological

assessments with regards to groundwater need to be aimed at chronic exposures at concentrations around the ng/L range (O'Dwyer et al., 2017). Moreover, it is at these lower concentrations where a selective pressure is exerted on microbes forming antibiotic resistance. For which the risk assessment is not concerned, this generally needs to change and assessments need to be made regarding ARG formation and occurrence in the environment and thus food/water.

The FOCUS_PEARL modelling predictions were found to surpass those of the measured concentrations for lincomycin and trimethoprim, although this was not the case for sulfadiazine. This is likely attributed towards the reliable degradation and adsorption data (radiolabelled) which was conducted on a similar soil type to that of the farm (Lamshöft et al., 2010). This phenomenon reiterates the points raised in Chapters 2 and 3 where degradation data needs to be thorough accurate and assessed over a range of properties. The FOCUS_PEARL modelling suite is routinely used within the risk assessment although does not consider numerous inputs from neighbouring farms. The consequence of this is potentially underestimating the concentrations present within the environment, although this trend was not observed in Chapter 6 it is a potential for other regions where husbandry is more intense and veterinary usage is higher.

6.6 Conclusions

In conclusion, the presented research within this thesis demonstrates that realistic environmental scenarios and processes can alter the fate of veterinary medicines in the environment. Pig slurry properties such as pH and redox have been identified to be important parameters in determining the fate of veterinary medicines during on farm storage. In order to accurately assess the concentrations that would be applied to land following on farm storage, it is critical to consider numerous manures with differing properties to be representative of farms as a whole. Currently this is not the case and is likely contributing towards the variability that is seen between studies and therefore potentially resulting in inaccurate exposure assessments. Moreover, the acidification and aeration of slurries is becoming increasingly popular and until now the influence this has on veterinary antibiotic degradation was unknown. For the majority of the

assessed analytes acidic slurries inhibited the degradation rate (florfenicol, tylosin and to a lesser extent oxytetracycline), therefore such practices are likely to increase the exposure of veterinary medicines in the environment and increase the risk. The influence of manure processing techniques on contaminant fate needs researching prior to application in the real-world, such processing should also be considered within the environmental risk assessment.

Following storage animal manures are typically applied to land, the presented research demonstrated the capability for veterinary medicines to be transported to waters in both a semi-field scenario and field scale. This research demonstrated the capability of modern application methods to enhance the retention of veterinary medicines to soils and therefore reduce their transport to waters. Therefore, to protect water sources advanced applications (injections) or incorporating immediately is advised as broadcasting alone exhibits a greater risk of exposure. It is essential to reduce the exposure of waters to veterinary medicines considering the toxic effect they have towards aquatic organisms and the formation of antimicrobial resistance. Moreover, antibiotics have been demonstrated to persist and accumulate within groundwaters following the repeated application of animal manures to land. This further exacerbates the environmental and societal concerns with these contaminants. Although veterinary medicines were detected in varying environmental compartments at the farm, they were identified to be substantially lower than that of other publications. This is thought to be a result of the farm utilizing good veterinary and husbandry practices which have reduced the burden of veterinary medicines in the environment. Future farm developments should adopt these innovative methods of animal husbandry in order to protect the environment but also improve the ethics of modern husbandry practices.

6.7 Recommendations for future research

The presented experimental chapters have produced novel and significant findings regarding the fate of veterinary medicines in the environment. During this research it was apparent that further research is required to address additional questions. Therefore, future work should consider the following:

- In Chapter 1 the influence of pig slurry pH on veterinary antibiotic degradation was investigated. This was found to have a compound specific effect which was driven by both biotic and abiotic processes. Therefore, it is crucial to determine whether similar effects are exhibited within other animal manures such as dairy and poultry litter. This information would be essential in order to fully understand the extent in which pH can drive changes within the risk assessment and the environment.
- Chapter 2 explored the effect of pig slurry redox potentials on veterinary medicine degradation. It would be beneficial to further the understanding of this through abiotic assessments such as sterile manure degradation trials and hydrolysis assessments. Moreover, future investigations could investigate whether anaerobic redox potentials alter the rate or route of transformation products.
- Currently there is little literature investigating the combined effect of redox potential and pH on the degradation of veterinary medicines. Ali et al., (2011) completed a study where this was assessed although realistic redox potentials (anaerobic) and more chemicals require assessing.
- For the recommended future research regarding veterinary medicine degradation in animal wastes, it would be beneficial to conduct future assessments using radiolabelled compounds. This would have several benefits and would provide insight into, degradation kinetics of metabolites, quantification of metabolite concentrations (known and unknown), definitive quantification of the amounts mineralized, and the quantitation of the percentage lost to sorption.
- Chapter 4 investigated the influence of manure application method on veterinary medicine losses to waters. In conducting this assessment, it was apparent that future research is required to fully understand the processes occurring within the environment. It would be beneficial for future assessments to incorporate numerous soils of differing textures (i.e., sandy soils, sandy silt loams, loams and heavy clays). Whilst the research was comprehensive what it was lacking was a detailed understanding of how these methods may differ in different geographical locations.

- Currently surface water runoff model predictions (FOCUS_SWASH) do not consider advanced manure application techniques. Future studies should investigate whether some parameters within the model (such as depth) can be altered to better predict surface water runoff concentrations.
- Chapter 5 contained a field monitoring study to investigate the fate of veterinary medicines at a specific field site. It would be beneficial to further this understanding with more field trials and comparisons to modelling predictions. This would provide a more robust answer to whether modelling predictions software's are comprehensive enough to be used within the risk assessment.
- Further investigations of veterinary medicine transport to groundwater should incorporate the analysis and quantification of breakdown products in groundwaters. Typically, there is very little literature regarding the presence of transformation products in groundwaters, a quantification of which will ultimately improve our understanding of the risk of veterinary antibiotics in groundwaters.

6.8 Generic advice for future studies

- Phase I and II metabolites are not considered in fate assessments, this is a precursor of laboratory assessments spiking the parent compound. This practice eliminates the potential assessment of Phase I or II metabolites which would have formed within the gastrointestinal tract of the animal (i.e., conjugates).
- It would also be beneficial for future studies to investigate whether other manure properties are of importance in regards the degradation of veterinary medicines.
- Currently there is very little information regarding the fate and effects of veterinary medicines in mixtures. These assessments are unrepresentative of reality in that these compounds will exist with other veterinary medicines, but also other agricultural contaminants such as biocides, pesticides, and herbicides. Therefore, future assessments should consider this if we wish to fully understand their fate and effects within the environment.
- Research should be more concerned with veterinary medicine metabolites and their fare in the environment but also their effect.

- Currently ecotoxicological effects studies are only conducted over a short duration and future research should be focussed on lower concentrations over longer timeframes but also looking at more sensitive endpoints.
- There is very little known regarding the concentrations that can form antimicrobial resistance within the environment, therefore future research should be concerned in addressing this so that we can fully understand the extent of the issue.

References

Ali, M., Wang, J.J., DeLaune, R.D., Seo, D.C., Dodla, S.K. and Hernandez, A.B., 2013. Effect of redox potential and pH status on degradation and adsorption behavior of tylosin in dairy lagoon sediment suspension. *Chemosphere*, *91*(11), pp.1583-1589.

Anses, 2018. Publicly available assessment report for a veterinary medicinal product tylosin. French Agency for Veterinary Medicinal Products Available at: https://www.vmd.defra.gov.uk/productinformationdatabase/files/UKPAR_Documents/UKPAR_1989958.PDF>.

Berendsen, B.J.A., Lahr, J., Nibbeling, C., Jansen, L.J.M., Bongers, I.E.A., Wipfler, E.L. and Van de Schans, M.G.M., 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. *Chemosphere*, 204, pp.267-276.

Bilal, M., Mehmood, S., Rasheed, T. and Iqbal, H.M., 2020. Antibiotics traces in the aquatic environment: persistence and adverse environmental impact. *Current opinion in environmental science & health*, *13*, pp.68-74.

Boguta, P. and Sokołowska, Z., 2020. Zinc binding to fulvic acids: Assessing the impact of pH, metal concentrations and chemical properties of fulvic acids on the mechanism and stability of formed soluble complexes. *Molecules*, *25*(6), p.1297.

Brain, R.A., Bestari, K.J., Sanderson, H., Hanson, M.L., Wilson, C.J., Johnson, D.J., Sibley, P.K. and Solomon, K.R., 2005. Aquatic microcosm assessment of the effects of tylosin on Lemna gibba and Myriophyllum spicatum. *Environmental Pollution*, 133(3), pp.389-401.

Busari, M.A., Kukal, S.S., Kaur, A., Bhatt, R. and Dulazi, A.A., 2015. Conservation tillage impacts on soil, crop and the environment. *International soil and water conservation research*, 3(2), pp.119-129.

Canh, T.T., Aarnink, A.J.A., Verstegen, M.W.A. and Schrama, J.W., 1998a. Influence of dietary factors on the pH and ammonia emission of slurry from growing-finishing pigs. *Journal of Animal Science*, *76*(4), pp.1123-1130.

Canh, T.T., Sutton, A.L., Aarnink, A.J.A., Verstegen, M.W.A., Schrama, J.W. and Bakker, G.C.M., 1998b. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. *Journal of Animal Science*, *76*(7), pp.1887-1895.

Carvalho, I.T. and Santos, L., 2016. Antibiotics in the aquatic environments: a review of the European scenario. *Environment International*, 94, pp.736-757.

Chemspider.com. 2022. Florfenicol | ChemSpider. [online] Available at: <http://www.chemspider.com/Chemical-Structure.102776.html> [Accessed 17 January 2022].

Chemspider.com. 2022. Tylosin | ChemSpider. [online] Available at: < http://www.chemspider.com/Chemical-Structure.4444097.html>.

Chen, K.L., Liu, L.C. and Chen, W.R., 2017. Adsorption of sulfamethoxazole and sulfapyridine antibiotics in high organic content soils. *Environmental Pollution*, 231, pp.1163-1171.

CTIC. 2004. National crop residue management survey. Available at http://www.ctic.purdue.edu/CRM/ (verified edition 26 Sept. 2010). Conservation Technology. Information Cent., West Lafayette, IN.

DEFRA, 2002. Extreme Rainfall and Flood Event Recognition. R&D Technical Report FD2201. [online] Available at: <http://file:///C:/Users/jnigh/AppData/Local/Temp/FD2201_708_TRP.pdf> [Accessed 27 September 2021].

ECD, 1984. Terrestrial Plants: Growth Test, OECD Guidance for Testing of Chemicals, NO. 208. OECD, Paris.

EMA (2016) Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 and GL38, EMA/EMA/ERA/418282/2005-Rev.1- Corr.1, Committee for Medicinal Products for Veterinary Use, European Medicines Agency, London, United Kingdom.

EMA (2018) Questions and Answers: Implementation of EMA Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 (Phase I) and GL38 (Phase II), Committee for Medicinal Products for Veterinary Use,EMEA/EMA/ERA/172074/2008-Rev.6, London, United Kingdom.

EMA, (2016). Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38. EMA/CVMP/ERA/418282/2005-Rev.1- Corr.1.

EMA, n.d. Draft for an OECD guidelines for the testing of chemicals. Anaerobic Transformation in Liquid Manure. [online] Available at: <https://www.oecd.org/env/ehs/testing/draft-test-guideline-anaerobic-transformation-liquid-manure.pdf> [Accessed 11 March 2022].

EMEA, 1997, Discussion Paper on Environmental Risk Assessment for Veterinary Medicinal Products Other Than GMO-Containing and Immunological Products. EMEA, London, UK.

EUROPEAN COMMISSION (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Official Journal 22 December 2000 L 327/1. Brussels: European Commission.

Fangueiro, D., Hjorth, M. and Gioelli, F., 2015. Acidification of animal slurry–a review. Journal of Environmental Management, 149, pp.46-56.

FDA, 2013. Environmental Assessment for Aquaflor (Florfenicol). Aquaflor for Freshwater-reared Finfish in Recirculating Aquaculture Systems. [online] EPA. Available at: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/113.

FOCUS (2001). FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC, Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2.

FOCUS (2009) Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU, Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 1, 604 pp.

Frey, S.K., Topp, E., Khan, I.U., Ball, B.R., Edwards, M., Gottschall, N., Sunohara, M. and Lapen, D.R., 2015. Quantitative Campylobacter spp., antibiotic resistance genes, and veterinary antibiotics in surface and ground water following manure application: influence of tile drainage control. *Science of the Total Environment*, *532*, pp.138-153.

Grenni, P., Patrolecco, L., Rauseo, J., Spataro, F., Di Lenola, M., Aimola, G., Zacchini, M., Pietrini, F., Di Baccio, D., Stanton, I.C. and Gaze, W.H., 2019. Sulfamethoxazole persistence in a river water ecosystem and its effects on the natural microbial community and Lemna minor plant. *Microchemical Journal*, *149*, p.103999.

Halling-Sørensen, B., 2000. Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere*, 40(7), pp.731-739.

Halling-Sørensen, B.N.N.S., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Lützhøft, H.H. and Jørgensen, S.E., 1998. Occurrence, fate and effects of pharmaceutical substances in the environment-A review. *Chemosphere*, 36(2), pp.357-393.

Heuer, H., Kopmann, C., Zimmerling, U., Krögerrecklenfort, E., Kleineidamm, K., Schloter, M., Top, E.M. and Smalla, K., 2011. Effect of veterinary medicines introduced via manure into soil on the abundance and diversity of antibiotic resistance genes and their transferability. *Antimicrobial Resistance in the Environment*. Hoboken, NJ, USA: John Wiley & Sons, Inc, pp.453-63.

Heuer, H., Kopmann, C., Zimmerling, U., Krögerrecklenfort, E., Kleineidamm, K., Schloter, M., Top, E.M. and Smalla, K., 2011. Effect of veterinary medicines introduced via manure into soil on the abundance and diversity of antibiotic resistance

genes and their transferability. Antimicrobial Resistance in the Environment. Hoboken, NJ, USA: John Wiley & Sons, Inc, pp.453-63.

Hetesi, Z., 2019. Systematic and Simple Cost-Benefit Investigation of Some Advantages of the Regenerative Agriculture. *Regional and Business Studies*, 11(1), pp.7-11.

Hjorth, M., Pedersen, C.Ø. and Feilberg, A., 2012. Redox potential as a means to control the treatment of slurry to lower H2S emissions. *Sensors*, *12*(5), pp.5349-5362.

Hu, S., Zhang, Y., Shen, G., Zhang, H., Yuan, Z. and Zhang, W., 2019. Adsorption/desorption behavior and mechanisms of sulfadiazine and sulfamethoxazole in agricultural soil systems. *Soil and Tillage Research*, *186*, pp.233-241.

Ingerslev, F., Vaclavik, E. and Halling-Sørensen, B., 2003. Pharmaceuticals and personal care products-A source of endocrine disruption in the environment?. *Pure and Applied Chemistry*, 75(11-12), pp.1881-1893.

Ingerslev, F., Vaclavik, E. and Halling-Sørensen, B., 2003. Pharmaceuticals and personal care products-A source of endocrine disruption in the environment?. *Pure and Applied Chemistry*, 75(11-12), pp.1881-1893.

ISO 8692, 1989. Water quality fresh water algal growth inhibition test with Scenedesmus subspicatus and Selenastrum Capricornutum. International Organization of Standardization, Geneva, Switzerland.

Jia, Y., Khanal, S.K., Zhang, H., Chen, G.H. and Lu, H., 2017. Sulfamethoxazole degradation in anaerobic sulfate-reducing bacteria sludge system. *Water research*, *119*, pp.12-20

Joubin, M., 2018. Animal slurry acidification: effects of slurry characteristics use of different acids, slurry pH buffering. *Uppsala, Sweden: RISE*, *40*.

Joy, S.R., Li, X., Snow, D.D., Gilley, J.E., Woodbury, B. and Bartelt-Hunt, S.L., 2014. Fate of antimicrobials and antimicrobial resistance genes in simulated swine manure storage. *Science of the Total Environment*, 481, pp.69-74.

Junker, T., Atorf, C., Berkner, S., Düring, R.A., Hennecke, D., Herrchen, M., Konradi, S., Merrettig-Bruns, U., Römbke, J., Wagner, J. and Weinfurtner, K., 2020. Development of a test method for transformation of veterinary pharmaceuticals and biocides in anaerobic liquid manure. *Environmental Sciences Europe*, 32(1), pp.1-21.

Kampouris, I.D., Alygizakis, N., Klümper, U., Agrawal, S., Lackner, S., Cacace, D., Kunze, S., Thomaidis, N.S., Slobdonik, J. and Berendonk, T.U., 2022. Elevated levels of antibiotic resistance in groundwater during treated wastewater irrigation associated with infiltration and accumulation of antibiotic residues. *Journal of Hazardous Materials*, *423*, p.127155.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological indicators*, 8(1), pp.1-13.

Knäbel, A., Bundschuh, M., Kreuzig, R. and Schulz, R., 2016. Runoff of veterinary pharmaceuticals from arable and grassland—a comparison between predictions from model simulations and experimental studies. *Agriculture, Ecosystems & Environment*, 218, pp.33-39.

Kolz, A.C., Moorman, T.B., Ong, S.K., Scoggin, K.D. and Douglass, E.A., 2005. Degradation and metabolite production of tylosin in anaerobic and aerobic swine-manure lagoons. *Water Environment Research*, *77*(1), pp.49-56.

Kreuzig, R., Höltge, S., Brunotte, J., Berenzen, N., Wogram, J. and Schulz, R., 2005. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. *Environmental Toxicology and Chemistry: An International Journal*, 24(4), pp.777-781.

Kreuzig, R., Höltge, S., Brunotte, J., Berenzen, N., Wogram, J. and Schulz, R., 2005. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. *Environmental Toxicology and Chemistry: An International Journal*, 24(4), pp.777-781.

Labite, H., Holden, N.M., Richards, K.G., Kramers, G., Premrov, A., Coxon, C.E. and Cummins, E., 2013. Comparison of pesticide leaching potential to groundwater under EU FOCUS and site specific conditions. *Science of the Total Environment*, 463, pp.432-441.

Lamshöft, M., Sukul, P., Zühlke, S. and Spiteller, M., 2010. Behaviour of 14C-sulfadiazine and 14C-difloxacin during manure storage. *Science of the Total Environment*, *408*(7), pp.1563-1568.

Liu, F., Ying, G.G., Tao, R., Zhao, J.L., Yang, J.F. and Zhao, L.F., 2009. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. *Environmental Pollution*, 157(5), pp.1636-1642.

Loke, M.L., Ingerslev, F., Halling-Sørensen, B. and Tjørnelund, J., 2000. Stability of tylosin A in manure containing test systems determined by high performance liquid chromatography. *Chemosphere*, 40(7), pp.759-765.

Loke, M.L., Tjørnelund, J. and Halling-Sørensen, B., 2002. Determination of the distribution coefficient (logKd) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. *Chemosphere*, 48(3), pp.351-361.

Maguire, R.O., Kleinman, P.J., Dell, C.J., Beegle, D.B., Brandt, R.C., McGrath, J.M. and Ketterings, Q.M., 2011. Manure application technology in reduced tillage and forage systems: A review. Journal of Environmental Quality, 40(2), pp.292-301.

Martins, A., Guimarães, L. and Guilhermino, L., 2013. Chronic toxicity of the veterinary antibiotic florfenicol to Daphnia magna assessed at two temperatures. *Environmental Toxicology and Pharmacology*, 36(3), pp.1022-1032.

Montforts, M.H.M.M. and Verschoor, A.J., 2003. Environmental Risk Assessment for Veterinary Medicinal Products Part 3. Validation of environmental exposure models.

Nguyen Dang Giang, C., Sebesvari, Z., Renaud, F., Rosendahl, I., Hoang Minh, Q., Amelung, W., 2015. Occurrence and dissipation of the antibiotics sulfamethoxazole, sulfadiazine, trimethoprim, and enrofloxacin in the Mekong Delta, Vietnam. PLoS One 10 (7), e0131855.

Niles, M.T., Horner, C., Chintala, R. and Tricarico, J., 2019. A review of determinants for dairy farmer decision making on manure management strategies in high-income countries. *Environmental Research Letters*, 14(5), p.053004.

OECD (2000) OECD Guidelines for the Testing of Chemicals, Test No 216, Soil Microorganisms: Nitrogen Transformation Test, Organisation for Economic Cooperation and Development, Paris, France.

OECD (2004) OECD Guidelines for the Testing of Chemicals. Test No 222: Earthworm Reproduction Test (Eisenia fetida / Eisenia Andrei), Organisation for Economic Co-Operation and Development, Paris, France.

OECD (2006b) OECD Guidelines for the Testing of Chemicals. Test No 208: Terrestrial Plant Growth Test: Emergence and Seedling Growth Test, Organization for Economic Cooperation and Development, Paris, France.

Osorio, V., Sanchís, J., Abad, J.L., Ginebreda, A., Farré, M., Pérez, S. and Barceló, D., 2016. Investigating the formation and toxicity of nitrogen transformation products of diclofenac and sulfamethoxazole in wastewater treatment plants. *Journal of Hazardous Materials*, *309*, pp.157-164.

Patrick Jr., W.H., Williams, B.G., Moraghan, J.T., 1973. A simple system for controlling redox potential and pH in soil suspensions. *Soil Science*. Soc. Am. Proc. 37, 331–332.

Pino, M.R., Val, J., Mainar, A.M., Zuriaga, E., Español, C. and Langa, E., 2015. Acute toxicological effects on the earthworm Eisenia fetida of 18 common pharmaceuticals in artificial soil. *Science of the Total Environment*, 518, pp.225-237.

Qiu, M., Hu, A., Yu-ming, M.H., Zhao, Y., He, Y., Xu, J. and Lu, Z., 2021. Elucidating degradation mechanisms of florfenicol in soil by stable-isotope assisted nontarget screening. *Journal of Hazardous Materials*, 403, p.123974.

Rabølle, M. and Spliid, N.H., 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere*, 40(7), pp.715-722.

Reis, A.C., Kolvenbach, B.A., Nunes, O.C. and Corvini, P.F., 2020a. Biodegradation of antibiotics: The new resistance determinants–part I. *New Biotechnology*, 54, pp.34-51.

Reis, A.C., Kolvenbach, B.A., Nunes, O.C. and Corvini, P.F., 2020b. Biodegradation of antibiotics: The new resistance determinants–part II. *New Biotechnology*, 54, pp.13-27.

Ricker, N., Trachsel, J., Colgan, P., Jones, J., Choi, J., Lee, J., Coetzee, J.F., Howe, A., Brockmeier, S.L., Loving, C.L. and Allen, H.K., 2020. Toward antibiotic stewardship: Route of antibiotic administration impacts the microbiota and resistance gene diversity in swine feces. *Frontiers in veterinary science*, *7*, p.255.

Rotz, C.A., Kleinman, P.J.A., Dell, C.J., Veith, T.L. and Beegle, D.B., 2011. Environmental and economic comparisons of manure application methods in farming systems. *Journal of Environmental Quality*, 40(2), pp.438-448.

Shao, S., Hu, Y., Cheng, J. and Chen, Y., 2018. Research progress on distribution, migration, transformation of antibiotics and antibiotic resistance genes (ARGs) in aquatic environment. *Critical Reviews in Biotechnology*, 38(8), pp.1195-1208.

Shao, S., Hu, Y., Cheng, J. and Chen, Y., 2018. Research progress on distribution, migration, transformation of antibiotics and antibiotic resistance genes (ARGs) in aquatic environment. *Critical Reviews in Biotechnology*, 38(8), pp.1195-1208.

Smith, K. A., & Williams, A. G. (2016). Production and management of cattle manure in the UK and implications for land application practice. *Soil Use and Management*, 32, 73–82. doi:10.1111/sum.12247

Spielmeyer, A., Höper, H. and Hamscher, G., 2017. Long-term monitoring of sulfonamide leaching from manure amended soil into groundwater. *Chemosphere*, 177, pp.232-238.

Srinivasan, P., Sarmah, A.K. and Manley-Harris, M., 2013. Co-contaminants and factors affecting the sorption behaviour of two sulfonamides in pasture soils. *Environmental Pollution*, *180*, pp.165-172.

Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils–a review. *Journal of Plant Nutrition and Soil Science*, 166(2), pp.145-167.

Tkaczyk, A., Bownik, A., Dudka, J., Kowal, K. and Ślaska, B., 2021. Daphnia magna model in the toxicity assessment of pharmaceuticals: A review. *Science of The Total Environment*, 763, p.143038.

VICH (2000). Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase I. London: CVMP/VICH, 2000. CVMP/VICH/592/98-final.

VICH (2003). Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase II Draft Guidance. London: CVMP/VICH GL 38. CVMP/VICH/790/03-Consultation.

VMD, 2021. Product Information Database. [online] Vmd.defra.gov.uk. Available at: <https://www.vmd.defra.gov.uk/productinformationdatabase/> [Accessed 17 January 2022].

Webb, J., Pain, B., Bittman, S. and Morgan, J., 2010. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—a review. *Agriculture, Ecosystems & Environment*, 137(1-2), pp.39-46.

Wehrhan, A., Streck, T., Groeneweg, J., Vereecken, H. and Kasteel, R., 2010. Long-term sorption and desorption of sulfadiazine in soil: Experiments and modeling. *Journal of Environmental Quality*, 39(2), pp.654-666.

Weinfurtner, K., 2011. Matrix parameters and storage conditions of manure.

Wellington, E.M., Boxall, A.B., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W. and Thomas, C.M., 2013. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. The Lancet infectious diseases, 13(2), pp.155-165.

Widyasari-Mehta, A., Suwito, H.R.K.A. and Kreuzig, R., 2016. Laboratory testing on the removal of the veterinary antibiotic doxycycline during long-term liquid pig manure and digestate storage. *Chemosphere*, *149*, pp.154-160.

Wohde, M., Berkner, S., Junker, T., Konradi, S., Schwarz, L. and Düring, R.A., 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. *Environmental Sciences Europe*, 28(1), pp.1-25.

Wollenberger, L., Halling-Sørensen, B. and Kusk, K.O., 2000. Acute and chronic toxicity of veterinary antibiotics to Daphnia magna. *Chemosphere*, 40(7), pp.723-730.

Yalkowsky, S.H. and He, Y., 2003. An extensive compilation of aqueous solubility data for organic compounds extracted from the AQUASOL database. Handbook of Aqueous Solubility Data, p.377.

Zainab, S.M., Junaid, M., Xu, N. and Malik, R.N., 2020. Antibiotics and antibiotic resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water Research*, p.116455.

Zheng, Y., Liu, Y., Qu, M., Hao, M., Yang, D., Yang, Q., Wang, X.C. and Dzakpasu, M., 2021. Fate of an antibiotic and its effects on nitrogen transformation functional bacteria in integrated vertical flow constructed wetlands. *Chemical Engineering Journal*, 417, p.129272.

Zou, H., He, J., Guan, X., Zhang, Y., Deng, L., Li, Y. and Liu, F., 2021. Microbial responses underlying the denitrification kinetic shifting exposed to ng/L-and μg/L-level lomefloxacin in groundwater. *Journal of Hazardous Materials*, 417, p.126093.

List of Abbreviations

Veterinary medicines

ALT – Altrenogest

ASA – Acetylsalicylic acid

AZA – Azaperone

CFT – Ceftiofur

ENR – Enrofloxacin

FLO – Florfenicol

LNC – Lincomycin

MLX – Meloxicam

OTC – Oxytetracycline

SDZ – Sulfadiazine

SMX – Sulfamethoxazole

TMP – Trimethoprim

TYL – Tylosin

Generic scientific terminology

bdl – Below detectable limits

MEC – Measured Environmental Concentration

DT_{50} – Half life

EC_{50} – Concentration at which 50% of the population is inhibited

LC_{50} – Lethal concentration towards 50% of the population

NOEC – No observed effect concentration

LC-MS/MS – Liquid chromatograph mass spectrometry – mass spectrometry

HR-MS – High resolution mass spectrometry

SFO – Single first order degradation kinetics

FOMC – First order multi compartmental degradation kinetics

ORP – Oxidative reduction potential

OM – Organic matter

OC – Organic carbon

DOC – Dissolved organic carbon

DIC – Dissolved inorganic carbon

PEC – Predicted environmental concentration

SPC – Summary of product characteristics

BC – Broadcast manure application

CS – Chisel sweep manure application (injection)

IC – Immediate manure incorporation (ploughing)

Appendix A: Supplementary materials for Chapter 1

Table A1: Manure storage and application regulations through EU member states. (Table amended and edited from Weinfurtner, 2011)

Country	Manure storage time	Information	Reference
Austria	6 months	Prohibited manure application from 15th October and 15th February for in-vegetated soils, and 15th November to 15th February for grasslands composed of peaty-clay soils.	Eu-Nitrates Directive 91/676/EWG-Österreichischer Bericht, 2004
Germany	6 months for manure 10 months of pig manure	Prohibited application times are between 11 November and 31st January. On tilled land and grassland, it is from 15th November and 31st of January. - Application limit for NVZ 230 kg/N/ha^{-1}	LandWritschaftskammer NRW, (2008) Van Grinsven et al., (2012)
The Netherlands	At least 6 months for all manure types	Application prohibited between 1st September and the 31st of January on sandy soils. 15th October to 12th of January for other soils types.	Third Dutch action programme (2004-2009) concerning the nitrates directive (2005)
Denmark	Manure storage capacity 6-9 months	Closed period is from November 15th to the 1st of February.	Liu et al., (2018)
Sweden	Manure storage capacity 6-10 months	Closed period is from November 1st to the 22nd of February.	Liu et al., (2018)

Norway	Manure storage capacity 8 months	Closed period is from November 1st to the 15th of February.	Liu et al., (2018)
Ireland	Storage for at least 6 months	Manure application banned between 15th October and 12th of January, depending on the region this may be extended to 31st of January (NVZ). - Application limit for NVZ 250 kg/N/ha^{-1}	Good Agricultural Practice for Protection of Water, (2008)
Italy		Manure application rate is 4 tonnes per hectare with no specification on manure type.	Montforts, (2008)
France	N/A	Applying manure banned from 1st November and 15th of November. This can be extended to 15th of January depending on arable crop and cultivation. - Application limit 170 kg N/ha^{-1}	Chambre d'Ágriculture Vienne, 2009
Great Britain	6 months for pigs And 5 months for other species	Sand and shallow soils with grass: 1st September and 31st December. Sand and shallow soils under tillage 14th October and 15th January. Other soils under grass: 15th October and 15th January. Shallow sandy soils on tilled land with crops sown before 15th September: 16th September to 31st December Other soils with tillage: 1st October to 15th January.	Defra, (2013) and NFU, (2017)

		- Application limit for NVZ 250 kg/N/ha^{-1}
Slovenia	Manure stored for at least 4 to 6 months	Dependent on region

Table A2: Pig manure and slurry properties.

Matrix and storage details	pH	Moisture content (A) Dry matter content (B)	Phosphorus	Total organic carbon	Total inorganic carbon	C:N ratio	TKN	NH_4^+ Or NH_4^+-N	Nitrogen	Organic matter	BOD (A)/COD (B)	Reference
N/A		2 (B)	3260 mg/L	41.1 g/kg					6830 mg/L			Blackwell et al., (2005)
Pig slurry	7.04		7.04 g/kg						29.63 g/kg			Chu et al., (2017)
Compost	5.11											Chu et al., (2017b)
Pig slurry	8		72.30 %	44.10 %		15.5			2.85 %			Hu et al., (2011)
		86 g/kg (B)	1.9±0.4 g/kg					4.5±0.6 g/kg (A)				Bousek et al., (2018)
Lactating sows	7.13	68 % (A)		399.60 g/kg		15.38	25.9 g/kg			745±25.6 g/kg	39.8 g/L (A) 60 g/L (B)	Chen et al., (2018)
	7.8-8.1	94 %-96.5 % (A)		34.4-16.8 g/L								Lamshoft et al., (2010)
N/A	7.68	66.3 % (A)	12.01 g/kg			9.01	30.36 g/kg			64.30 %		Wu et al., (2018)
Pig slurry	7.13	74 % (A)		30.74 %		7.6	4.03 %			72.10 %		Selvam et al., (2012)
Pig slurry		44.3 g/L (B)	1.7 g/L	422.2 g/L			153.35 g/L	98.2 g/L (B)				Ingerslav and Halling-Sørensen 2001
Pig slurry	7.3	95 % (A)		34.28 %					4.11 %			Kim et al., (2012b)

Pig slurry	7.4	68.70 % (A)		33.80 %				2.60%			Lin et al., (2017)
Fattening pigs	7.94			448.8 mg/kg				46.1 mg/kg			Stone et al., (2009)
Liquid slurry	8			36%							Kreuzig and Holtge, (2005)
								25.6 ± 0.08 g/kg	61 ± 0.7 %.		Shi et al., (2012)
Sow manure	6.97	74.3 % (A)		35.50%	12.90%						Wang et al., (2015A)
Pig manure	5.56	78.07 % (A)			698.21 mg/kg (T)			36.10 mg/kg			Wang et al., (2015B)
Pig slurry	6.7							2 g/kg		4.3 g/kg (A), 10 g/kg (B)	Kreuzig, (2010)
41 commercial swine farms Korea (average)			2 g/L					4g/L		20 g/L (A), 40 g/L (B)	Suresh et al., (2009)
Farrowing sows (Fresh and stored)	7.09 -7.9	94.9% and 95.9% (A)				2.4 g/kg, 2.2 g/kg (A)		3.5 g/kg, 3 g/kg,			Blanes-Vidal et al., (2009)
Finishing pigs (Fresh)	7.0-7.2	92.9 %, 93.5 % (A)				3.3 g/kg, 3.2 g/kg (A)		5.2 g/kg, 4.8 g/kg			Blanes-Vidal et al., (2009)
Weaning pigs (Fresh)	6.3-6.6	95.1 %, 95.5 % (A)				2.3 g/kg and 2.3 g/kg (A)		3.7 g/kg, 3.4 g/kg			Blanes-Vidal et al., (2009)

Swine effluent (totals)	7.9		62 mg/L				335 mg/L(B)	392 mg/L	Adeli et al., (2008)
Total slurry		96.8 % (A)			5.8			0.5 % (A) and 0.28 % (B)	Ndayrgamiye and Cote, (1989)
Liquid slurry	6.3-6.5	94.75 % (A)	1.7-3 %					6..3-9.1% (A), 3.3-5.7% (B)	Coudhary et al., (1996)
Swine slurry			3.10 %	48.10 %			10% (A)	48.1% (A), 5.8 g/kg	Larkin and Honeycutt, (2006)
Pig slurry (T)		81g/kg (B)	1.05 g/kg					Available : 1.1 g/kg	Sommer et al., (2007)
Swine slurry	8.1	95% (A)		37					Bunning (1997)
Pig slurry	7.7-7.59	14.5-28.6%B			0.064-0.313 mol/L				Sommer & Husted, 1995
Pig slurry	7.46	17.82% B				187 g/kg			Martinez-Suller et al., 2008
Pig slurry	5.5-8.17								Martinez-Suller et al., 2008
Pig slurry	7.43	2.27 %B				2.58 kg N m-3	2.01 kg N m^{-3}(A)		Moral et al., 2005
Pig slurry	6.3						206.9 mmol/kg (A+B)		Paul & Beauchamp, 1989
Fattening pigs	6.31 - 8.65								Canh et al. 1998/1
Finishing pigs	8.07 -8.9	73.4-76.5g/kg (B) DM					0.56-1.13g/kg(A)		Canh et al., 1998/2

Finishing pigs	6.3-8.4	290-402g/kg (B) DM				0.8-2.65g/100g(A)	9.5-16g/kg (T)	Canh et al., 1998/3
Finishing pigs	7.21-9.14	75.3-81.2g/kg (B) DM				5.43-8.83g/100g(A)	7.65-11.3g/kg(T)	Canh et al., 1998/4
Finishing pigs	7.57-8.92	4.4-5.9% (B)				1.92-4.32g N/kg(A)	3.05-5.48g/kg(T)	Dourmad & Jondreville , 2007
Finishing pigs	6.68-8.42	13.76-97.6 g/kg(B)				62.9-78% of total N	657.2-78.2g/kg DM	Kreuzer et al. 1998
Finishing pigs	7.26-7.77					2.6-2.7g/kg(B)		Le et al., 2009
Finishing pigs	7.75-7.89							Le et al., 2008
Finishing pigs	6.47	116.2 mg/L total OP			2.88 g/l			Luo et al., 2002
Finishing pigs (Lombardy)	6.7-8.17							Martinez-Suller et al., 2008
Farrowing sows	7.46	15.02 kg/m^{-3} (B)			2.29kg/m^{-3}			Moral et al., 2005
Sow	6.88-7.77	0.9-1.07 kg/P/m^{-3}	0.42-0.78kg/N/m^{-3} (OC)		1.8-3.42 kg N/m^{-3}			Martinez-Suller et al., 2008
Fattening pigs	4.8	4%B	1.75% OC	12.60%			0.14% N (total)	Thiele Bruhn and Aust, (2003)

Pig slurry	5.11	70.36% A	7.04g/kg (total)	16.76	29.63g/kg (total)	Shan et al (2017)
Fattening pigs	6.91		1.12mg/g (organic)			Huang et al (2011

Table A3: Property data for poultry litter.

Matrix	pH	EC µS/cm	Moisture content (A) Dry matter content (B)	Phosphorus	Organic carbon	C:N ratio	TKN	$NH4^+$ Or NH_4^+-N	Nitrogen	Organic matter	Reference
Fresh poultry manure	6.5			27.1 g/kg	461 g/kg	8.47		4.2 g/kg	54.3 g/kg		Mahimairaja et al., (1994)
Broiler chicken	6			7.55 g/kg	325.3 g/kg				34.1 g/kg		Bao et al., (2009)
									34.52 g/kg		Bao et al., (2009)
Broiler chicken - hay	6.95	10.8 dS/cm	55%	18.4 g/kg	35%	8.1			43.7 g/kg		Ho et al., (2013)
Broiler chicken (9 farms)			57.67-82.12%	0.03-1.51%	32.64 - 38.73 % (TC)				3.93-7.11 %	63.41-71.44 %	Quiroga et al., (2010)
Poultry	7.73					5.1		0.1 g/kg	53 g/kg		Huang et al., (2017)

Poultry manure (T)	9.64	5.02 dS/m	38%		288.4 g/kg (TOC)	9.72		27.5 g/kg		Dias et al., (2010)
Water diluted poultry manure (T)	7.4	93.9 %				3.9	5.55 g/L (B)	6.81 g/L		Gelegenis et al., (2006)
Layer hens (T)	8.37 ± 0.06	3.94 ± 0.10 dS/m	72.59 ± 0.97%		43.37 % (dw)	9.53		4.55% (dw)	78.07 ± 1.83 % (dw)	Petric et al., (2009)
Layer hens	7			9.36 g/kg^{-}	313.1 g/kg			34.52 g/kg		Bao et al., 2009
Layer hens 28 w old	8.06 ± 0.2		71.4 ±.7%				4±0.5 g/kg (B)	13.7±1.2 g/kg (Total)		Chepete et al., (2011)
Layer hens 29 w old	8.5 ± 0.2		75±1.7%				4.0 ±0.5 g/kg^{-1}	8.1±1.4 g/kg (organic) 12.1±1.2 g/kg (total)		Chepete et al., (2011)

Layer hens 30 w old	8.5 ± 0.2	71.6±1.7%			4.8±0.5 g/kg^{-1}	10±1.4 g/kg (organic) 14.8±1.2 g/kg (total)	Chepete et al., (2011)
Layer hens 31 w old	8.9 ± 0.2	73.6±1.7%			5.0±0.5 g/kg^{-1}	7.9±1.4 g/kg (organic) 12.9±1.2 g/kg (total)	Chepete et al., (2011)

Table A4: Property data for cattle manures (dairy or beef)

Matrix	pH	Moisture content (A), Dry matter content (B)	phosphorus	Organic carbon	C:N ratio	NH_4+-N	Nitrogen	Organic matter	BOD (A)/COD (B)	Reference
	8.2			41 %	36.7		1.12 %			Mitchel et al., (2015)

Dairy Slurry (T)									
Dairy manure (T)	8.1-8.6	187-232 g/kg				80.6-139.8 g/kg			Storteboom et al., (2007)
Dairy (manure heap)	8.3	68.30 %	0.20 %	13.90 %	25.5		0.60 %		Arikan et al., (2009b)
Dairy slurry	5.95		0.50%	8 g/kg	16.2		0.5 g/kg		Ali et al.., (2013)
Dairy slurry (T)	7.8		0.68 g/kg			1.3 g/kg (B)		56 g/kg	Lithourgidis et al., (2007)

Dairy manure (T)				17.90 %		0.9 % (B)	1.60 %	Larkin and Honeycutt, (2006)
Dairy FYM (T)	7.6		65.50 %	218g/kg		8.99 g/kg	14.2 g/kg	Aust et al., (2008)
Beef FYM bedding mix			72.40 %	6.8 mg/g	12.90 %	1.9 mg/g	0.40 %	Arikan et al., (2007)
Cattle FYM (T)	6.8	45 kg/kg		9g/kg	249.3 g/kg (T)	2.9g/kg	22.8 g/kg	Whalen et al., (2000)
Solid cattle FYM			80.40 %		8.20 %	20.5 0.075 % (B)	0.40 %	Ndayegamiye and Cote, (1989)
Beef FYM (T)			38.62 %		31.80 %		1.33 %	Liu et al., 2011
Beef FYM (T)	8.8	539 g/kg		4 g/kg	133.1 g/kg	0.48 g/kg	10.2 g/kg	Eghball, (2000)

Cattle FYM (T)	7.1	8.8 g/kg			19 g/kg	457 g/kg		Celik et al., (2004)
Cattle slurry	7.7	0.42 kg/m		0.7 kg/t (A)	2.55 kg/t			Shepherd et al., (2002
Survey (29)				35 kg/m				
Cattle FYM	8.5	1 kg/m		0.26 kg/t (A)	5.2 kg/t			Shepherd et al., (2002
Survey (29)				5.5% (B)				
Cattle manure (T)	7.1		9.47 g/L				27.6 g/L (A), 25.8 g/L (B)	Ermawati et al., (2007)
Cattle FYM (T)	7.3		67.35 ± 0.045 g/kg	22.37 ± 0.14				Zhai et al., (2015)

Cattle FYM (T)	7.9			308.2 g/kg (T)	10.7	0.02 g/kg (B)	28.7 g/kg		Huang et al., (2017)
Fresh cattle slurry (T)		90.10%					3.96 g/kg		Petersen et al., (2012)
Cattle slurry 3 months (T)		94.40%					3.1 g/kg		Petersen et al., (2012)
Cattle manure fresh (T)	9.2		0.60%	OC 29.7%	23.2		1.30 %	50.10 %	Gholamhoseini et al., (2013)
Cattle FYM with	8.4	601g/kg (A)		330.5g/kg (TC)	16.6	2.09 g/kg (B)			Hao et al., (2004)

straw bedding							
Cattle FYM with straw bedding	8.1		282 g/kg^{-1} (TC)	13.4	0.99 g/kg (B)		Hao et al., (2004)
Cattle manure fed	7.9	95 g/kg	34.2 g/kg (TC)	7.8	2.3 g/kg (B)	4.4 g/kg	Velthof et al., (2005)
Cattle manure	8.4	110 g/kg^{-}	36.5 g/kg (TC)	5.4	4.1 g/kg (B)	6.8 g/kg	Velthof et al., (2005)

Cattle manure	8	82 g/kg	28.9 g/kg (TC)	5.8	3.1 g/kg (B)	5 g/kg		Velthof et al., (2005)

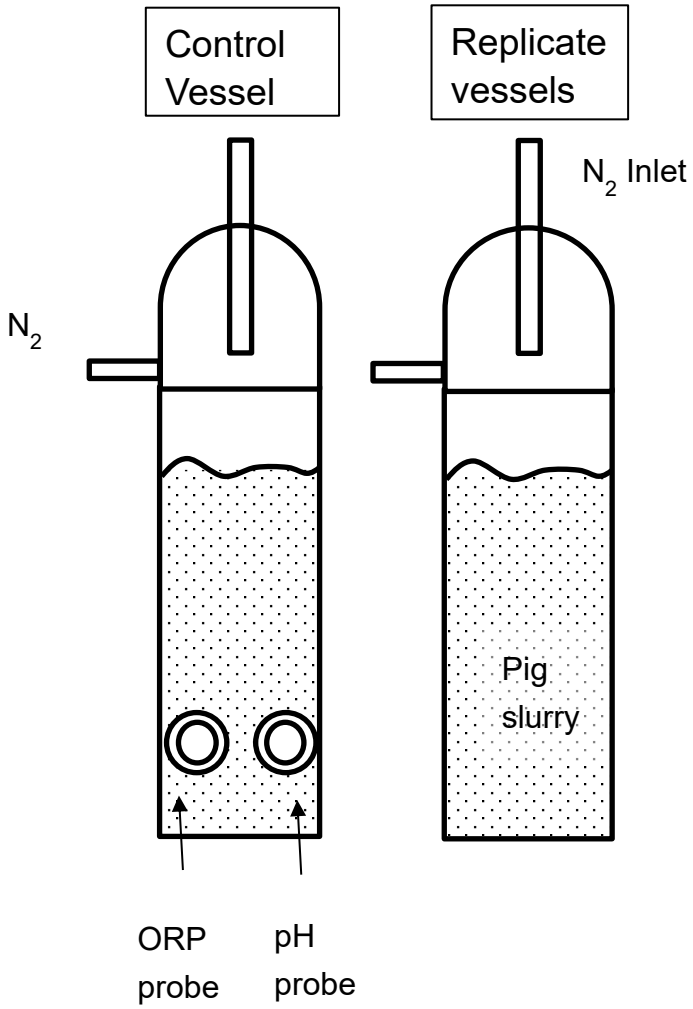
Appendix B: Supplementary information for Chapter 2

A 1.0 Pig slurry characteristics methodology

Fresh pig manure was adjusted to the moisture content of pig slurries (95%) using tap water, the samples were then left to equilibrate at 23ºC for 10 d. In order to assess the dissolved and available, nutrients, carbon and metals, that are present within the slurries the samples were centrifuged at 3,250 rpm for 2 hours and decanted. The supernatant was collected and passed through a G/F Whatman and 1Ps filter paper, the liquid fraction was then filtered using a 20µm GF syringe filter then a 0.45µm nylon syringe filter. Nutrients (NH_4-N , NO_2-N and PO_4) were analysed using an auto analyser. Dissolved organic and inorganic carbon were analysed using a Analytik Jena Multi NC2100 the calibration standards that were used were VKIWW4A and Cranberry-05. Samples for metal analyses were acidified to a pH 2 using nitric acid for preservation purposes (EPA, 2008), the samples were then analysed for Co, Zn, Cd and Pb using an ICP-OES (iCAP 7400 Radial).

A 1.1 Quality control and LC-MS/MS

In order to ensure accuracy and precision of the analysis of veterinary medicines varying steps of quality control were utilized. This entailed using blanks (methanol and blank control) to ensure that no carry over occurred throughout the run (> 5% of the LOQ), calibration standards were bracketed every 25 injections, and a sample of a known concentration was analysed throughout the sequence to check for retention time drift and sensitivity drop.



SI Figure B1: Schematic drawing of specialized incubation vessels to facilitate continuous pH and ORP monitoring.

SI Table B1: Summary of dosage concentrations each selected veterinary antibiotic and their associated veterinary SPC document.

Summary of Product Characteristics	Compound	PEC (mg/kg) (Spaepen, 1997)	Dosage concentration (mg/kg)
Amphen	Florfenicol	5.53	1
Teramin 200	Oxytetracycline	33.16	5
T.S-Sol	Sulfamethoxazole	19.34	3
Pharmasin	Tylosin tartrate	22.11	3.3

Curacef	Ceftiofur	1.66	3

SI Table B2: Validated extraction recoveries for the analytes assessed using 0.1M Na2EDTA-Mcilvaine buffer (pH 4) (50:50), the extractive efficiency of the method is displayed in %. The method was validated to 100%, 10% and 1% of the dosage concentration.

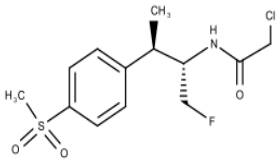
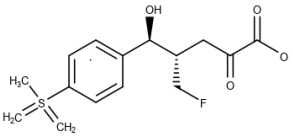
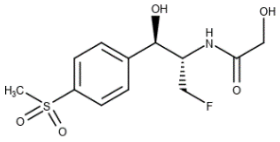
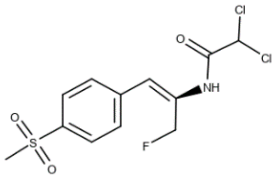
	Recovery (%)				
Dosage	**Ceftiofur**	**Florfenicol**	**Oxytetracycline**	**Sulfamethoxazole**	**Tylosin**
1%	19.05±50.67	bdl	104.7±5.79	91.82±20.00	60.39±11.92
10%	55.50±29.32	77.71±23.10	110.7±7.90	91.33±17.89	92.72±17.00
100%	66.62±24.25	118.65±6.60	96.20±11.3	91.82±22.84	93.36±13.35

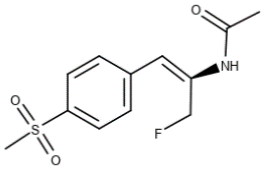
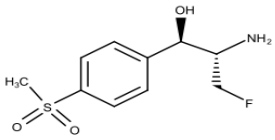
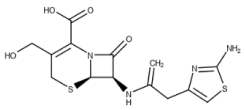
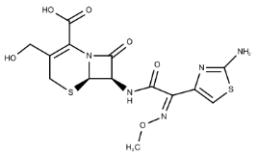
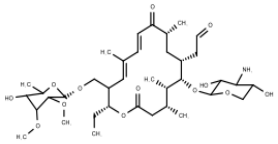
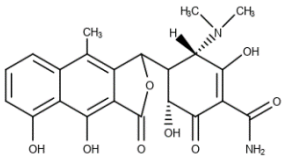
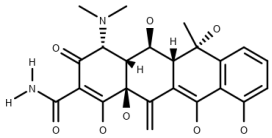
SI Table B3: Mass spectrometer details for each analyte within the study. Obtained using a SCIEX 5500+ quadrupole MS system.

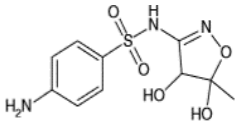
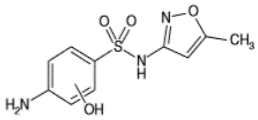
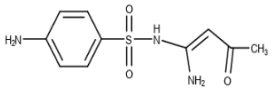
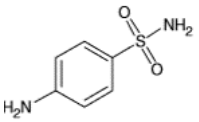
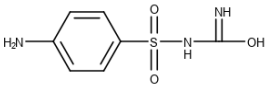
Analyte	Polarity	Q1	Q3	Dwell time	Entrance potential	Collision cell exit potential	Collision energy	Pig slurry LOD (mg/kg)	Pig slurry LOQ (mg/kg)
			125.3	50	10	10	83	0.05	0.073
CFT	+	525.1	126.2	50	10	10	74		
			127.2	50	10	10	64		
			340.1	20	10	12	12	0.012	0.073
FLO	+	358	241.1	20	10	12	24		
			130.2	20	10	12	69		
			444.8	50	10	10	24	0.006	0.006
OTC	+	461.8	427.2	50	10	10	31		
			201.4	50	10	10	56		
			156.1	50	10	10	22	0.000025	0.0006
SMX	+	254.3	108	50	35	10	10		

			92	50	10	10	37		
			774	50	12	12	47	0.0002	0.073
TYL	+	917.8	174.8	50	12	12	55		
			88.3	50	12	12	230		

SI Table B4: Summary of identified TPs, structures, and accurate masses.

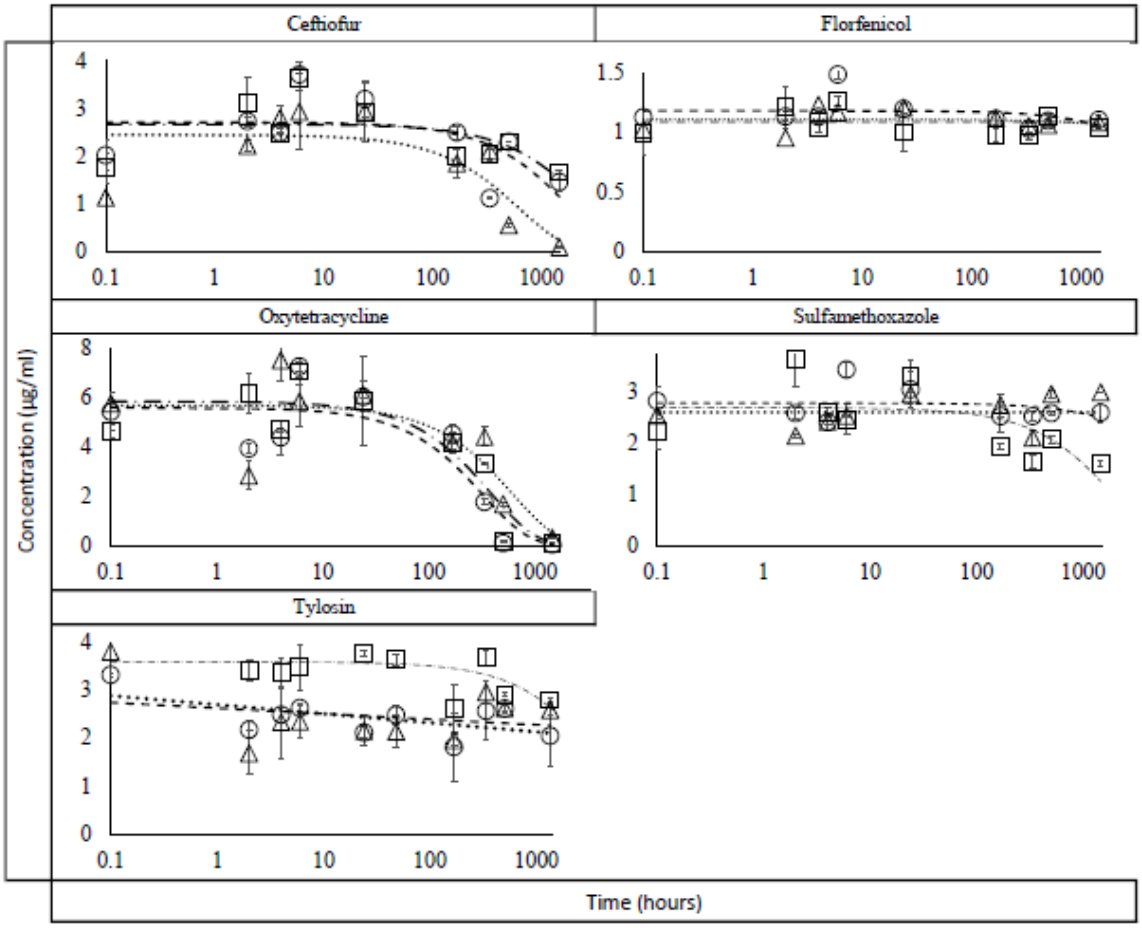
Parent	Transformation product (tentatively identified)	ID	Proposed Chemical structure	Structural change	Monoisotopic mass (*m/z*)	Ionization
FLO	Monochloroflorfenicol $C_{12}H_{15}ClFNO_4S$	MCF		-Cl	323.03942	H^+
	Florfenicol oxamic acid $C_{12}H_{14}FNO_6S$	FOA		+O, -Cl_2	319.05258	NH_4^+
	Florfenicol alcohol $C_{12}H_{16}FNO_5S$	FOH		+O, +H_2, -Cl_2	305.07330	NH_4^+
	C_{12} H_{12} Cl_2 F N O_3 S	FLO_M_338		+H2+O	338.98990	H^+

	$C_{12}H_{14}NO_3FS$	FLO_M_271		-Cl_2, -O	271.06784	H^+
	Florfenicol amine $C_{10}H_{14}FNO_3S$	FA		-Cl, -O, -Cl_2	247.06784	H^+
CFT	Deacetyl-cefotaxime $C_{14}H_{15}N_5O_6S_2$	DCX		-C_2, -H_2, -O_2, -S	413.04636	Na^+
	Desfuroylceftiofur $C_{14}H_{15}N_5O_5S_3$	DCFT		-C5, -H2, -O2	429.02353	H^+
TYL	Tylosin B $C_{39}H_{65}NO_{14}$	TYL_B		-C_7, -H_{12}, -O_3	771.44050	H^+
OTC	B-Apo-Oxytetracycline/A-APO $C_{22}H_{22}N_2O_8$	A-APO		-H_2, -O	442.13760	H^+
	4-*epi*-Oxytetracycline $C_{22}H_{24}N_2O_9$	4-*epi*-OTC			460.14816	H^+
SMX	$C_{10}H_{11}N_3O_6S$	SMX_M_301	Unknown	+O_3	301.03690	H^+

$C_{10}H_{13}N_3O_5S$	SMX_ M_287		+H_4	287.05760	H^+
$C_{10}H_{11}N_3O_4S$	SMX_ M_269		+O	269.05430	H^+
$C_8H_7N_3O_3S$	SMX_ M_255	Unknown	+H4	255.06780	NH_4^+
$C_8H_5N_3O_4S$	SMX_ M_239	Unknown	-C2-H6+O	239.00010	H^+
$C_8H_9N_3O_3S$	SMX_ M_227	Unknown	-C_2-H_2	227.03650	H^+
$C_8H_7N_3O_3S$	SMX_ M_225	Unknown	-C2-H4	225.02080	NH_4^+
$C_8H_5N_3O_4S$	SMX_ M_239		-C3-H6+O	239.00010	H^+
$C_6H_8N_2O_2S$	SMX_ M_173		-C2, -H3,	173.01467	Na^+, H^+
$C_7H_9O_3N_3S$	SMX_ M_215		-C3, -H2,	215.03646	H^+

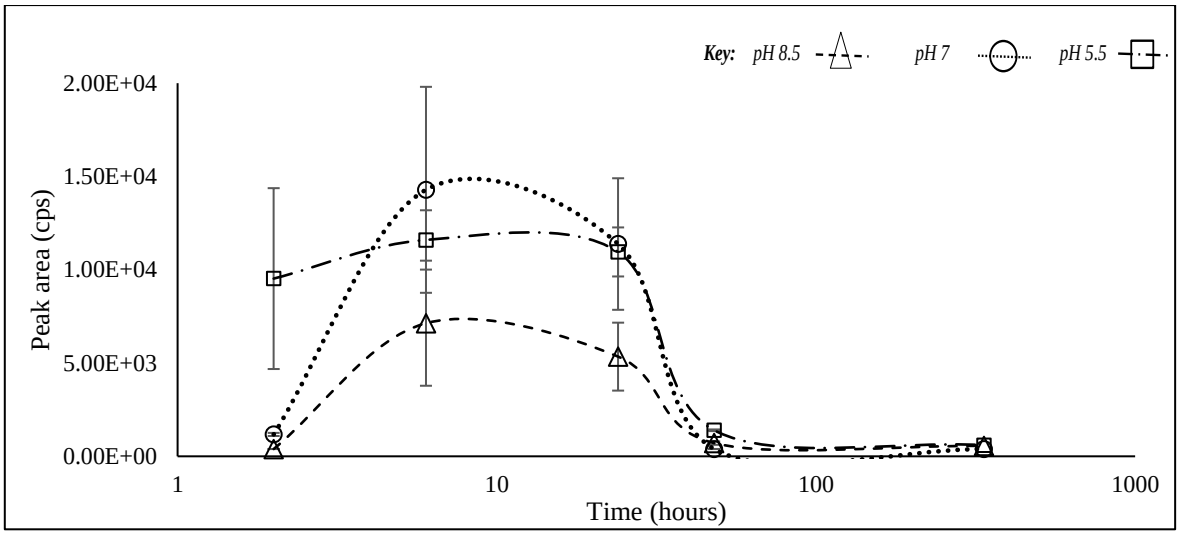
SI Table B5: DT_{50} values of identified TPs found at varying pHs for non-sterile pig slurry. DT_{50} values were derived by plotting mass spectrometer peak areas over time.

	Non-Sterile Pig Slurry		
Transformation Product	**pH 8.5**	**pH 7**	**pH 5.5**
MCF	9.22 h	9.37 h	N/D
FLO_M_338	N/D	6.4 h	33 h
TYL_B	7.1 h	13.4 h	31.4 h

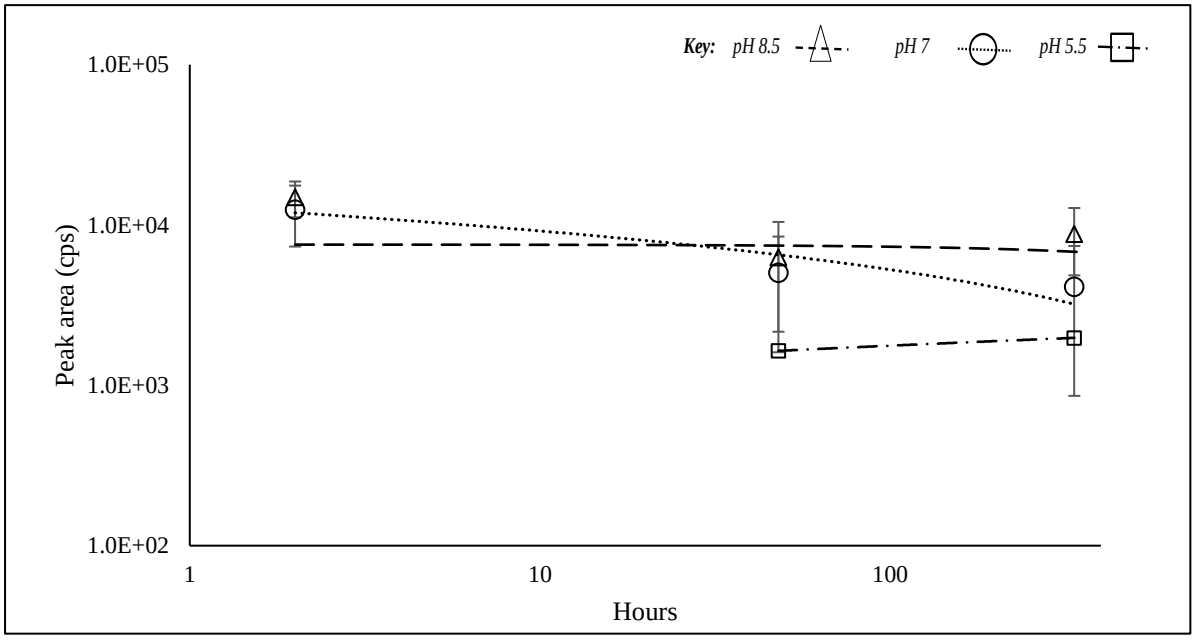


SI Figure B2: Hydrolysis as a function of pH for five selected veterinary antibiotics plotted over time (X-axis logarithmic).

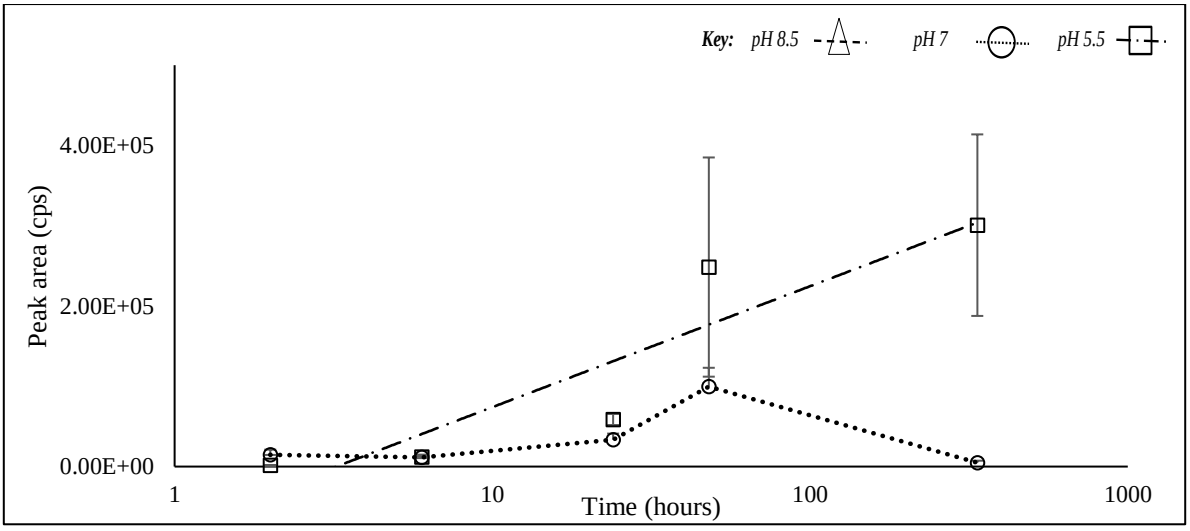
Key: *pH 8.5* *pH 7* *pH 5.5*



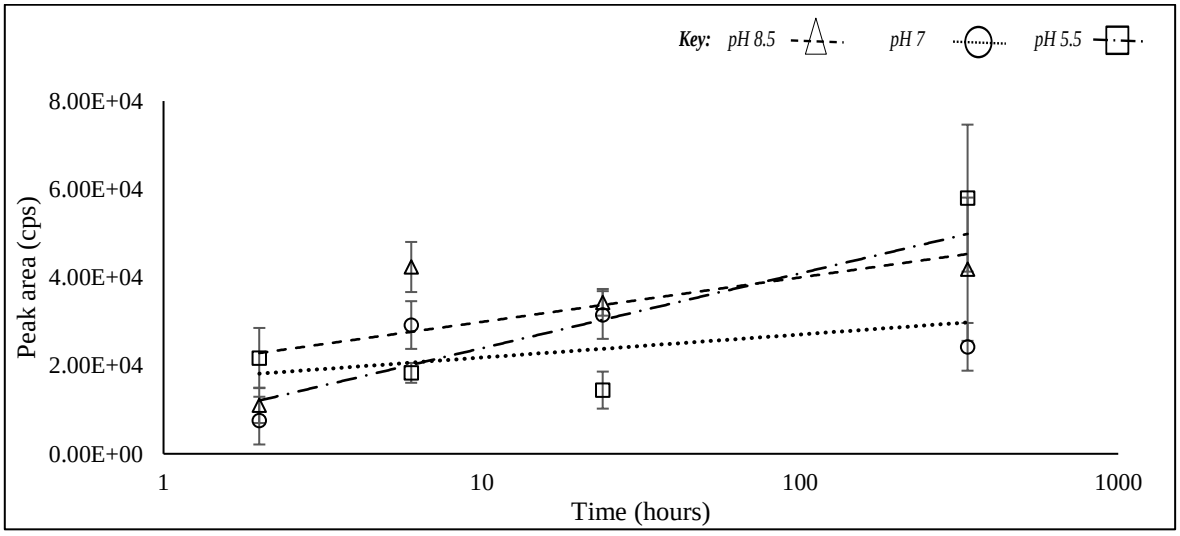
SI Figure B3: Formation and degradation of florfenicol alcohol within three slurry pHs.



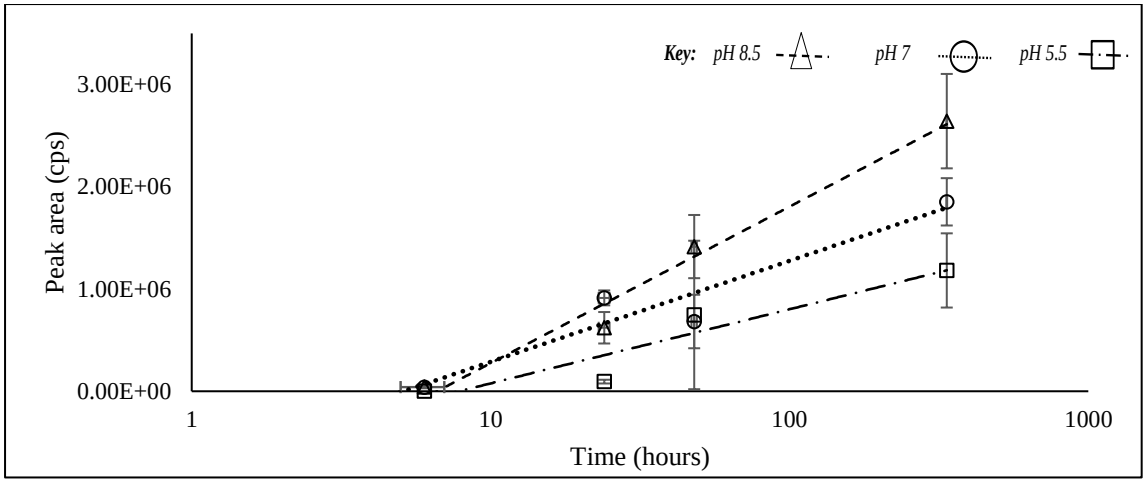
SI Figure B4: Formation and degradation of florfenicol amine pig slurries at varying pHs. Plotted logarithmically with time on the x-axis and peak area on the Y.



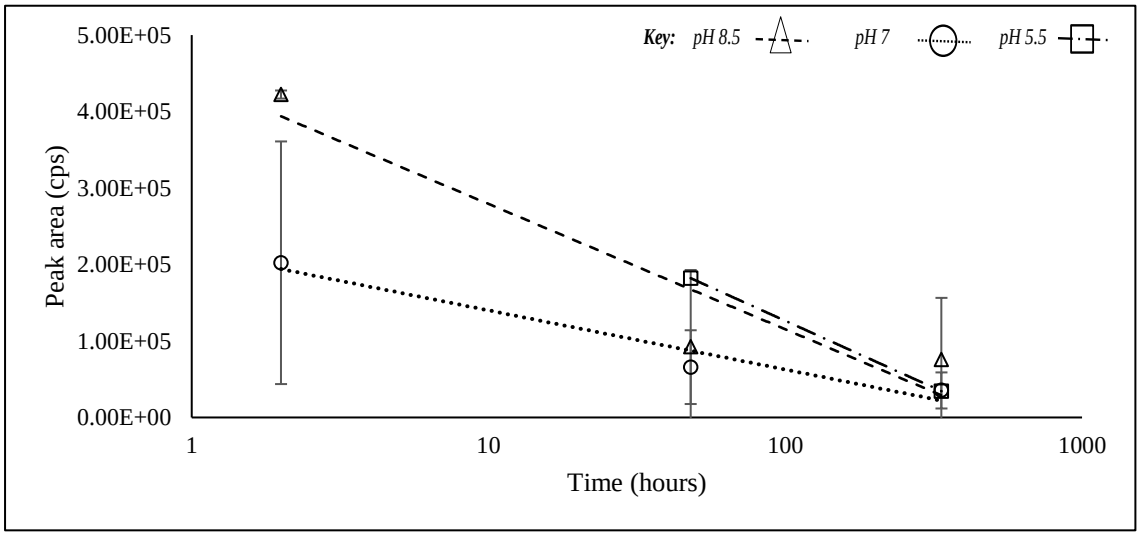
SI Figure B5: Formation of SMX_M_255 (ketone to alcohol transformation) within three slurry pHs. Plotted logarithmically with time on the x-axis and peak area on the Y.



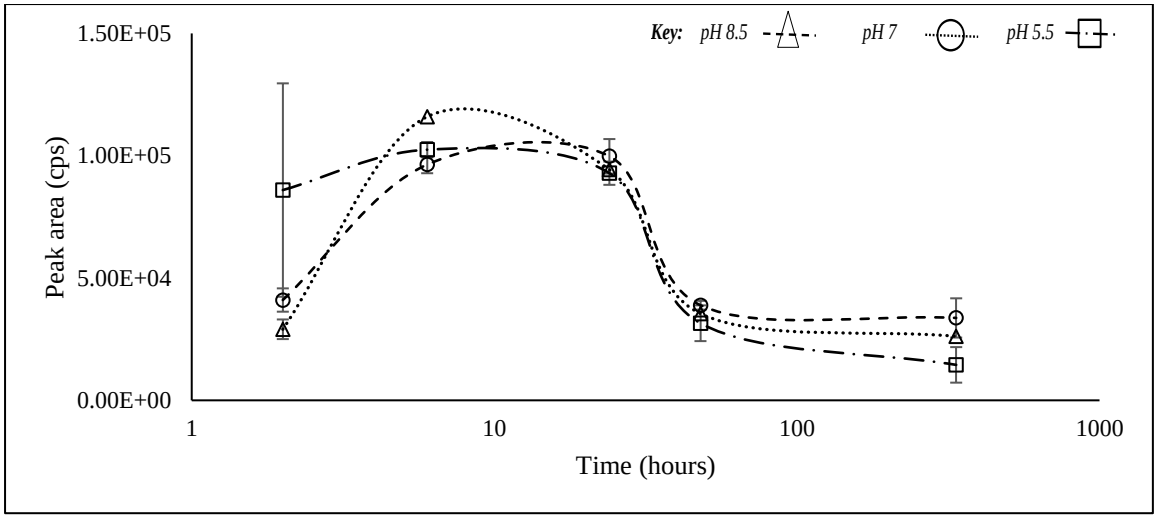
SI Figure B6: Formation of SMX_M_301 within three slurry pHs. Plotted logarithmically with time on the x-axis and peak area on the Y.



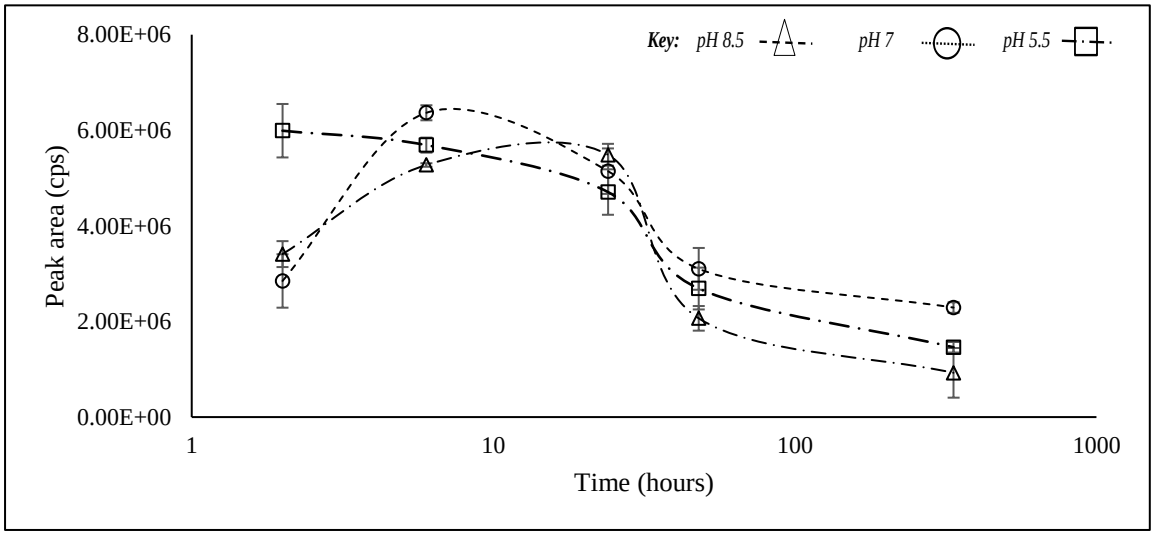
SI Figure B7: Degradation of SMX_M_227 within three slurry pHs. Plotted logarithmically with time on the x-axis and peak area on the Y.



SI Figure B8: Formation of SMX_M_269 within three slurry pHs. Plotted logarithmically with time on the x-axis and peak area on the Y.



SI Figure B9: Degradation of APO-OTC within three slurry pHs. Plotted logarithmically with time on the x-axis and peak area on the Y.



SI Figure B10: Degradation of 3 Epi-OTC within three slurry pHs. Plotted logarithmically with time on the x-axis and peak area on the Y.

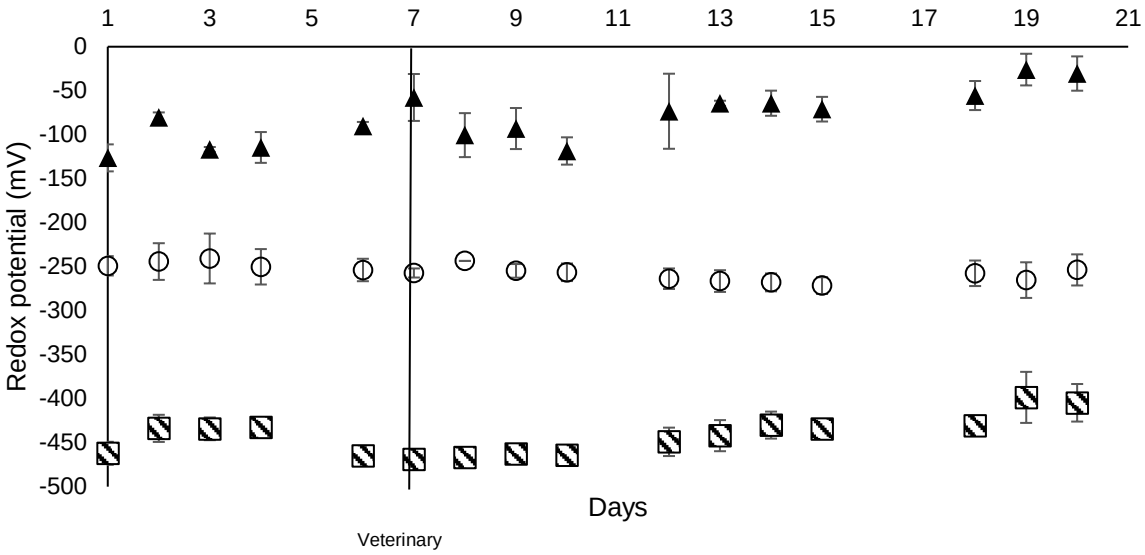
Appendix C: Supplementary information for Chapter 3

SI Table C1: Physiochemical properties of the selected veterinary antibiotics and their respective chemical structures.

Veterinary medicine	Solubility in water at 20°C (mg/L)	Log K_{ow}	pKa	K_d (mL/g)	K_{oc} (g/kg)	K_f
Acetyl-salicylic acid	10000 [a]	1.19 [b]	2 [c]			
Ceftiofur	23 [a]	1.6 [d]	2.68 [e]	19.82 [f]	845.19 [f]	
Florfenicol	1320 [g]	0.37 [g]	9.03 [g]	0.07-0.59 [g]	18.38 [g]	
Oxytetracycline	414 [a]	-1.22 [h]	3.27, 7.32. 9.11 [i]	680 [j]	42500 [j]	2.49-2.24 [j]
Sulfamethoxazole	610 [a]	0.89 [i]	1.4, 5.7 [b]	pH dependent 7.62-2383.33 [k]	219 [l]	
Tylosin	5000 [a]	-3.41 [h]	7.73 [h]	45.7-240 [h]	553-7988 [j]	2.3-7 [j]

References: *a Yalkowsky, and Dannenfeslser, (2016), b Hansch et al., (1995), c Papadopoulos et al, (1991), d US EPA, (2004), e Ribeiro et al, (2018), f An et al, (2021), g FDA, (2013), h Loke et al., (2002), I Stephens, (1956), j Rabølle and Spliid, (2000), k Chen et al., (2017), l Nguyen Dang Giang et al., (2015).*

Adsorption/desorption details: *f geomean of sandy loam, loam and clay soil, g geomean of four soil types, j geomean of four sandy soils,*



SI Figure C1: Redox potential values throughout the duration of the study. The values presented here are the average redox potentials for the first experiment. The line indicates dosing of the vessels. The ORP values presented do not show the entire acclimation period.

SI Table C2: Calculated PEC manures for selected analytes and their actual dosage concentration.

SPC	Compound	PEC (mg/kg) (Spaepen, 1997)	Dosage concentration (1/5th of PEC) (mg/kg)
Suispirin	Acetyl-salicylic acid	6.63	1.33
Amphen	Florfenicol	5.53	1
Teramin 200	Oxytetracycline	33.16	5
T.S-Sol	Sulfamethoxazole	19.34	3
Pharmasin	Tylosin tartrate	22.11	3.3
Curacef	Ceftiofur	1.66	3

SI Table C3: Validation of veterinary medicine extraction method to three dosage levels (1%, 10% and 100%) using 0.1M Na_2EDTA-Mcilvaine buffer (pH 4).

Dosage	Acetylsalicylic acid	Ceftiofur	Florfenicol	Oxytetracycline	Sulfamethoxazole	Tylosin
1%	12.47±42.33	19.05±50.67	91.24±18.46	104.7±5.79	91.82±20	60.39±11.92
10%	38.88±14.48	55.5±29.32	77.71±23.1	110.7±7.9	91.33±17.89	92.72±17
100%	44.29±8.19	66.62±24.25	118.65±6.6	96.2±11.3	91.82±22.84	93.36±13.35

SI Table C4: Mass spectrometer details for each analyte within the study. Obtained using a SCIEX 5500+ quadrupole MS system.

Analyte	Polarity	Retention time (minutes)	Q1	Q3	Dwell time	Entrance potential	Collision cell exit potential	Collision energy	Pig slurry LOD (mg/kg)	Pig slurry LOQ (mg/kg)
ASA	-	1.5-1.6	178.9	137	50	-10	-10	-15	0.0002	0.0024
				93	50	-10	-10	-46		
				59	50	-10	-10	-38		
CFT	+	3.0-3.1	525.1	125.3	50	10	10	83	0.05	0.073
				126.2	50	10	10	74		
				127.2	50	10	10	64		
FLO	+	4-4.7	358	340.1	20	10	12	12	0.012	0.073
				241.1	20	10	12	24		
				130.2	20	10	12	69		
OTC	+	2.0-2.1	461.8	444.8	50	10	10	24	0.006	0.006
				427.2	50	10	10	31		
				201.4	50	10	10	56		

				156.1	50	10	10	22	0.000025	0.0006
SMX	+		254.3	108	50	35	10	10		
		2.3-2.6		92	50	10	10	37		
				774	50	12	12	47	0.0002	0.073
TYL	+		917.8	174.8	50	12	12	55		
		3.34-3.36		88.3	50	12	12	230		

SI Table C5: Summary of the sulfamethoxazole properties that were used in the FOCUS_PEARL modelling.

Substance	**Molecular mass (g/mol)**	**Water solubility (mg/L)**	**Dissociation constant range K_f or K_{oc} (geomean)**	**Vapour pressure (Pa)**	**Octanol-water partition coefficient (Log K_{ow})**
Sulfamethoxazole	358.21	1320 a	Range of four soil types K_{oc} 10 -27 (18.38) a	18.26 estimated – EPI suite b	0.37 a

Appendix C References

An, B., Xu, X., Ma, W., Huo, M., Wang, H., Liu, Z., Cheng, G., Huang, L., 2021. The adsorption-desorption characteristics and degradation kinetics of ceftiofur in different agricultural soils. *Ecotoxicology and Environmental Safefty*. 222, 112503.

Chen, K.L., Liu, L.C. and Chen, W.R., 2017. Adsorption of sulfamethoxazole and sulfapyridine antibiotics in high organic content soils. *Environmental Pollution*, 231, pp.1163-1171.

FDA, 2013. Environmental Assessment for Aquaflor (Florfenicol). Aquaflor for Freshwater-Reared Finfish in Recirculating Aquaculture Systems [online] EPA Available at: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/113. (Accessed 7 May 2020).

Hansh, C., Leo, A. and Hoekman, D., 1995. Exploring QSAR: hydrophobic, electronic, and steric constants. ACS, Washington, DC.

Loke, M.L., Tjørnelund, J. and Halling-Sørensen, B., 2002. Determination of the distribution coefficient (logKd) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. *Chemosphere*, 48(3), pp.351-361.

Luo, A., Zhu, J., Ndegwa, P.M., 2002. Removal of carbon, nitrogen, and phosphorus in pig manure by continuous and intermittent aeration at low redox potentials. *Biosystems and Engineering*. 82, 209–216.

Nguyen Dang Giang, C., Sebesvari, Z., Renaud, F., Rosendahl, I., Hoang Minh, Q., Amelung, W., 2015. Occurrence and dissipation of the antibiotics sulfamethoxazole, sulfadiazine, trimethoprim, and enrofloxacin in the Mekong Delta, Vietnam. PLoS One 10 (7), e0131855.

Papadopoulos, N., Avranas, A., 1991. Dissociation of salicylic acid, 2, 4-, 2, 5-and 2, 6-dihydroxybenzoic acids in 1-propanol-water mixtures at 25 C. *Journal of Solution Chemistry*. 20 (3), 293–300.

Rabølle, M., Spliid, N.H., 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere* 40 (7), 715–722.

Ribeiro, A.R., Lutze, H.V., Schmidt, T.C., 2018. Base-catalyzed hydrolysis and speciation-dependent photolysis of two cephalosporin antibiotics, ceftiofur and cefapirin. *Water Research*. 134, 253–260. doi:10.1016/j.watres.2017.12.048.

Stephens, C.R., Murai, K., Brunings, K.J., Woodward, R.B., 1956. Acidity constants of the tetracycline antibiotics. *Journal of the American Chemical Society* 78 (16), 4155–4158.

US EPA. Estimation program interface (EPI) suite. Ver.3.12. Available from, as of Apr 18, 2006:https://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, 2004.

Yalkowsky, S.H., He, Y. and Jain, P., 2016. Handbook of aqueous solubility data. CRC press.

Appendix D: Supplementary materials for Chapter 4

Section D1: Pig slurry characterisation methodology.

The dissolved properties of the pig slurry were then analysed, this was achieved by centrifuging at 3,250rpm for 2h, the supernatant was then sequentially filtered through varying filter grades; G/F Whatman, 1Ps filter paper, 20µm GF syringe and a 0.45 µm nylon syringe filter. The dissolved nutrients and carbon were then analysed using a Autoanalyser and a Anakytik Jena Multi NC2100 (carbon).

SI Table D1: Collated physical-chemical property data for the selected veterinary medicines.

Veterinary Medicine	Florfenicol	Enrofloxacin	Lincomycin	Meloxicam	Oxytetracycline	Sulphadiazine	Trimethoprim	Tylosin
Molar mass	358.2 a	359.4 a	406.5 a	351.4 a	460.4 a	250.28 a	290.32 a	916.1 a
Solubility in water mg/L (20ºC)	44000 b	70 c	408 d	0.022 e	195 d	77 d	17.52 d	0.005 d
Half-life soil (20ºC) (days)	7.35 f	4.5d g	9d h	3.62-6.22 i	15.91 j	3 k	>84d l	
Freundlich isotherm (Kf)	0.94 m	0.85 n	0.85 o		1.06 p	0.83 q	1.06 r	15.94 s
Adsorption-desorption coefficient KOM l/kg KOC l/kg	K_{oc} 122.3 K_{om} 210.36 m	K_{om} 397.3 K_{oc} 230.99 n	K_{om} 90.27 K_{oc} 52.36 0	15.31 K_{oc} i	K_{oc} 148.92 K_{om} 256.14 p	32.68 k_{oc} 56.21 K_{om} q	622 K_{oc} 1069 K_{om} r	553-7988 k_{oc} v

References: a Hertfordshire, (2013); b Chem Spider; c Seedher and Agarwal, (2009); d Yalkowsky; e Meloxicam Product Description, (2017); f Qiu *et al*., (2021); g Chung *et al*., (2017); h Albero *et al*., (2018); i Jiménez *et al.,* 2018; j Chen *et al*., (2014); k Kreuzig and Holtge, (2005); l Lin and Gan, (2011); m Ma *et al.,* 2021; n Álvarez -Esmorís *et al.*, 2020; o Wang *et al.,* 2012; p Kim *et al.,* 2012; q Doretto and Rath, 2013; r Zhang *et al.,* 2014; s Sassman *et al*., (2007); v Loke *et al*., 2002.

Type: f – sandy loam; g sandy soil; h - range of agricultural soils from Spain; i - river sediment; j - geomean of 3 unknown soils; k - geomean of six differing soils from varying regions; l - medium loam; m - geomean of clay, sandy-loam and loam; n - geomean of 16 soils from Spain; o - sandy clay loam, sandy loam and clay loam; p - geomean of sandy, clay, and sandy clay; q - loamy sand; s - geomean of six soils, clay loam-sandy soils; p - geomean of silt loam and sandy loam (pH dependent); v - Range of four soil types.

SI Table D2: Summary the pig slurry properties that were utilized within this study, specifically the table relates to the dissolved available fraction of the slurry.

Redox Potential (mV)	pH	DOC, DIC, DC (mg/L)	NH_4^{-1}, NO_2 (mg/L)	PO_4 (mg/L)
-324	7.15	1642±101.18, 559.78±20.76, 2201.78.±121.91	195.96±8.72, 0.27±0.04	2.39±0.49

Table D3: Irrigation validation utilizing 15 cups over the boxes surface area. (See Figure C1).

Cup	Irrigator 1	Irrigator 2	Irrigator 3	Irrigator 4	Irrigator 5	Irrigator 6
1	37.2	42.53	40.00	36.69	38.71	44
2	45.38	47.63	47.12	43.33	45.08	31.88
3	48.57	48.02	53.37	45.38	52.16	56.79
4	42.67	45.45	41.95	37.59	41.57	45.86
5	47.11	49.05	48.05	43.23	46.75	35.99
6	47.37	49.53	53.49	44.94	54.49	53.57
7	44.59	47.6	43.58	38.06	43.85	46.73
8	48.98	49.01	49.2	44.08	47.9	39.44
9	44.43	48.32	52.66	44.49	55.36	52.26
10	47.25	47.42	43.09	40.08	45.19	51.41
11	46.95	48.04	47.42	44.55	49.31	45.55
12	45.74	46.02	48.93	44.32	54.05	51.6
13	47.32	46.65	40.72	41.48	44.04	48.22
14	46.81	45.14	44.81	45.75	49.1	49.8
15	43.57	42.14	43.13	44.98	52.03	49.59
Total	683.94	702.55	697.52	638.95	719.59	702.69

Table D4: Total percentage difference in rainfall applied from the irrigators.

	Irrigator 1	Irrigator 2	Irrigator 3	Irrigator 4	Irrigator 5	Irrigator 6
Box 1		2.65	1.95	6.58	4.95	2.67
Box 2	2.65		0.72	9.05	2.37	0.02
Box 3	1.95	0.72		8.40	3.07	0.74
Box 4	6.58	9.05	8.40		11.21	9.07
Box 5	4.95	2.37	3.07	11.21		2.35
Box 6	2.67	0.02	0.74	9.07	2.35	

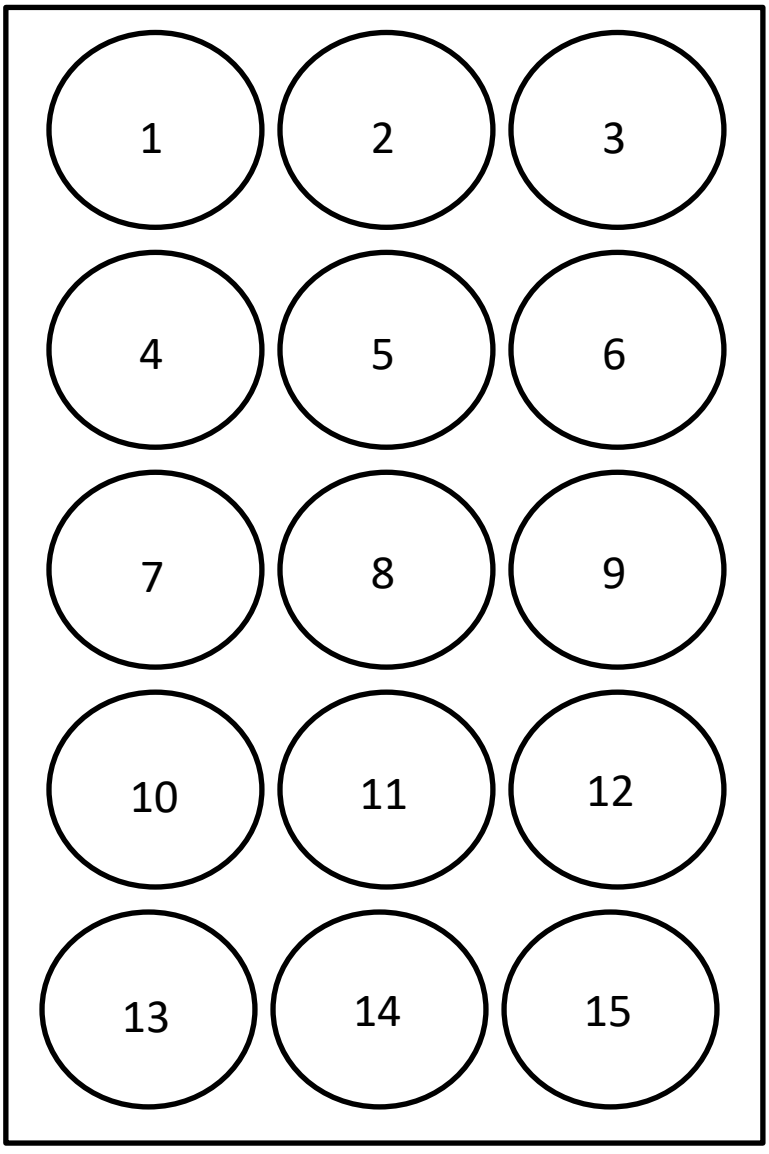


Figure D1: Irrigation validation and the cups placement over the surface area of the boxes used in the study.

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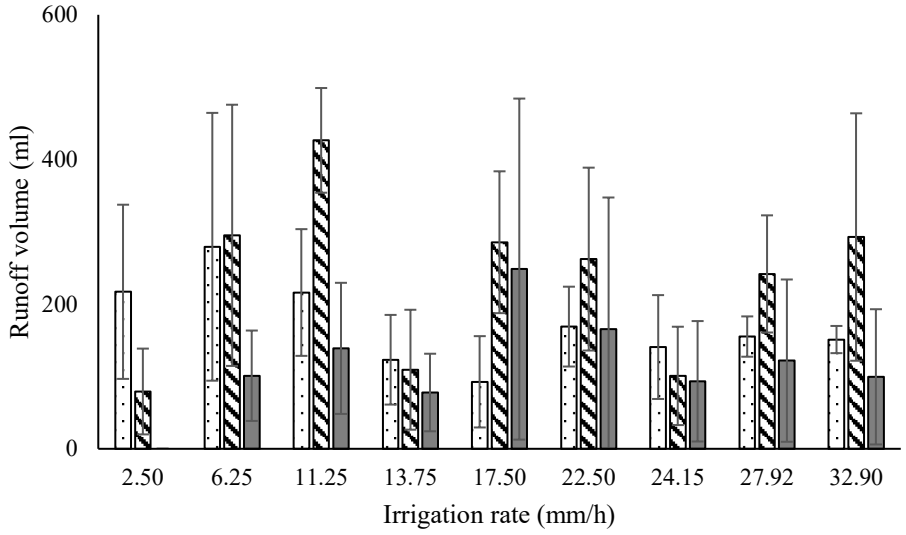
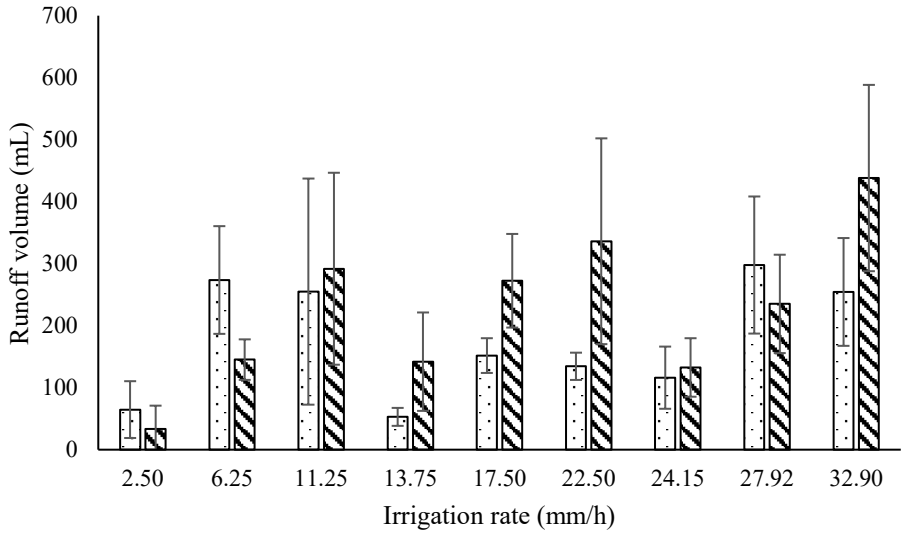
a SCIEX 5500+ quadrupole MS system.

Analyte	Retention time (minutes)	Q1	Q3	Dwell time	Entrance potential	Collision cell exit potential	Collision energy	LOD (µg/mL)	LOQ (µg/mL)
ENR	4-4.7	360.1	342.3	20	6	31	25	0.000005	0.00001
			316.3	20	6	25	25		
			245	20	8	38	25		
FLO		358	340.1	20	10	12	12	0.00001	0.0001
			241.1	20	10	12	24		
			130.2	20	10	12	69		
LNC		407.6	357.3	20	10	25	10	0.00001	0.0001
			359.9	20	10	27	10		
			126.3	20	10	34	10		
MLX		252.1	141.1	20	10	35	10	0.00001	0.005
			115.1	20	10	40	10		
			73	20	10	80	10		
OTC	2.0-2.1	461.8	444.8	50	10	10	24	0.0001	0.0005
			427.2	50	10	10	31		
			201.4	50	10	10	56		
SDZ		251.1	156.1	20	10	32	10	0.00001	0.00005
			106.2	20	10	35	10		
			92.1	20	10	35	10		
TMP		291.1	275.2	20	10	34	15	0.000001	0.00001
			261.2	20	10	33	15		
			230.2	20	10	33	15		
			774	50	12	12	47		

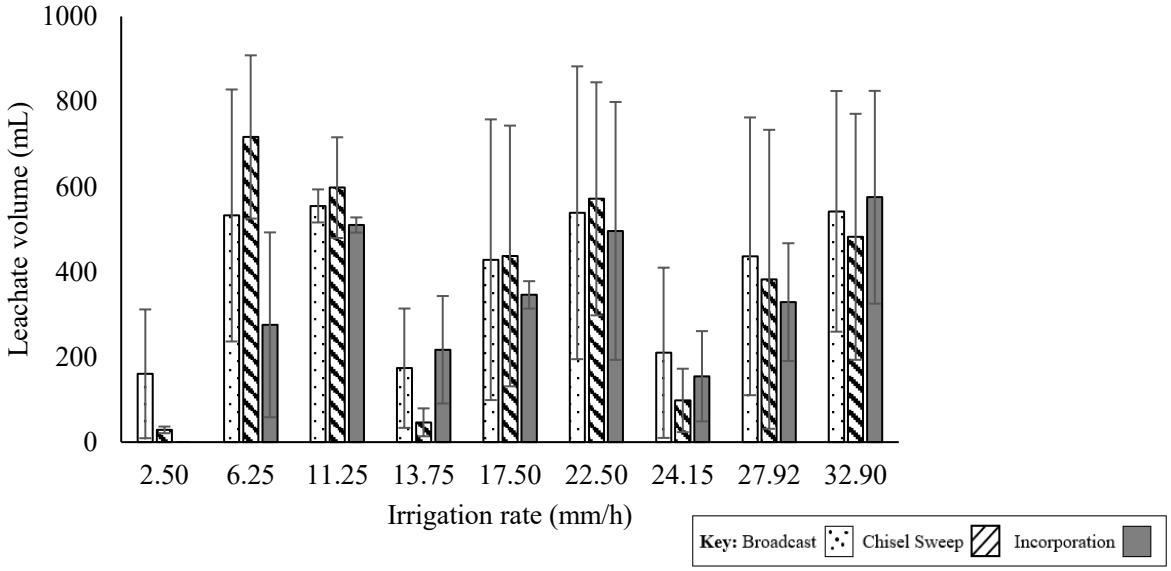
TYL	3.34-3.36	917.8	174.8	50	12	12	55	0.00002	0.0001
			88.3	50	12	12	230		



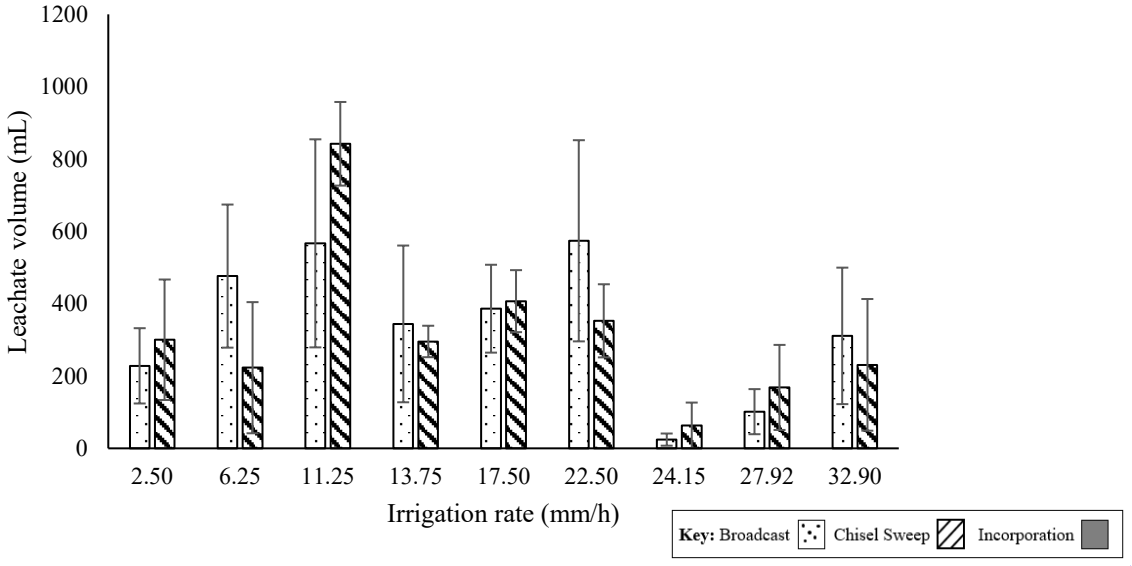
SI Figure D2: Surface runoff volumes for manured soils under varying application techniques over three irrigation events (days). Key: Broadcast Chisel Sweep Incorporation



SI Figure D3: Surface runoff volumes for manured pasture under varying application techniques over three irrigation events (days). Key: Broadcast Chisel Sweep Incorporation



SI Figure D4: Leachate volumes for manured soils under varying application techniques over three irrigation events (days).



SI Figure D5: Leachate volumes for manured pasture under varying application techniques over three irrigation events (days).

SI Table D6: **Bare soil runoff and leachate volumes collected throughout the irrigation events under varying application techniques.**

			Soil Runoff (mL)			Soil Leachate (mL)		
Replicate	Irrigation event	Timepoint	BC	CS	IC	BC	CS	IC
1		2.5	271.67	93.49	0	6.57	39.67	0
2		2.5	329.36	143.62	0	108.73	24.92	0
3		2.5	50.07	0.48	0	366.11	21.68	0
1		6.25	78.85	388.75	45.01	597.57	873.83	145.4
2	1	6.25	525.34	454.04	69.8	141.73	446.92	99.68
3		6.25	233.61	42.6	188.19	857.91	829.93	581.48
1		11.25	262.85	426.34	162.54	502.57	483.06	500.2
2		11.25	93.34	515.04	17.9	595.73	760.63	535.15
3		11.25	292	337.78	236.04	566.28	549.78	494.74
1		13.75	210.5	93.04	5.9	40.04	2.81	377.09
2		13.75	85.01	217.98	92.96	113.67	81.86	205.35
3		13.75	73.82	16.89	134.59	367.25	54.936	68.52
1		17.5	174.32	305.49	134.96	93.81	137.2	391.01
2	2	17.5	82.93	394.43	33.66	314.58	316.86	317.8
3		17.5	20.61	156.78	576.64	877.07	857.78	328.82
1		22.5	182.16	256.81	2.97	168.89	351.01	247.92
2		22.5	95.67	419.73	73.15	450.23	406.29	317.8
3		22.5	228.92	110.23	419.62	997.28	957.38	922.61
1		24.15	139.98	154.1	2.97	6.89	9.97	247.92
2		24.15	53.07	143.39	73.15	140.79	91.88	6.44
3		24.15	228.92	4.81	203.84	481.88	192.11	209.9
1		27.915	142.76	239.5	25.17	101.65	85.82	424.92
2	3	27.915	128.99	342.05	61.31	329.02	185.85	133.27
3		27.915	193.74	143.73	279.17	878.82	875.72	428.51
1		32.9	172.9	342.55	12.75	211.47	296.37	576.19
2		32.9	153.12	472.82	56.43	512.82	260.08	268.68
3		32.9	127.03	62.87	229.21	902.03	890.42	880.83

Footnote: Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC).

Table D7: Pasture surface runoff and leachate volumes collected throughout the irrigation events under varying application techniques.

			Grass Runoff (mL)		Grass Leachate (mL)	
Replicate	**Irrigation event**	**Irrigation rate mm/h**	**BC**	**CS**	**BC**	**CS**
1		2.50	127.59	8.05	104.89	494.23
2		2.50	46.39	86.46	219.98	318.94
3		2.50	19.96	6.328	359.43	87.54
1		6.25	304.11	139.04	545.55	123.88
2	**1**	6.25	155.34	108.67	676.28	477.29
3		6.25	361.54	188.03	206.85	67.69
1		11.25	417.3	320.81	196.02	894.62
2		11.25	347.3	89.43	607.18	949.91
3		11.25	0.27	465.29	897.09	681.73
1		13.75	50.57	51.89	245.13	244.34
2		13.75	71.68	128.96	644.62	291.43
3		13.75	36.44	244.98	142.27	350.69
1		17.50	170.69	169.26	256.28	462.78
2	**2**	17.50	172.43	300.36	353.59	472.3
3		17.50	112.19	347.80	548.50	285.93
1		22.50	109.30	159.83	457.86	292.03
2		22.50	131.20	290.29	306.37	270.70
3		22.50	162.87	558.47	957.29	495.00
1		24.15	172.97	177.85	3.15	15.42
2		24.15	124.42	67.50	44.31	152.69
3		24.15	51.00	152.47	24.94	21.36
1		27.92	454.00	130.11	79.50	15.02
2	**3**	27.92	226.26	253.46	186.28	189.56
3		27.92	213.23	321.82	38.35	300.67
1		32.90	169.85	649.91	64.69	121.96
2		32.90	373.99	346.79	345.19	487.30
3		32.90	219.41	317.60	523.04	83.27

Footnote: Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC).

SI Table D8: The total cumulative mass of veterinary medicines transported from manured soils into surface runoff and leachate.

		Runoff (µg)			Leachate (µg)		
Veterinary Medicine	**Irrigation Rate (m/h)**	**BC**	**CS**	**IC**	**BC**	**CS**	**IC**
	2.5-11.25	411.70	197.50	61.27	76.14	9.79	3.32
	13.75-22.50	331.57	132.18	9.76	17.72	7.96	0.65
ENR	24.15-32.90	246.70	118.25	5.85	6.24	16.95	2.28
	2.5-11.25	5541.04	2886.48	1420.33	2317.67	986.00	441.00
	13.75-22.50	2081.17	1232.80	1268.67	1315.67	434.01	437.23
FLO	24.15-32.90	1486.50	780.23	652.06	550.52	367.00	473.13
	2.5-11.25	2635.00	1190.50	345.67	1390.13	498.63	69.73
	13.75-22.50	626.10	770.50	232.43	553.97	180.08	49.49
LNC	24.15-32.90	450.60	511.93	111.26	183.21	177.40	75.23
	2.5-11.25	2873.00	955.00	660.47	4194.37	1643.65	390.75
	13.75-22.50	617.10	514.77	468.48	3155.65	603.62	30.52
MLX	24.15-32.90	621.30	948.70	253.75	300.85	628.39	269.71
	2.5-11.25	74.77	54.02	6.32	37.71	12.13	5.08
	13.75-22.50	70.02	37.95	0.33	8.42	3.56	0.18
OTC	24.15-32.90	71.65	35.13	0.16	3.79	5.71	0.88
	2.5-11.25	1489.33	997.67	769.63	1297.13	491.87	149.90
	13.75-22.50	565.37	740.00	518.40	539.20	297.25	421.53
SDZ	24.15-32.90	360.80	373.20	167.89	175.56	238.40	175.37
	2.5-11.25	531.13	213.92	81.40	633.90	141.00	48.76
	13.75-22.50	362.10	164.55	34.01	195.86	62.57	4.29
TMP	24.15-32.90	197.79	100.50	8.57	59.39	102.98	21.47
	2.5-11.25	11560.00	29165.00	4.35	26979.00	2490.50	3847.50
	13.75-22.50	7518.00	4069.50	0.92	2644.60	643.10	37.80
TYL	24.15-32.90	3229.50	1798.35	0.00	382.75	322.88	77.55

Footnote: *Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC). Enrofloxacin (ENR), florfenicol (FLO), lincomycin (LNC), meloxicam (MLX), oxytetracycline (OTC), sulfadiazine (SDZ), tylosin (TYL), trimethoprim (TMP).*

SI Table D9: The total cumulative mass of veterinary medicines transported from manured pasture into surface runoff and leachate.

		Runoff (µg)		Leachate (µg)	
Veterinary Medicine	**Irrigation Rate (mm/hr)**	**BC**	**CS**	**BC**	**CS**
	2.5-11.25	74.40	46.66	44.30	10.72
	13.75-22.50	82.80	84.63	27.78	25.49
ENR	24.15-32.90	44.20	58.11	0.70	7.66
	2.5-11.25	1152.67	1029.00	653.08	375.78
	13.75-22.50	1160.00	1216.67	295.67	348.23
FLO	24.15-32.90	662.00	597.67	114.06	100.09
	2.5-11.25	627.00	687.33	176.82	82.75
	13.75-22.50	551.93	594.67	66.23	93.09
LNC	24.15-32.90	315.53	305.33	20.72	25.84
	2.5-11.25	287.55	140.73	244.67	40.92
	13.75-22.50	694.73	2206.60	145.45	85.37
MLX	24.15-32.90	613.17	377.54	17.67	21.65
	2.5-11.25	129.89	127.31	84.72	52.18
	13.75-22.50	82.00	93.70	28.70	24.08
OTC	24.15-32.90	48.17	55.48	1.35	4.97
	2.5-11.25	132.76	79.70	113.82	64.95
	13.75-22.50	55.43	39.38	28.66	35.71
SDZ	24.15-32.90	11.85	10.06	11.85	10.06
	2.5-11.25	82.43	57.51	16.81	3.31
	13.75-22.50	91.17	121.57	12.04	15.71
TMP	24.15-32.90	54.20	74.17	0.23	2.74
	2.5-11.25	5650.00	3627.33	2244.83	1291.00
	13.75-22.50	4907.67	2904.33	1251.00	1353.33
TYL	24.15-32.90	1819.67	1600.67	926.67	963.67

Footnote: *Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC). Enrofloxacin (ENR), florfenicol (FLO), lincomycin (LNC), meloxicam (MLX), oxytetracycline (OTC), sulfadiazine (SDZ), tylosin (TYL), trimethoprim (TMP).*

SI Table D10: The percentage difference between veterinary medicine mass loads between manure application treatments on bare soils and grasses and their statistical significance (p value) calculated via an analysis of variance (three-way).

			Veterinary Medicine							
	Treatments	**Parameter**	**ENR**	**FLO**	**LNC**	**MLX**	**OTC**	**SDZ**	**TMP**	**TYL**
Bare Soil runoff	**BC-IC**	Percentage	92%	60%	82%	66%	97%	40%	89%	87%
		p value	9.90E-08	8.10E-07	9.90E-09	0.016	9.9E-08	0.097	1.05E-05	0.00102
	BC-CS	Percentage	55%	48%	37%	41%	41%	13%	56%	29%
		p value	1.07E-07	1.60E-05	7.30E-06	0.107	3.40E-05	0.465	0.51	0.00688
	CS-IC	Percentage	83%	24%	72%	43%	95%	31%	74%	91%
		p value	0.00035	0.59	7.00E-07	0.709	3.30E-05	0.628	0.000044	0.00014
Bare Soil leachate	**BC-IC**	Percentage	94%	70%	91%	91%	88%	66%	92%	87%
		p value	4.00E-05	1.20E-07	0.00001	0.0078	0.00011	0.00027	0.714	0.00273
	BC-CS	Percentage	65%	60%	63%	62%	57%	49%	67%	88%
		p value	0.00068	4.70E-07	0.0003	0.0074	0.00135	0.126	0.846	0.00261
	CS-IC	Percentage	17%	24%	24%	24%	29%	34%	24%	13%
		p value	0.01	0.0074	0.0046	0.0099	0.00552	0.77	0.97	0.0099
Grass runoff	**BC-CS**	Percentage	15%	2%	8%	25%	72%	12%	14%	8%
		p value	0.76	0.12	0.32	0.52	0.56	0.77	0.79	0.10
Grass leachate	**BC-CS**	Percentage	37%	27%	23%	61%	44%	28%	26%	97%
		p value	0.13	0.06	0.20	0.08	0.02	0.10	0.19	0.01

Footnote: *Broadcast application (BC), Chisel Sweep (CS), Incorporation (IC). Enrofloxacin (ENR), florfenicol (FLO), lincomycin (LNC), meloxicam (MLX), oxytetracycline (OTC), sulfadiazine (SDZ), tylosin (TYL), trimethoprim (TMP).*

Appendix D references

Albero, B., Tadeo, J.L., Escario, M., Miguel, E. and Pérez, R.A., 2018. Persistence and availability of veterinary antibiotics in soil and soil-manure systems. *Science of the Total Environment*, 643, pp.1562-1570.

Álvarez-Esmorís, C., Conde-Cid, M., Ferreira-Coelho, G., Fernández-Sanjurjo, M.J., Núñez-Delgado, A., Álvarez-Rodríguez, E. and Arias-Estévez, M., 2020. Adsorption/desorption of sulfamethoxypyridazine and enrofloxacin in agricultural soils. *Science of The Total Environment*, 706, p.136015.

Álvarez-Esmorís, C., Conde-Cid, M., Ferreira-Coelho, G., Fernández-Sanjurjo, M.J., Núñez-Delgado, A., Álvarez-Rodríguez, E. and Arias-Estévez, M., 2020. Adsorption/desorption of sulfamethoxypyridazine and enrofloxacin in agricultural soils. *Science of The Total Environment*, 706, p.136015.

Chemspider. 2022. Florfenicol | C12H14Cl2FNO4S | ChemSpider. [online] Available at: <http://www.chemspider.com/Chemical-Structure.102776.html> [Accessed 15 February 2022].

Chen, G.X., He, W.W., Wang, Y., Zou, Y.D., Liang, J.B., Liao, X.D. and Wu, Y.B., 2014. Effect of different oxytetracycline addition methods on its degradation behavior in soil. *Science of the Total Environment*, 479, pp.241-246.

Chung, H.S., Lee, Y.J., Rahman, M.M., Abd El-Aty, A.M., Lee, H.S., Kabir, M.H., Kim, S.W., Park, B.J., Kim, J.E., Hacımüftüoğlu, F. and Nahar, N., 2017. Uptake of the veterinary antibiotics chlortetracycline, enrofloxacin, and sulphathiazole from soil by radish. *Science of the total Environment*, 605, pp.322-331.

Doretto, K.M. and Rath, S., 2013. Sorption of sulfadiazine on Brazilian soils. *Chemosphere*, 90(6), pp.2027-2034.

Hertfordshire, U.O., 2013. o. The Veterinary Medicine Properties DataBase (VMDB) developed by the Agriculture & Environment Research Unit (AERU). *University of Hertfordshire, 2006-2013*.

Jiménez, Juan J.; Muñoz, Beatriz E.; Sánchez, María I.; Pardo, Rafael (2018). Forced and long-term degradation assays of tenoxicam, piroxicam and meloxicam in river water. Degradation products and adsorption to sediment. *Chemosphere, 191(), 903–910.* doi:10.1016/j. *Chemosphere.*2017.10.056.

Kim, Y., Lim, S., Han, M. and Cho, J., 2012. Sorption characteristics of oxytetracycline, amoxicillin, and sulfathiazole in two different soil types. Geoderma, 185, pp.97-101.

Kreuzig, R. and Höltge, S., 2005. Investigations on the fate of sulfadiazine in manured soil: laboratory experiments and test plot studies. Environmental Toxicology and Chemistry: *An International Journal*, 24(4), pp.771-776.

Lin, K. and Gan, J., 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. *Chemosphere*, 83(3), pp.240-246.

Ma, W., Xu, X., An, B., Zhou, K., Mi, K., Huo, M., Liu, H., Wang, H., Liu, Z., Cheng, G. and Huang, L., 2021. Single and ternary competitive adsorption-desorption and degradation of amphenicol antibiotics in three agricultural soils. *Journal of Environmental Management*, 297, p.113366.

Meloxicam Product Description., 2017. Chemicalbook.com. Available from: https://www.chemicalbook.com/ChemicalProductProperty_US_CB2191355.aspx.

Qiu, M., Hu, A., Yu-ming, M.H., Zhao, Y., He, Y., Xu, J. and Lu, Z., 2021. Elucidating degradation mechanisms of florfenicol in soil by stable-isotope assisted nontarget screening. *Journal of Hazardous Materials*, 403, p.123974.

Sassman, S.A., Sarmah, A.K. and Lee, L.S., 2007. Sorption of tylosin A, D, and A-aldol and degradation of tylosin A in soils. Environmental Toxicology and Chemistry: *An International Journal*, 26(8), pp.1629-1635.

Seedher, N. and Agarwal, P., 2009. Various solvent systems for solubility enhancement of enrofloxacin. *Indian Journal of Pharmaceutical Sciences*, 71(1), p.82.

Wang, C., Teppen, B.J., Boyd, S.A. and Li, H., 2012. Sorption of lincomycin at low concentrations from water by soils. *Soil Science Society of America Journal*, 76(4), pp.1222-1228.

Yalkowsky, S.H. and He, Y., 2003. An extensive compilation of aqueous solubility data for organic compounds extracted from the AQUASOL database. Handbook of Aqueous Solubility Data, p.377.

Zhang, Y.L., Lin, S.S., Dai, C.M., Shi, L. and Zhou, X.F., 2014. Sorption–desorption and transport of trimethoprim and sulfonamide antibiotics in agricultural soil: effect of soil type, dissolved organic matter, and pH. *Environmental Science and Pollution Research*, 21(9), pp.5827-5835.

Appendix E: Supplementary materials for Chapter 5

SI Table E1: Mass spectrometer details for each analyte within the study. Obtained using a SCIEX 5500+ quadrupole MS system.

Analyte	Retention time (minutes)	Q1	Q3	Dwell time	Entrance potential	Collision cell exit potential	Collision energy
AZA	2.5-2.6	311.3	227.3		10	150	30
			165.2	20	10	150	91
			115.1		10	150	117
ALT	2.1-2.2	328.5	165.2		10	120	32
			123.1	20	10	120	60
			121.1		10	120	30
CFT	2.22-2.43	525.1	125.3	50	10	10	83
			126.2	50	10	10	74
			127.2	50	10	10	64
ENR	2.71-2.75	360.1	342.3	20	6	31	25
			316.3	20	6	25	25
			245	20	8	38	25
FLO	4-4.7	358	340.1	20	10	12	12
			241.1	20	10	12	24
			130.2	20	10	12	69
LNC	2.51-2.57	407.6	357.3	20	10	25	10
			359.9	20	10	27	10
			126.3	20	10	34	10
MLX	3.91-3.95	252.1	141.1	20	10	35	10
			115.1	20	10	40	10
			73	20	10	80	10
OTC	2.0-2.1	461.8	444.8	50	10	10	24
			427.2	50	10	10	31
			201.4	50	10	10	56
		251.1	156.1	20	10	32	10

SDZ	2.26-2.33		106.2	20	10	35	10
			92.1	20	10	35	10
TMP	4.2-4.35	291.1	275.2	20	10	34	15
			261.2	20	10	33	15
			230.2	20	10	33	15

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Si Table E2: Summary of LOQs and LODs for the compounds detected in this study

	Pig slurry		Soil		Groundwater	
Compound	**LOQ (ug/kg)**	**LOD (ug/kg)**	**LOQ (ug/kg)**	**LOD (ug/ml)**	**LOQ (ug/ml)**	**LOD (ug/ml)**
Sulfadiazine	0.01	0.05	0.25	0.01	0.0001	0.0001
Lincomycin	0.01	0.05	0.01	0.005	0.0001	0.000001
Trimethoprim	0.005	0.0001	0.01	0.005	0.0001	0.000001
Oxytetracycline	1	0.05	0.01	0.005	0.0001	0.000001

SI Table E3: Pig slurry extraction validation to three concentration levels.

Analyte	**1.75 mg/kg**	**RSD**	**1 mg/kg**	**RSD**	**0.1 mg/kg**	**RSD**
Azaperone	6.88	39.86	7.39	9.75	4.06	71.11
Altrenogest	26.22	26.27	35.99	12.26	35.52	23.46
Ceftiofur	82.73	6.19	79.69	7.15	76.85	19.47
Enrofloxacin	18.63	36.26	17.63	12.74	10.73	25.34
Meloxicam	38.10	6.72	39.47	10.45	33.56	37.52
Oxytetracycline	77.18	4.88	83.94	4.71	84.19	11.67
Florfenicol	81.27	8.72	85.48	6.83	82.21	13.49
Lincomycin	89.37	2.01	95.85	3.26	90.04	14.26
Sulfadiazine	100.52	3.09	70.96	2.16	94.83	9.86
Trimethoprim	59.37	7.58	94.35	1.01	84.79	3.75

Footnote: *RSD = relative standard deviation (n=5).*

SI Table E4: Validation of soil extraction method to three concentrations for both a clay and a sandy soil.

	Clay soil						Sandy soil					
	0.5		0.05		0.005		0.5		0.05		0.005	
Analyte	mg/kg	RSD	mg/kg	RSD	mg/kg	RSD	mg/kg	RSD	mg/kg	RSD	mg/kg	RSD
Azaperone	0.87	0.72	8.72	1.27	bdl		41.74	6.38	33.50	10.84	67.16	24.35
Altrenogest	99.89	8.55	93.56	4.34	106.62	13.40	87.91	5.16	98.33	8.57	92.75	4.98
Ceftiofur	74.79	8.49	66.12	13.04	bdl		89.74	14.63	102.56	5.92	bdl	
Enrofloxacin	2.02	0.59	2.29	7.93	bdl		3.22	6.46	2.11	20.01	bdl	
Meloxicam	96.35	10.47	85.80	11.81	110.81	9.46	101.85	7.02	71.73	45.61	104.25	5.95
Oxytetracycline	31.13	0.45	27.66	0.2	28.98	2.12	103.4	8.17	78.72	6.49	101.43	5.38
Florfenicol	84.37	9.34	105.99	9.94	99.84	5.19	84.39	9.41	106.16	10.14	105.84	17.80
Lincomycin	45.07	3.03	46.10	2.38	50.84	6.38	102.24	4.61	105.04	4.54	106.94	7.95
Sulfadiazine	95.86	10.52	97.42	2.63	85.37	1.14	79.04	5.79	99.82	4.82	109.26	6.08
Trimethoprim	13.24	91.37	21.81	1.79	27.62	6.08	39.60	3.98	40.38	4.27	37.67	4.20

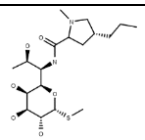
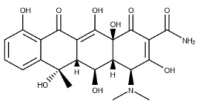
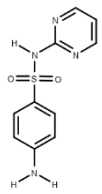
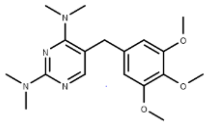
Footnote: *RSD = relative standard deviation (n=5). bdl = Below Detectable Limits*

SI Table E5: Groundwater SPE validation to three concentration levels.

Analyte	20 ng/L	RSD%	10 ng/L	RSD%	1 ng/L	RSD%
Azaperone	9.39	28.85	20.50	36.02	20.50	36.02
Altrenogest	77.27	3.92	bdl		bdl	
Ceftiofur	106.76	7.07	97.75	12.76	85.40	21.52
Enrofloxacin	37.82	12.10	14.67	4.30	77.12	22.25
Meloxicam	105.90	17.46	108.19	32.79	60.61	11.23
Oxytetracycyline	96.06	7.99	129.01	22.45	70.54	3.95
Florfenicol	96.40	7.45	96.41	11.69	100.23	5.54
Lincomycin	100.02	10.68	101.10	7.46	105.75	3.09
Ssulfadiazine	80.35	5.56	71.23	4.73	79.88	3.29
Trimethoprim	101.52	5.76	78.94	6.04	110.17	5.64

Footnote: *RSD = relative standard deviation (n=5). bdl = Below detectable limits.*

SI Table E6: Summary of the manure degradation data used to derive the PECsoil refined.

Veterinary medicine	Structure	Pig slurry DT_{50} (days)
Lincomycin		100 Loftin, (2006)
Oxytetracycline		79 (Blackwell et al., 2005)
Sulfadiazine		20 (Lamshöft et al., 2010)
Trimethoprim		76.78 Wu et al., (2012)

Appendix E References

Lamshöft, M., Sukul, P., Zühlke, S. and Spiteller, M., 2010. Behaviour of 14C-sulfadiazine and 14C-difloxacin during manure storage. *Science of the Total Environment*, 408(7), pp.1563-1568.

Blackwell, P.A., Boxall, A.B., Kay, P. and Noble, H., 2005. Evaluation of a lower tier exposure assessment model for veterinary medicines. *Journal of Agricultural and Food Chemistry*, 53(6), pp.2192-2201.

Loftin, K.A. 2006. Th e effects and fate of selected veterinary antibiotics in two Missouri anaerobic swine lagoons. Ph.D. diss. Univ. of Missouri, Rolla, MO.

Wu, Yang; Williams, Mike; Smith, Lester; Chen, Donghui; Kookana, Rai (2012). Dissipation of sulfamethoxazole and trimethoprim antibiotics from manure-amended soils. *Journal of Environmental Science and Health*, Part B, 47(4), 240–249. doi:10.1080/03601234.2012.636580