

Chapter 10

Fate of Veterinary Pharmaceuticals in Agroecosystems



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Abstract Veterinary pharmaceuticals, which are increasingly used in animal production practices, can enter surface and groundwater after land application of animal manures or animal wastewater. The presence of veterinary pharmaceuticals can result in negative environmental impacts including the proliferation of environmental antibiotic resistance and endocrine-disrupting effects in aquatic organisms. The efficacy of manure application strategies to limit the occurrence of veterinary pharmaceuticals in runoff and best management practices to remove these compounds from runoff prior to entering surface water should be investigated to mitigate the impact of these compounds on the environment.

10.1 Introduction

Veterinary pharmaceuticals are used regularly in the livestock industry as growth promoters, to improve feed efficiency, for disease prevention, or as part of therapeutic treatment. Biologically active pharmaceuticals used in animal production include antimicrobials, steroid hormones, and beta agonists, such as ractopamine which is used in swine and cattle production. The amount of antimicrobials used in the agriculture industry in the United States has been estimated between 8.5 million kg [31] and 12.6 million kg. Although reliable data regarding the usage of antibiotics in animal production are difficult to find, over half of the antibiotics consumed in the United States are used in animal agriculture [27]. Similarly, steroid hormones are given to nearly all of the approximately 32 million beef cattle produced in the United States annually, with dosage amounts up to hundreds of milligrams, depending on the implant or feed additive administered [24].

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Veterinary pharmaceuticals administered to animals are excreted in manures, and up to 90% of certain pharmaceuticals may be excreted unmetabolized [18]. Pharmaceutical concentrations in manure have been reported to range from trace levels to hundreds of milligrams per kilogram [35, 37]. For centuries, minimally treated animal manures have been applied to agricultural fields as a soil conditioner and fertilizer. In 2015, the United States produced a record high 94 billion pounds of red meat and poultry, despite an 80% decline in the number of animal production facilities since the 1950s [14]. This trend of increasing geographic density of animal production in the United States unavoidably results in increased water quality degradation by conventional contaminants such as nutrients and pathogens as well as veterinary pharmaceuticals due to runoff from fields where animal manures are land applied [40]. The presence and activity of antimicrobials in manure can increase antimicrobial resistant bacteria, even at low antimicrobial concentrations [15], while the occurrence of other veterinary pharmaceuticals, such as steroid hormones, can lead to endocrine-disrupting effects in aquatic organisms [1, 34, 42].

The fate pathways for veterinary pharmaceuticals in agroecosystems are shown in Fig. 10.1. Veterinary pharmaceuticals are released into animal manure or wastewater, which is typically stored on site in manure pits (swine), in stockpiles or compost piles (beef cattle, dairy, and poultry), or in wastewater lagoons. Both animal manures and wastewaters are routinely land applied as an organic fertilizer and soil conditioner. Once applied to land, the veterinary pharmaceuticals can be transported to surface water via runoff or infiltrate into soil and be transported to groundwater. This chapter reviews the occurrence of veterinary pharmaceuticals in surface water and describes practices that may limit their transport after land application of manure to crop fields.

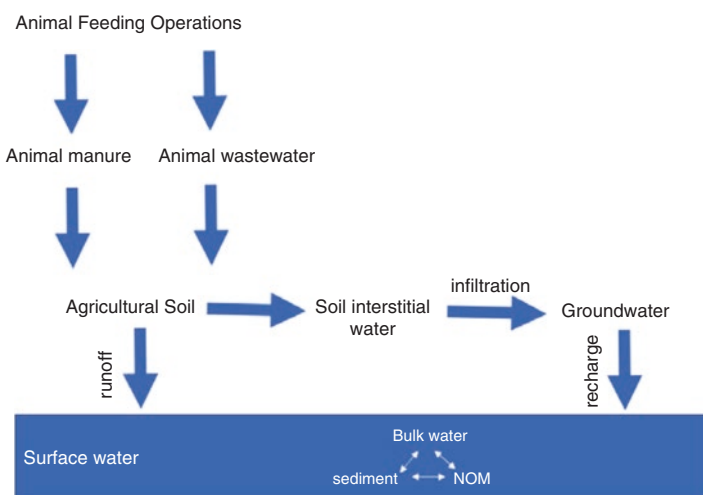


Fig. 10.1 Fate pathways for veterinary pharmaceuticals in the agro-ecosystem

10.2 Occurrence of Veterinary Pharmaceuticals in Agroecosystems

The occurrence of veterinary pharmaceuticals in animal manures has been well-documented. Comprehensive reviews regarding the concentrations and types of veterinary pharmaceuticals in animal manures are provided by Sarmah et al. [35] and Song and Guo [37]. Numerous classes of antimicrobials have been detected in manures from swine, cattle, and poultry production at concentrations ranging from trace levels to hundreds of mg/kg [37]. Although there is significant evidence showing that pharmaceuticals can be transported to surface water in runoff from land-applied manure, to date, a limited number of studies have evaluated the fate and persistence of antibiotics in surface waters in intensively agricultural watersheds with minimal municipal wastewater inputs.

Jaimes Correa et al. [17] previously documented the occurrence and persistence of pharmaceuticals in an intensively agricultural watershed in. In this study, the occurrence of pharmaceuticals was monitored in the Shell Creek watershed in east-central Nebraska. This watershed is approximately 1200 km², and the five communities within the watershed have a combined population of 1675 people. By contrast, the counties comprising the watershed include 1550 farms with over one million head of swine, cattle, and poultry. Cultivated land cover within the watershed is 78.2%, while urban developed areas are only 4.4%. During this monitoring study, occurring from September 2008 through October 2009, the presence of 12 veterinary pharmaceuticals was detected using a LC-MS/MS analysis method in at least one sampling event with concentrations ranging from 0.0003 to 68 ng/L (Fig. 10.2). As shown in Fig. 10.2, ANOVA reveals significant differences in mean concentrations between antibiotics ($p < 0.01$). Results from Tukey's multiple comparison test are represented by letters. Antibiotics with similar letters (e.g., "a" and "ab") have no significant differences in mean concentrations ($p > 0.05$) while antibiotics with different letters ("a" and "b") have significant differences in mean concentrations ($p < 0.05$). The compounds detected at the highest time-weighted average (TWA) concentrations in Shell Creek were lincomycin (68 ng/L) and monensin (49 ng/L). Tiamulin, sulfadimethoxine, and sulfamethazine had maximum concentrations of 2.6, 3.9, and 13 ng/L, respectively. Dissolved concentrations of the beta agonist, ractopamine, three sulfonamide-group antibiotics sulfachloropyridazine, sulfamethazole, sulfamethoxazole, and the macrolide tylosin were all detected at average concentrations less than 1 ng/L [17]. In this study, increased antibiotic concentrations were identified in the summer months and were likely driven by rainfall-runoff events [17]. This finding is consistent with other studies that have identified increases in antibiotic concentrations in agricultural watersheds in the summer months [30]. Although some temporal trends were observed, it should be noted that antibiotics were detected in each monthly sampling event, indicating that pharmaceuticals can persist in surface water, even if they are introduced via episodic runoff events. In urban or suburban watersheds, the predominant source of veterinary pharmaceuticals is from municipal wastewater effluents, which are more continuous sources. The

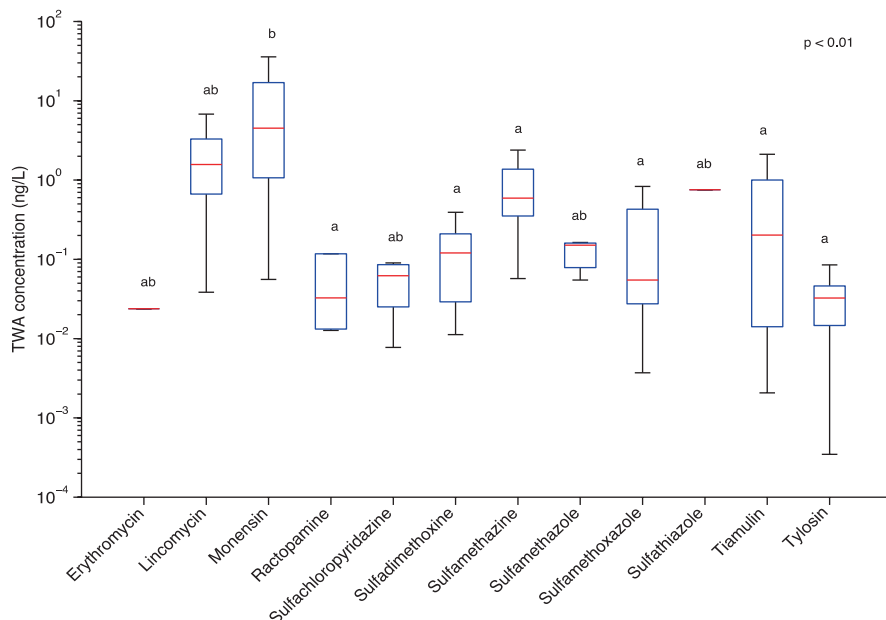


Fig. 10.2 Distribution of time-weighted average (TWA) pharmaceutical concentrations in Shell Creek from Jaimes Correa et al. [17]. The split box shows the 25th, 50th, and 75th, whereas whiskers shows the 5th and 95th percentiles

results indicate that although agricultural ecosystems are less likely to contain significant veterinary pharmaceutical loadings from municipal wastewater, the occurrence of pharmaceuticals in surface waters within these watersheds is persistent.

10.3 Influence of Manure Handling Practices

Cattle and swine manure accumulates within the production facility during the animal production period. In cattle feedlots, manure accumulates within the animal pen and then is typically scraped out at the end of the animal production period, prior to the introduction of new animals. This manure is typically stockpiled for a period of months prior to land application onto crop fields. In swine production systems, typically one of three waste handling systems is used: flush systems, pit recharge, or deep pits [35]. In deep pit systems, manure falls from a slatted floor into a pit below the animal housing facility and typically uses less water than either flush or pit recharge systems [35]. Manure may be stored in these pits for up to a year. Deep pit systems are commonly used in colder climates such as the upper Midwest in the United States, and manure accumulating in deep pits provides an environment for anaerobic microbial activities.

The fate of antimicrobials during anaerobic swine manure storage was evaluated in a previous study [20]. In this study, manure was obtained from an operating swine production facility that contained chlortetracycline, tylosin, and bacitracin A. After collection, manure and water were mixed in a 2:1 (w/w) ratio in 100 mL amber glass reactors, sparged with nitrogen, and incubated at 37 °C for up to 40 days to monitor the persistence of the antimicrobials. The parent antimicrobials tylosin and chlortetracycline were detected in swine manure reactors at initial concentrations of 10 mg/kg (dry weight basis) and 300 mg/kg (dry weight basis), respectively [20]. Bacitracin A was not detected in the manure at any time, but bacitracin F, a metabolite of bacitracin A, was detected at an initial concentration of 50 mg/kg (dry weight basis) in the manure.

Observed antimicrobial concentrations were fit with a first-order reaction equation to determine rate constants and first-order half-lives (Table 10.1). The first-order reaction rate constant for tylosin, chlortetracycline, and bacitracin F were -0.07 d^{-1} ($R^2 = 0.34$), -0.6 d^{-1} ($R^2 = 0.79$), and -0.36 d^{-1} ($R^2 = 0.94$), respectively. The half-life for chlortetracycline measured in Joy et al. [20] is shorter than that reported previously in studies of chlortetracycline degradation in swine manure or soil [6, 28, 39]. In contrast, the tylosin half-life reported in Joy et al. [20] is consistent with previous studies that measured tylosin half-lives on the order of 4.4 days [6, 25].

In Joy et al. [20], the occurrence of the antimicrobials and their corresponding antibiotic resistance genes (ARGs) were monitored. Although the antibiotic concentrations at the end of the 40 day experiments were $\sim 10\%$ of the initial concentration, the relative abundance of certain ARGs were more persistent, with approximately 50% of the initial abundance at the end of the storage period. The differences in observed behavior between the antimicrobials and corresponding ARGs indicates the importance of identifying not only the occurrence of the parent antimicrobial but also any biologically active degradation products, which could continue to exert a selective pressure allowing for the observed proliferation of resistance genes in manure storage systems.

Cattle manure and poultry litter handling have also been evaluated to determine the influence of practices such as composting or stockpiling on veterinary pharmaceutical concentrations. A number of studies have demonstrated the efficacy of composting for reducing the concentrations of nutrients and veterinary pharmaceuticals such as antibiotics and steroid hormones [2–4, 8, 22, 33].

Table 10.1 Antimicrobial degradation rates in simulated swine manure storage from Joy et al. [20]

| Antimicrobial | Measured degradation rate (d^{-1}) | Measured half-life (d) | Reported half-life (d) |
|-------------------|---|------------------------|------------------------|
| Chlortetracycline | -0.6 | 1 | 20–70 d [6, 39] |
| Tylosin | -0.07 | 9.7 | 0.02–4.4 [25, 6] |
| Bacitracin F | -0.36 | 1.9 | Not available |

10.4 Practices to Control Veterinary Pharmaceutical Transport After Land Application of Manures

Contaminants present in the manure including nutrients, pathogens, and trace compounds such as veterinary pharmaceuticals can be transported to surface water following land application. Although the concentrations of conventional pollutants in runoff from crops fertilized with animal manures have been routinely documented, there are few studies investigating the fate and transport of antimicrobials in soil and in runoff following land application of manure. Once animal manure is land applied, the fate of manure-borne compounds in soil and subsequent transport in runoff will be affected by the compounds' sorption properties [7, 23, 36] and susceptibility to biotic and abiotic degradation process such as photolysis [11, 16, 41].

Several studies have investigated the influence of manure application strategy on antimicrobial concentrations in runoff. One study found no statistically significant differences in concentrations of chlortetracycline, monensin, and tylosin in infiltration water and surface runoff when manure was applied using two different land application methods [9]. In contrast, other studies suggest that soil tillage leads to reduced vertical transport of antimicrobials after broadcast application of liquid manure [21], and manure incorporation (i.e., mixing manure into the top soil) could lead to reduced antimicrobial concentrations in runoff [26]. Joy et al. [19] published a study evaluating the influence of manure application methods on the concentration of antimicrobials in soil and runoff after land application of swine manure. In this study, a rainfall simulation study was conducted using test plots (0.75 m × 2.0 m) where swine manure was land applied using one of three land application methods: broadcast, incorporation, or injection. The plots were established using a randomized block design, and on each plot, three sequential rainfall simulation experiments were performed. Control plots with no manure amendment were also subjected to rainfall simulation experiments.

Broadcast manure generally resulted in higher antimicrobial concentrations in runoff than did incorporated and injected manures (Fig. 10.3). Because swine slurry

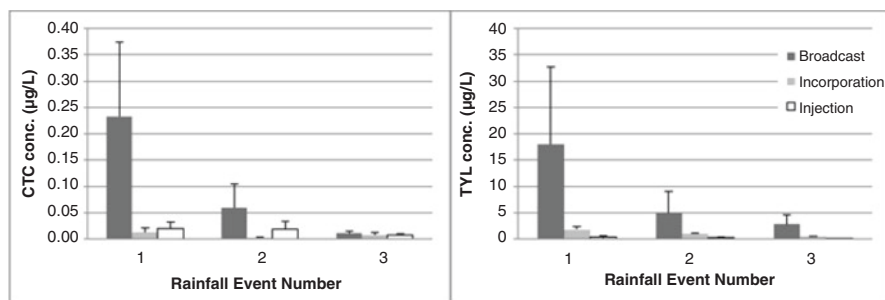


Fig. 10.3 Aqueous concentrations of chlortetracycline (CTC) and tylosin (TYL) in runoff from manure-amended plots receiving broadcast, incorporation, and injection treatments over three rainfall events. Error bars show the standard errors over triplicate field experiments. (Figure reprinted with permission from Joy et al. [19])

was spread on the soil surface in the broadcast application, the antimicrobials were readily available for transport to runoff during rainfall events. In contrast, mixing manure slurry with surface soil to various extents (i.e., injection and incorporation) resulted in reduced transport of antimicrobials to runoff. Although the main treatment factor, application method, was not considered statistically significant according to the rANOVA tests ($p = 0.26$ for chlortetracycline and $p = 0.31$ for tylosin), this is likely due to large variation in observed concentrations among the triplicate plots, which are not uncommon in field-scale experiments. The differences in sorption partition coefficients between chlortetracycline and tylosin to soil might account for the differences in runoff concentrations. It was not surprising to observe that the aqueous chlortetracycline concentrations in the runoff were low (Fig. 10.3) because of its sorptive nature ($\log K_{ow}$ for chlortetracycline is -0.62). By contrast, the range of tylosin concentrations in runoff measured in this study was 0.087 – $18 \mu\text{g/L}$ ($\log K_{ow}$ for tylosin is 1.63).

In addition to manure application practices, other best management practices that have been used historically to control the movement of conventional contaminants such as nutrients or pathogens can also be used to mitigate veterinary pharmaceutical transport, although this remains an underinvestigated research area. Soni et al. [38] investigated the use of narrow grass hedges, a type of vegetative barrier, in controlling antimicrobial runoff from plots amended with swine manure.

Vegetative barriers (VBs) are strips of densely growing plants used primarily on croplands adjacent to surface water. Vegetative barriers can reduce both dissolved and sediment-bound compounds in runoff by reducing runoff volume and capturing sediment [29]. VBs reduce the kinetic energy of the runoff, which can lead to enhanced settling of particulate contaminants. Dissolved contaminants can be reduced by enhanced infiltration and improved water-holding capacity of the surface soil within VBs [29].

Narrow grass hedges (NGH) are one type of vegetative barrier and are constructed using stiff stemmed grass strips that are ~ 1.5 m wide. Narrow grass hedges have been demonstrated to be effective in removing both dissolved [10, 13, 32] and sediment-bound [12] nutrients from runoff. The potential efficacy of narrow grass hedges for removal of antimicrobials from runoff was evaluated [38]. Similar to as in Joy et al. [19], test plots were established which were amended with swine manure and were established in a randomized block design. Three treatment factors were tested for their effects on runoff water quality: manure amendment (manure application to meet zero vs. three times annual N demand by corn or control vs. amended plots), NGH (plots with and without a NGH), and rainfall events (day 1, 2, and 3). In this set of field experiments, the only antimicrobial measured in the manure that was land applied was tylosin.

ANOVA analysis indicates that both manure amendment and the presence of a NGH had significant effects on the presence of tylosin in runoff ($p < 0.0001$). Although tylosin concentrations in runoff decreased with successive rainfall events (Fig. 10.4), the impacts of this treatment factor were not statistically significant. Prior to this study, little was known about the effectiveness of NGHs on reducing

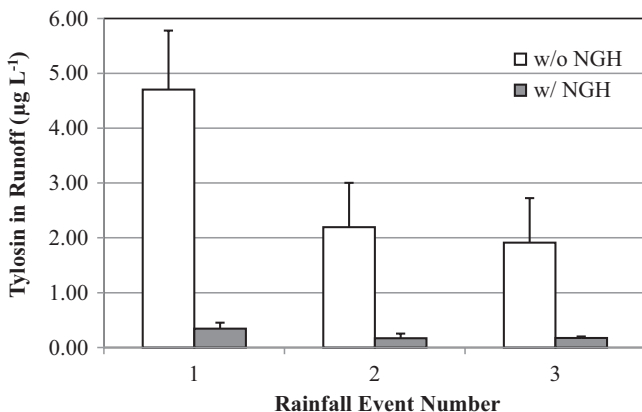


Fig. 10.4 Concentrations of dissolved tylosin in runoff from manure amended plots with and without NGH. Error bars represent standard errors from triplicate field experiments. (Reprinted with permission from Soni et al. [38])

Table 10.2 Mass loadings of tylosin in runoff from the amended plots with and without NGH during three rainfall events (average \pm standard error). Averages and standard errors were calculated based on triplicate field experiments. Reprinted with permission from Soni et al. [38]

| Rainfall event | Tylosin | |
|------------------|----------------------------------|---------------------------------|
| | w/o NGH ($\mu\text{g m}^{-2}$) | w/ NGH ($\mu\text{g m}^{-2}$) |
| 1 | 48.5 \pm 23.3 | 2.74 \pm 1.77 |
| 2 | 33.7 \pm 13.4 | 3.61 \pm 3.29 |
| 3 | 20.5 \pm 12.6 | 2.48 \pm 0.59 |
| Sum | 103 | 8.87 |
| Fraction from #1 | 0.47 | 0.31 |

dissolved antimicrobial loadings and concentrations in runoff. In this study, NGHs lowered tylosin loadings in runoff by more than an order of magnitude (Table 10.2).

Another study also demonstrated that vegetative buffer strips made of tall fescue could reduce tylosin in runoff [29]. Enhanced infiltration or adsorption of tylosin within the NGH system likely accounted for increased removal of dissolved tylosin loadings in runoff. The dissolved tylosin concentrations in runoff decreased with successive rainfall events for plots without a NGH, whereas no such trend was observed for plots with a NGH (Fig. 10.4 and Table 10.2). As a cost-effective best management practice, NGHs have been demonstrated to be effective in reducing contaminant loads in agricultural runoff, and the results from Soni et al. [38] also demonstrate that NGH can reduce dissolved antimicrobials in agricultural runoff following land application of swine manure.

10.5 Conclusions

Antibiotics and steroid hormones are regularly used in animal production and are excreted in animal manures. Although land application of manure provides benefits in agricultural production, including the reduction in use of commercial fertilizers, trace organics contained in manure can run off from cropland and contaminate surface and groundwater. The results presented here quantify antibiotic loading in runoff from cropland amended with manure and in surface water within watersheds with significant animal and crop production facilities. It is important to understand the impact of manure management practices on limiting antimicrobial impacts to surface and groundwater. Management practices such as manure storage and composting, manure incorporation into soil during land application, and the use of vegetated buffer strips can all reduce the loading of antibiotics and steroid hormones to the environment; however, more research is needed to evaluate the transformation of trace organics in agricultural production systems, as well as the relationship between the occurrence of antibiotics and antibiotic resistance genes, which can lead to the proliferation of environmental antibiotic resistance.

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