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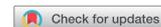
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## Transport of oxytetracycline, chlortetracycline, and ivermectin in surface runoff from irrigated pasture

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### ABSTRACT

The transport of oxytetracycline, chlortetracycline, and ivermectin from manure was assessed via surface runoff on irrigated pasture. Surface runoff plots in the Sierra Foothills of Northern California were used to evaluate the effects of irrigation water application rates, pharmaceutical application conditions, vegetative cover, and vegetative filter strip length on the pharmaceutical discharge in surface runoff. Experiments were designed to permit the maximum potential transport of pharmaceuticals to surface runoff water, which included pre-irrigation to saturate soil, trimming grass where manure was applied, and laying a continuous manure strip perpendicular to the flow of water. However, due to high sorption of the pharmaceuticals to manure and soil, less than 0.1% of applied pharmaceuticals were detected in runoff water. Results demonstrated an increase of pharmaceutical transport in surface runoff with increased pharmaceutical concentration in manure, the concentration of pharmaceuticals in runoff water remained constant with increased irrigation flow rate, and no appreciable decrease in pharmaceutical runoff was produced with the vegetative filter strip length increased from 30.5 to 91.5 cm. Most of the applied pharmaceuticals were retained in the manure or within the upper 5 cm of soil directly beneath the manure application sites. As this study evaluated conditions for high transport potential, the data suggest that the risk for significant chlortetracycline, oxytetracycline, and ivermectin transport to surface water from cattle manure on irrigated pasture is low.

### ARTICLE HISTORY

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Veterinary pharmaceuticals; surface runoff; irrigated pasture; vegetative filter strip; environmental exposure; ivermectin; oxytetracycline; chlortetracycline; manure; sorption

### Introduction

The extensive use of veterinary pharmaceuticals in animal husbandry has raised environmental concern due to the large percentage of administered doses that pass unaltered in animal waste.<sup>[1]</sup> Recent studies have focused on the use of pharmaceuticals in concentrated animal feed operations, but there is limited information on the fate and transport of these veterinary medicines in pasturelands. The production of beef cattle on pasturelands is a common agricultural practice in the western United States. With over 43.2 million acres in California alone dedicated to cattle grazing, the potential for pharmaceutical entry into waterways is quite high. Livestock grazing, in general, can adversely impact water quality and ecosystem health in the absence of proper management practices,<sup>[2]</sup> with the release of pharmaceuticals in animal waste to agricultural watersheds presenting an additional ecosystem health risk. Therefore, an understanding of the fate and transport of pharmaceutical compounds from beef cattle manure during runoff events is needed for the evaluation and development of sustainable grazing practices to limit pharmaceutical transport from grazed watersheds to downstream aquatic ecosystems.

Three widely used pharmaceuticals for cattle on grazed watersheds are chlortetracycline, oxytetracycline and ivermectin. Chlortetracycline and oxytetracycline are broad-spectrum

antibiotics used to treat a wide variety of diseases such as pneumonia, foot-rot, anaplasmosis, and bacterial scours, with over 100 formulations approved by the US Food and Drug Administration for use in cattle. Slow-release formulations of these antibiotics, that are effective over a long period of time, are most commonly used in cattle on grazed watersheds. Ivermectin, a member of the larger family of avermectins, is a macrocyclic lactone anthelmintic primarily used in injectable and topical formulations for cattle. Ivermectin has a broad spectrum of activity, low toxicity and long duration of action and is widely used in cattle to control endo- and ectoparasites. Ivermectin undergoes little metabolism, so most of the dose is excreted unchanged. The excretion of ivermectin from various methods of application including subcutaneous injection, intraruminal, and sustained-release bolus ranged from 62 to 90% via feces, with <2% appearing in urine.<sup>[3]</sup> Chlortetracycline and oxytetracycline are more water soluble than ivermectin and are primarily excreted in urine.<sup>[4]</sup> A 2002 review of pharmaceutical contaminants in the environment reports that the overall excretion rates of unmetabolized chlortetracycline, oxytetracycline, and ivermectin in feces and urine in veterinary treatment are 17–75%, 20%, and 40–75%, respectively.<sup>[1]</sup>

Numerous studies have been conducted on the environmental fate of avermectins with a focus on effects on dung

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arthropods,<sup>[5]</sup> soil invertebrates,<sup>[6–7]</sup> and aquatic ecosystems.<sup>[8]</sup> Studies looking at the mobility<sup>[9]</sup> and dissipation<sup>[10]</sup> of ivermectin in different soil types have shown that pH and cation exchange capacity of the soil can affect the ionic binding potential of ivermectin, and that the dissipation of ivermectin is slow in many soils under aerobic conditions. To our knowledge, there are no studies examining the transport of ivermectin in pastureland surface waters.

The sorption, leaching and transport of tetracyclines including chlortetracycline and oxytetracycline in a variety of soils are well documented.<sup>[11–15]</sup> These studies showed that despite having relatively high water solubility, tetracyclines can bind to sediments and manure, forming strong complexes via divalent cations. A nationwide survey of rivers across the United States revealed that these compounds are commonly detected, with chlortetracycline and oxytetracycline detected in approximately 1–2.5% of samples.<sup>[16]</sup> Due to the high sorption affinity of tetracyclines for soil, they are more commonly detected in waterways downstream from point sources of waste discharge than in runoff water from agricultural land. For example, tetracyclines were detected in surface water samples downstream of water treatment facilities,<sup>[17]</sup> and oxytetracycline and chlortetracycline were detected in manure slurries from confined animal rearing facilities.<sup>[18–19]</sup> However, the transport of oxytetracycline in pasture using simulated rainfall to generate surface water runoff after the application of a slurry of urine and feces demonstrated oxytetracycline concentrations in runoff water was quite low, and did not exceed 0.07% of the initial slurry concentration.<sup>[20]</sup> Irrigated pasture presents a very different scenario where runoff potential is increased due to surface flood irrigation, often on sloping landscape, and thus the risk of increased transport of tetracyclines to waterways may also be greater.

Although the production of beef cattle on pasturelands is a common agricultural practice in the western United States, there is a dearth of information on the fate and transport of pharmaceuticals under irrigated pasture conditions. Therefore, the primary objective of this study was to evaluate the transport of oxytetracycline, chlortetracycline and ivermectin via surface runoff from irrigated pasture. Surface runoff plots were used to evaluate the effects of pharmaceutical concentration in manure, irrigation water application rates, pharmaceutical application conditions, vegetative cover, and vegetative filter strip length on pharmaceutical discharge in surface runoff.

## Materials and methods

### Chemicals

Chlortetracycline, oxytetracycline, and ivermectin were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 4-epitetracycline (IS, internal standard), 4-epianhydrochlorotetracycline (recovery standard) were purchased from Acros Organics (Fair Lawn, NJ, USA). The selected chemical and physical properties of the pharmaceuticals can be found in Table A1. Optima grade methanol and 18.2  $\Omega$ -cm water (Thermo Scientific Barnstead Nanopure) were used in the chemical analyses. Solvents and all other chemicals were purchased from Sigma-Aldrich or Thermo Fisher Scientific (Pittsburgh, PA, USA).

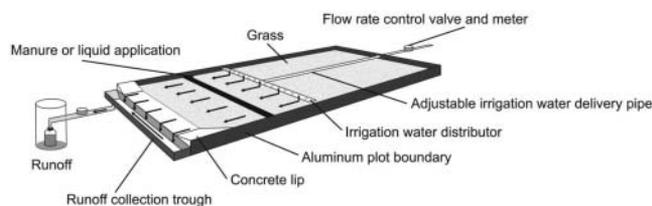


Figure 1. Experimental setup of runoff plots. Arrows represent the flow of irrigation water.

### Study site

Field experiments were conducted on irrigated pasture runoff plots at the University of California, Division of Agriculture and Natural Resources, Sierra Foothills Research and Extension Center (SFREC) in Browns Valley, CA. The center is located on the western slope of the Sierra Nevada foothills at an elevation of approximately 350 m. The site has a xeric moisture regime, with an average of 65 cm annual precipitation primarily during the months of October to March, and a maximum daily summer temperature of 31°C. Forage for pasture cattle is maintained using flood irrigation during the dry summer months.<sup>[21]</sup> The USDA taxonomic class of the soil is a fine, mixed, superactive, thermic Mollic Haploxeralfs and is assigned to the Argonaut soil series. The soil texture is loam with basic metavolcanic residuum parent material with 1.0% organic carbon (OC). Additional soil characteristics and properties can be found in Table A2.

Two-meter-wide irrigated pasture runoff plots were used for this study (Fig. 1). Each plot was seeded with a mixed pasture grass blend. Each plot contained a flow rate control valve with an attached flow meter to control and measure water input to the plot. A concrete lip connected to an aluminum collection trough was located at the end of each plot (Fig. 1). The plots were flood irrigated with a perforated 0.5 in (1.27 cm) PVC pipe to simulate flood irrigation used on grazed, irrigated pasture across the western United States, with the placement of irrigation pipe (length of vegetative filter strip) and irrigation flow rates set according to the specific experiments. Additional plot description can be found in the Appendix.

### Experiments

A series of experiments were conducted using the irrigated pasture runoff plots between June and August of 2012. Each experiment was designed to independently assess a single treatment, and each treatment was replicated three times within the runoff plots. Treatments were (1) pharmaceutical concentration (dose) in manure; (2) irrigation water application rate; (3) pharmaceutical application conditions and vegetative cover (as manure applied to plots with 5 cm tall grass, as manure applied to plots with grass cut to soil surface, as liquid applied to plots with 5 cm tall grass); and (4) length of vegetative filter strip at the end of each runoff plot.

Fresh manure was obtained from the Sierra Foothills Research and Extension Center cattle each sampling day. Manure was collected from range cows that were not treated with oxytetracycline, chlortetracycline, or ivermectin for several months prior to collection and the manure was analyzed to verify that there were no detectable levels of these pharmaceuticals.

The moisture content of manure was adjusted to 80–85% using deionized water.

For each irrigation event with all trials, plots were pre-irrigated (until soil saturation) to maximize runoff potential immediately prior to each trial. Control water runoff samples were taken prior to application of manure and pharmaceuticals. The grass was cut to the soil surface where the manure and liquid (when applied) pharmaceutical matrices were applied. Manure was applied across each runoff plot in a continuous strip perpendicular to runoff, 2 m in length, immediately after pre-irrigation and before the trial (irrigation event) was initiated. When examining the effect of pharmaceutical application conditions, the pharmaceuticals were also applied as a liquid in the same manner as the manure for the other treatments. The intent of this treatment was to introduce the maximum concentration of pharmaceuticals to the soil by eliminating binding to the manure. Surface water samples were collected over a 60-minute period for each irrigation event. The surface water discharge rate ( $\text{L s}^{-1}$ ) from the plots was measured and water samples were collected for pharmaceutical concentration determinations at 0, 5, 10, 15, 30, 45, and 60 minutes from the initiation of plot runoff (0 minute). Cumulative pharmaceutical discharge from each plot was then calculated. Statistically significant ( $P < 0.05$ ) differences in cumulative pharmaceutical discharges between treatment levels in each experiment were determined using negative binomial regression (Table A3). All statistical analyses were conducted in Stata/SE 11.1 (StataCorp LP, College Station, TX, USA).

#### Effect of concentration

Three pharmaceutical concentrations (0.5, 5, 50  $\text{mg kg}^{-1}$  of manure) were used to determine the effect of concentration on pharmaceutical discharge as surface runoff. Fresh manure (2 kg) was spiked with each pharmaceutical at each concentration level (in methanol, less than 0.1% w/w of final manure sample), homogenized, and placed in a continuous strip perpendicular to runoff flow, 61 cm from end of runoff plots. Grass height within plots was 5 cm and irrigation water application rate was set at a constant 3.8  $\text{L min}^{-1}$  (1  $\text{gal min}^{-1}$ ) for this experiment. After the first irrigation event, manure pats were left on the plots to determine possible transport of pharmaceuticals from the manure during a subsequent irrigation event two weeks later. No additional manure or pharmaceuticals were added to the plots for this subsequent irrigation event.

#### Effect of irrigation rate

The effect of irrigation water application rate on pharmaceutical discharge as surface runoff was investigated by setting irrigation rates to 1.9, 3.8, and 7.6  $\text{L min}^{-1}$ . Two kg of manure spiked with each pharmaceutical (0.5  $\text{mg kg}^{-1}$ ) was applied to the experimental runoff plots as described above. Grass height was 5 cm for this experiment.

#### Effect of pharmaceutical application conditions and vegetative cover

To evaluate the effect of application conditions on transport of pharmaceuticals we examined pharmaceutical discharge as surface runoff from plots with pharmaceuticals applied as manure to plots with 5 cm tall grass, as manure applied to plots with

grass cut to soil surface, and as liquid applied to plots with 5 cm tall grass. Two kg of spiked manure with each pharmaceutical (0.5  $\text{mg kg}^{-1}$ ) was applied consistent with other experiments. Liquid solutions were produced with stock methanol solutions of pharmaceuticals diluted to 1700 mL with 18.2  $\Omega\text{-cm}$  water, corresponding to the water content of the manure applied (80–85%). The final concentration of methanol accounted to less than 0.1% by volume. Irrigation water application rate was set at a constant 3.8  $\text{L min}^{-1}$  for this experiment.

#### Effect of vegetative filter strip length

To determine the effect of vegetative filter strip length manure strips were placed at 30.5, 61 and 91.5 cm (i.e., 1, 2, ft) from end of runoff plots. One mg of each antibiotic was spiked and homogenized into 2 kg of manure. Grass height was 5 cm within plots. Irrigation water application rate was set constant at 3.8  $\text{L min}^{-1}$  for this experiment.

### Chemical analysis

#### Water samples

Water samples were collected at 0, 5, 10, 15, 30, 45, and 60 min after application of spiked manure to runoff plots, stored in 1 L amber glass bottles with Teflon lined caps at 4°C, and analyzed within 48 hours. All water samples were filtered through 1.6  $\mu\text{m}$  and 0.7  $\mu\text{m}$  glass microfiber filters (Whatman, Buckinghamshire, UK) to remove colloidal particles. Water filtrates were acidified to pH 4.0 with 1  $\text{mol L}^{-1}$  HCl. Ethylenediaminetetraacetic acid (EDTA; 0.25  $\text{g L}^{-1}$ ) was added to each sample to prevent complexation of tetracyclines with divalent cations.

#### Soil samples

Upon completion of the experiments, soil samples were taken from each plot. Five soil samples were collected using a 1 inch diameter soil probe directly under the manure deposit to a depth of 5 cm. Based on the previously published runoff data, the vertical transport of studied pharmaceuticals from surface applied manure is limited to the soil directly under (0–5 cm) the manure and declined with increased depth.<sup>[22]</sup> Control soil samples were taken before irrigation and manure application. The field-moist soil samples were sieved (2-mm sieve), mixed thoroughly, and then kept at 4°C until analysis. Soil samples were analyzed within 48 h after processing. The soil samples were extracted for antibiotic content by pressurized liquid extraction (PLE) and analyzed using high performance liquid chromatography-tandem mass spectrometry (HPLC/MS/MS). Soils were extracted before the experiment to validate that no target pharmaceuticals were originally present in the soil. Soil pH values were determined by the soil saturated paste method.<sup>[23]</sup> Total OC content of air dried soil was determined using a Costech ECS 4010 nitrogen/protein analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) by dry combustion. Soil moisture content was determined gravimetrically after drying at 105°C for 48 h.

#### Manure samples

Manure was collected from each plot, upon the completion of the experimental treatments. Wet manure samples were

homogenized and dry manure samples were broken up into smaller pieces and sieved (2 mm sieve), and then stored at 4°C until analysis. Manure samples were extracted for antibiotic content by PLE and analyzed using HPLC/MS/MS within 48 h of processing.

### Pressurized liquid extraction

Pharmaceuticals were extracted from soils and manure using a Dionex ASE 150 (Sunnyvale, CA, USA) PLE system. Samples were loaded into 22 mL stainless steel extraction cells as follows. An EDTA washed cellulose filter (1.9  $\mu\text{m}$  pore size) followed by 1 g of diatomaceous earth was placed at the bottom of each cell. Five grams of the soil or manure sample mixed with 2 g diatomaceous earth was then added (Hydromatrix, Agilent Technologies, Inc., Palo Alto, CA, USA). The remaining cell void volume was filled with diatomaceous earth and another EDTA washed cellulose filter was placed on top. Extraction was carried out at  $10^7$  Pa and 40°C. Static extraction time was 20 min, with a flush volume of 60%, and purge volume of 60%. Two extraction cycles were performed using methanol:water (3:1, v/v) containing 0.25 mmol L<sup>-1</sup> EDTA and 50 mmol L<sup>-1</sup> sodium chloride at pH 8.0. Extracts were diluted with sufficient water to reduce the organic solvent concentration to less than 5% by volume. The solution pH was subsequently adjusted to 4.0 with 1 M HCl. Pharmaceuticals were extracted by solid-phase extraction (SPE) as described below and analyzed via HPLC MS/MS.

### Solid-phase extraction

Pharmaceuticals were extracted from water and diluted PLE extracts using a Discovery<sup>®</sup> DSC-SAX cartridge in tandem with an Oasis HLB phase cartridge. Oasis HLB cartridges (30  $\mu\text{m}$ , 3 mL, 60 mg sorbent) were purchased from Waters Inc. (Milford, MA, USA). DSC-SAX cartridges (56  $\mu\text{m}$ , 3 mL, 500 mg sorbent) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cartridges were conditioned with 6 mL of methanol followed by 3 mL of DI water and 3 mL of 0.04 M citric acid in water (pH 4.0). The extracts were loaded on the SPE cartridges at 5 mm Hg negative pressure. After loading, the SPE cartridges were washed with 6 mL of 0.04 M citric acid (pH 4.0). The SAX cartridges were then removed, and the HLB cartridges were allowed to dry for 15 min at 5 mm Hg negative pressure. Pharmaceuticals were eluted from the HLB cartridge with 4 mL of 100% methanol by gravity. The methanol extracts were evaporated to dryness under a gentle steam of nitrogen in a water bath at 45°C. The dry extracts were reconstituted in 300  $\mu\text{L}$  of 50% aqueous methanol (v/v) with 0.1% formic acid and subsequently analyzed with HPLC/MS/MS.

### HPLC MS/MS analysis

HPLC-MS/MS analysis was performed using an Agilent series 1200 HPLC with diode-array and Agilent 6320 ion trap mass spectrometer detectors (Agilent Technologies, Palo Alto, CA, USA). A detailed description of the analytical protocols and method quality assurance and quality control procedures can be found in Popova et al.<sup>[22]</sup>

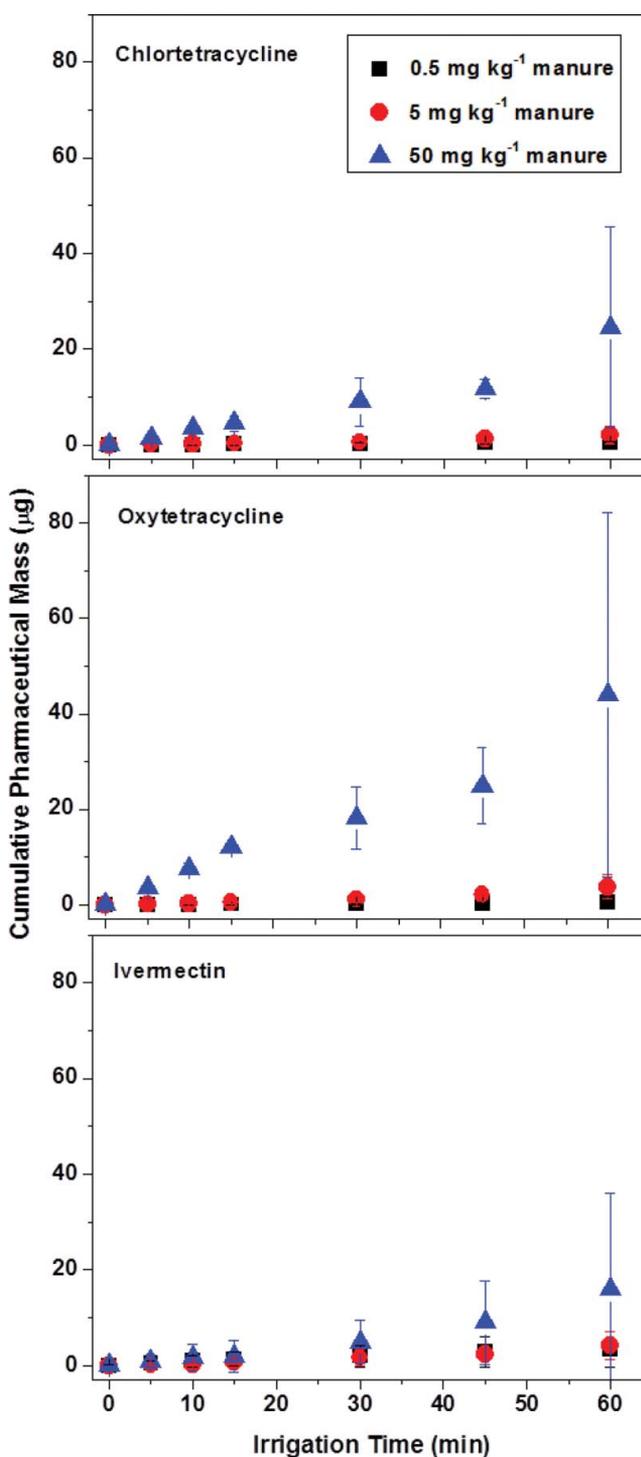


Figure 2. Cumulative mass of chlortetracycline, oxytetracycline and ivermectin in exported runoff water as a function of concentrations of pharmaceuticals in applied manure for initial (week 1) runoff event. Plots received 2 kg manure per plot and were irrigated at a rate of 3.8 L min<sup>-1</sup>.

## Results and discussion

Variation in irrigation conditions and practices can greatly affect the transport of pharmaceuticals from manure to runoff water (Figs 2–4). The heterogeneity of the soil surface in pasture land can also affect the contact or exposure of the manure to the irrigation water. The conditions of the experiment were set up to investigate a system highly vulnerable to contaminant

transport in runoff from irrigated grazed pasture. The research plots were pre-irrigated to saturate soil and maximize runoff potential during the experiments. The grass was cut to the soil surface where the manure was to be placed and the manure pats were laid in a strip perpendicular to the flow of water to maximize contact with surface water. A relatively high manure load was also used (2 kg manure at 80–85% moisture content per plot), resulting in 10, 100, and 200 mg m<sup>-2</sup> loading of each pharmaceutical. According to the U.S. Environmental Protection Agency recommendations, the stock density for pasture grazed cattle should be 1 animal unit per 1.6–3.2 hectares of land. Thus, the field relevant manure load is only 0.5–1.0 g manure m<sup>-2</sup>. In the present study, manure load was 20 kg manure per m<sup>2</sup> to represent extreme loading conditions and worst case scenarios.

Chlortetracycline, oxytetracycline, and ivermectin are used for both prophylactic and treatment purposes; thus their concentrations in manure differ based on the specific animal operation considered. Reported concentrations of chlortetracycline, oxytetracycline, and ivermectin in manure are 8–400, 800 and 90–360 µg kg<sup>-1</sup> manure, respectively.<sup>[19, 24–26]</sup> In combination with the variation in the composition of a herd and the herd stocking density, the amount of manure deposited pharmaceuticals on pasture can vary significantly.

The shape and consistency of manure pats can differ significantly. The shape of a pat is dependent on moisture content/consistency of the manure and is a function of feed moisture content and the time feed remains in the animal.<sup>[27]</sup> Other factors that affect manure moisture content include animal age and health, type of feed, and less directly, environmental conditions such as precipitation and air temperature. In the following experiments the manure (adjusted to 80–85% moisture content) was applied uniformly across treatments in a continuous strip perpendicular to irrigation water flow to avoid differences associated with manure shape or consistency. This application provided maximum contact area between irrigation water and manure. The “strip” application also helped reduce variability with runoff water pathways and channeling associated with the microtopography of pastureland.

### Effect of concentration

Analysis of runoff water collected 61 cm from the manure strip demonstrated a similar pattern in transport of chlortetracycline, oxytetracycline, and ivermectin (Fig. 2). Transport of pharmaceuticals was steady over the 60 min sampling period and no spikes in the detected concentrations were observed. The constant release or partitioning of the compounds from manure is consistent with our previous study on the transport of the same pharmaceuticals in a more controlled system using soil boxes.<sup>[22]</sup>

Elevated concentrations of the pharmaceuticals in the manure led to increased transport via surface runoff, with the highest dosing rates resulting in appreciable amounts of pharmaceuticals in the runoff water. The cumulative amount of chlortetracycline, oxytetracycline, and ivermectin leached from manure during 60 min were 38, 71, and 38 µg respectively, for the highest pharmaceutical spiking level (Fig. 2). Concentrations of all three pharmaceuticals did not exceed 1 µg L<sup>-1</sup> in the runoff water, and represent only a small fraction of the total

amount of pharmaceuticals spiked into manure (50 mg kg<sup>-1</sup>). Considering the fact that flood irrigation is typically carried out until runoff is generated, the simulated scenario where runoff water is collected for 60 min represents an extreme case. Under the typical irrigated pasture conditions, the current data suggest that the concentrations of pharmaceuticals transported in runoff water under similar conditions (e.g., soil, slope) would be lower than what is observed in the current study.

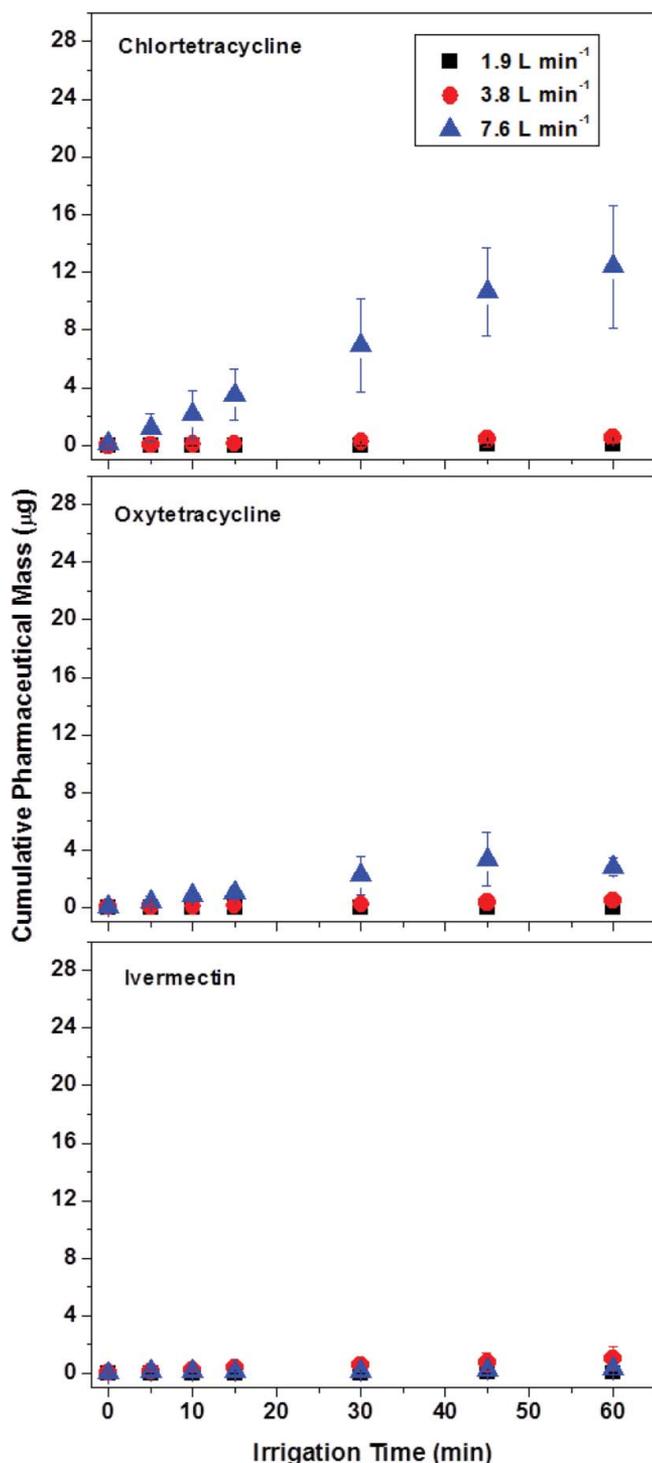
The applied manure was left on the plots for two additional weeks. After the two-week period the plots were re-irrigated. During the irrigation event the manure pats were barely rewetted. This was partially due to growth of the grass under the manure pat, effectively lifting the pat and resulting in reduced contact with the soil surface. Another factor affecting the rewetting of the manure was the increased hydrophobicity exhibited by the manure pat when dry. Over that time period, the moisture content of the manure was reduced by more than 86%. The amount of the pharmaceuticals in runoff water from these two subsequent irrigations accounted for less than 17% of the cumulative amount transported with the initial irrigation (Fig. 2). These data demonstrate that with the drying of manure over the two-week period the transport of pharmaceuticals into runoff water becomes even more limited.

### Effect of irrigation rate

The flow rate of irrigation water is an important parameter in determining the potential for contamination of water resources as lower flow rates have been shown to significantly decrease amounts of antibiotics in runoff as compared to higher flow rates.<sup>[28]</sup> When irrigation flow rates were increased from 1.9 to 7.6 L min<sup>-1</sup>, the concentration of the pharmaceuticals in water remained the same, however, there was a net increase in pharmaceutical transport due to the increase in total water volume. Plots with the highest flow rate (7.6 L min<sup>-1</sup>) showed increased runoff loads for oxytetracycline and chlortetracycline (cumulative mass) (Fig. 3). The mass of chlortetracycline recovered from runoff samples was greater than oxytetracycline. While the flow rate and thus total volume of water applied increased only by a factor of four from 1.9 to 7.6 L min<sup>-1</sup>, the cumulative amount of chlortetracycline recovered from the highest flow rate was more than 10 times greater than for the lowest flow rate. Average pharmaceutical transport rates of 1–14 µg pharmaceutical h<sup>-1</sup> of irrigation were detected. At this dissipation rate, the total possible amount of antibiotics transported would not account for a significant portion of the applied amount as the typical irrigation times would not exceed 2–10 h per irrigation event. Limited transport of tetracyclines is consistent with previous soil column and field monitoring studies where oxytetracycline was not detected in soil leachate.<sup>[29–30]</sup>

### Effect of pharmaceutical application conditions, vegetative cover, and filter strip length

The extent of pharmaceutical application conditions on pasture affects the mitigation and transport of pharmaceuticals. Under good pasture management, the presence of vegetation cover is



**Figure 3.** Cumulative mass of chlortetracycline, oxytetracycline and ivermectin in exported runoff water as a function of irrigation water application rates. Plots received 2 kg manure per plot containing 0.5 mg of each pharmaceutical kg<sup>-1</sup> manure.

typical. However, in areas of high traffic, such as around water sources, shady areas or along fences, areas of highly compacted soils with reduced or no vegetative cover are likely to be present. Vegetation cover serves several purposes. It prevents erosion of soil during irrigation and rainfall events.<sup>[31–32]</sup> It also acts as a filter, to slow and broaden the flow of surface water, decreasing its erosive potential.<sup>[31]</sup> When soil erosion occurs, pharmaceuticals can potentially be transported with the soil

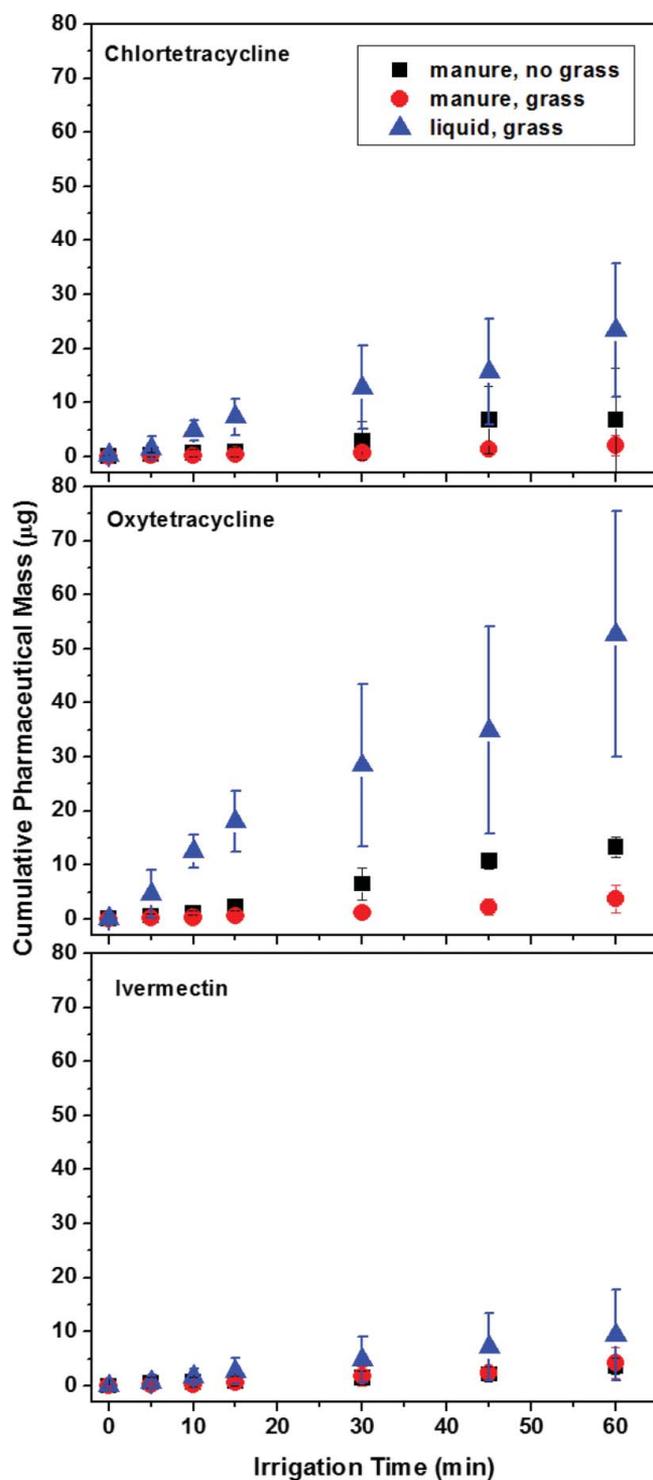
colloids in addition to water based transport thus increasing the total pharmaceutical loss.<sup>[33–35]</sup> However, colloid-associated transport depends on the chemistry of the specific compound, the soil type, and irrigation conditions. While studied pharmaceuticals were demonstrated to sorb strongly with particulate matter, their colloid-associated transport is limited by the absolute amount of colloids that can be carried with the water flow. Specifically, the transport of colloidal particles (< 1.3 µm) from the studied bare plots at typical irrigation flow rates of 40–80 gal acre<sup>-1</sup> accounted for less than 0.016 – 0.022 g gal<sup>-1</sup> of runoff water. Similarly, low particle associated transport of tetracyclines was demonstrated in silt loam and gravely silt loam soils.<sup>[22,36]</sup> Colloidal transport of these pharmaceuticals including the bioavailability and activity of colloid-bound pharmaceuticals warrants further investigation.

While the vegetation cover prevents the erosion of soil and transport of pharmaceuticals associated with soil colloids, it also prevents the dissipation and disintegration of a manure pile. When manure is deposited on the bare soil before or during an irrigation event, manure soluble solids and the particulate matter fraction are transported along with surface water flow. This potentially leads to the higher transport of pharmaceuticals, as they are strongly associated with the manure organic constituents through hydrophobic interactions and interactions with divalent ions within the manure matrix (Wang et al., 2008; Loke 2002). It was estimated that up to 95% of applied pharmaceuticals is irreversibly sorbed to the manure.<sup>[22,32]</sup> On average, beef manure contains up to 30% of soluble solids and 20–40% of particulates by weight.<sup>[37]</sup> Therefore, the potential loss of pharmaceuticals through manure disintegration during an irrigation event on bare soil is likely to be significant.

When pharmaceutical-spiked manure was applied on the soil runoff plots with no vegetation cover, no pronounced contrast in the transport of antibiotics was observed with an exception to a small increase in oxytetracycline (Fig. 4). The amount of pharmaceuticals detected in the runoff water coincides with the amount of particulate matter transported. Transport of soil particulate matter was more pronounced according to the turbidity of the water samples during the first 30 min of irrigation and steadily declined over next 30 min. Lab studies showed that 98% of these pharmaceuticals remain in manure and therefore transport would be limited by desorption.<sup>[22]</sup>

Pharmaceuticals delivery by adding them in water directly (i.e., no manure present) to the saturated soil in the plots with vegetative cover demonstrated higher pharmaceutical load in runoff water compared to the treatments where pharmaceuticals were added with manure (Fig. 4). In 60 min, the amount of pharmaceuticals in the water fraction was at least two times greater than in the plots where manure was applied with vegetative cover. The most pronounced effect was observed for oxytetracycline being the most water soluble compound. There was much greater transport of the pharmaceuticals by adding them in water directly to the saturated soil, though the runoff concentrations were still low in comparison to the initial concentration of the spike.

The presence of vegetation likely impacted the transport of the pharmaceuticals through two mechanisms. First, it may have provided a filter for the pharmaceutical bound organic



**Figure 4.** Cumulative mass of chlortetracycline, oxytetracycline and ivermectin in exported runoff water as a function of pharmaceutical application conditions and vegetative cover. Plots received either 0 or 2 kg manure per plot containing 0.5 mg of pharmaceutical  $\text{kg}^{-1}$  manure and were irrigated at a rate of  $3.8 \text{ L min}^{-1}$ .

material that detached from the manure, providing binding sites for soluble pharmaceuticals.<sup>[38]</sup> Secondly, vegetation constrained the flow of the surface water and allowed for additional time and opportunity for pharmaceuticals to sorb. In the cur-

**Table 1.** Concentration range of pharmaceuticals detected in runoff water, soil and manure.

	Chlortetracycline	Oxytetracycline $\mu\text{g kg}^{-1}$	Ivermectin
Runoff water	ND*–0.7	ND*–1.3	ND* – 0.556
Soil (extracted)	10–24	8–15	1–14
Manure (extracted)	72–175	24–66	54–118

\*ND = below detection limit

rent study, the net effect of these two mechanisms was not extensive.

A difference in vegetative filter strip length of 30.5 to 91.5 cm did not produce any appreciable increase or decrease in concentration of these pharmaceuticals in runoff water (data not shown). This may be largely attributed to the increased sorption of these compounds to manure and soil. Ivermectin, in particular, has a high sorption affinity to manure with a sorption coefficient ( $K_d$ ) of  $63020 \text{ L kg}^{-1}$ .<sup>[22]</sup> Additionally, studies have shown that the antibiotics, oxytetracycline and chlortetracycline, demonstrate high sorption to soils regardless of particle size distribution.<sup>[15,29]</sup> In sandy and sandy loam soil,  $K_d$  values of oxytetracycline were found to be 420 and  $1030 \text{ L kg}^{-1}$ , respectively.<sup>[39]</sup>

### Evaluating environmental concern

Each of the factors evaluated in the current study (i.e., effect of pharmaceutical concentration in manure, irrigation water application rate, pharmaceutical application conditions, and vegetative filter strip length on pharmaceutical discharge in surface runoff) demonstrate limited transfer of the tested pharmaceuticals in runoff water. The concentration range of ivermectin detected in runoff water in all experiments was  $0.0\text{--}0.556 \mu\text{g L}^{-1}$ . The concentration ranges of the antibiotics, chlortetracycline and oxytetracycline were  $0.001\text{--}0.7$  and  $0.001\text{--}1.3 \mu\text{g L}^{-1}$  respectively. For all pharmaceuticals, the concentrations are less than 0.1% of the applied pharmaceuticals to the manure and thus represent very low potential for their transport in runoff waters.

Higher concentrations of the pharmaceuticals were detected in the soil beneath the manure and in the manure pat itself, highlighting the strong affinity of these compounds for non-polar domains in these materials. The extractable concentrations of the pharmaceuticals detected in the soil below the manure pats ranged from  $1\text{--}24 \mu\text{g kg}^{-1}$  while the extractable concentrations from manure ranged from  $24\text{--}175 \mu\text{g kg}^{-1}$  (Table 1). Sorbed pharmaceuticals may still be biologically active, though in the case of tetracycline, dissipation and sorption of  $200 \text{ mg kg}^{-1}$  tetracycline to soil was sufficient to neutralize the antibiotic's effectiveness.<sup>[40]</sup> Furthermore, the 90% minimum inhibitory concentrations (MIC) for tetracyclines are  $16,000 \mu\text{g kg}^{-1}$ .<sup>[41]</sup> Due to the limited transport of these compounds in water and retention in soil continued research and emphasis on local

environmental impacts may be more meaningful than studies examining off-site transport.

## Conclusions

Chlortetracycline, oxytetracycline, and ivermectin introduced to irrigated pasture on a series of runoff plots showed limited transport in runoff water due to their high sorption to manure and soil. Even with conditions for high transport potential, less than 0.1% of the applied pharmaceuticals were detected in runoff water from field plots with most of the analytes retained in the manure or in the soil directly beneath the manure application site. A difference in vegetative filter strip length of 30.5 to 91.5 cm did not produce any appreciable decrease in concentration of these pharmaceuticals in runoff water. Based on the results the use of at least one foot of vegetative strips would be beneficial to reduce the concentration of pharmaceuticals in runoff water. The concentrations of chlortetracycline and oxytetracycline detected in surface water are more than four orders of magnitude lower than the minimum inhibitory concentration (MIC) for these antibiotics. Although there may be other ecological concerns regarding the presence of chlortetracycline, oxytetracycline, and ivermectin within manure on irrigated pasture, this data demonstrates that the potential for these compounds to reach surface waters via surface runoff is low.

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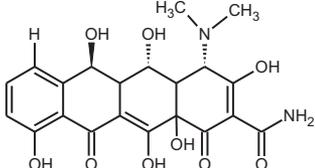
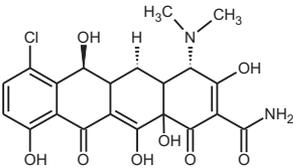
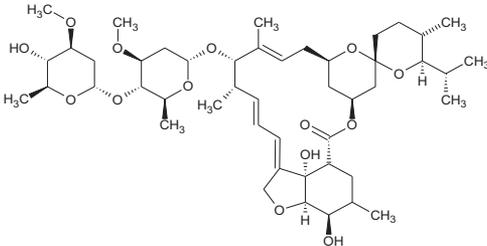
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## Appendix

### Experimental plots

The experimental plots were 2 m wide on a 2–4% slope. The vegetative buffer strip length was adjusted according to the experimental setup (30.5, 61, 91.5 cm). To ensure contact with runoff water the grass was cut down to the soil surface (6-cm-wide strip across the width of the plots) where the manure or liquid was applied. The irrigation delivery pipes were adjustable and the distributors (see Figure 1) were set 15 cm behind the application site.

**Table A1.** Chemical structure and selected properties of the studied pharmaceuticals.<sup>[1–5]</sup>

	Oxytetracycline	Chlortetracycline	Ivermectin
Chemical Structure			
Molecular Formula	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>9</sub>	C <sub>22</sub> H <sub>23</sub> ClN <sub>2</sub> O <sub>8</sub>	C <sub>48</sub> H <sub>74</sub> O <sub>14</sub>
pKa	4.5, 10.8	4.5, 11.0	NA
Solubility in water, g L <sup>-1</sup>	25 (pH 1), 0.17 (pH 6), (pH 9)	4.8 (pH 1), 0.048 (pH 6), (pH 10)	0.004
K <sub>ow</sub>	-2.1	-0.6	3.2
Kd, L kg <sup>-1†</sup>	2580	280	63020

<sup>†</sup>Single point Kd values calculated at an initial pharmaceutical concentration of 0.01 mmol L<sup>-1</sup> with Argonaut soil from SFREC.

**Table A2.** Soil properties from the Argonaut soil series profile from the runoff plots at the University of California Sierra Foothill Research Center.<sup>[6]</sup>

Depth Range	Horizon Designation	Clay, %	Sand, %	Organic Matter, %	pH	K sat., mm hr <sup>-1</sup>	CEC, cmol charge kg soil <sup>-1</sup>
0–18	H1	17.5	43	1.5	6.1	32.4	15
18–36	H2	25	38.5	0.25	6.5	9.72	15
36–60	H3	27.5	34.7	0.25	6.5	9.72	15
60–89	H4	47.5	23.3	0.25	7	0.774	25
89–150	H5	0	NA	0	NA	0	NA

**Table A3.** Mean cumulative pharmaceutical discharges ( $\mu\text{g}$ ) by treatment levels for independent experiments examining effects of irrigation water application rate, pharmaceutical application conditions, and pharmaceutical concentrations in manure.

Compound	Irrigation Water Application Rate			Pharmaceutical Application Conditions			Pharmaceutical Concentration in Manure		
	1.9 L min <sup>-1</sup>	3.8 L min <sup>-1</sup>	7.6 L min <sup>-1</sup>	Manure, grass	Manure, no grass	Liquid, grass	0.5 mg kg <sup>-1</sup>	5 mg kg <sup>-1</sup>	50 mg kg <sup>-1</sup>
	Mean cumulative pharmaceutical discharge ( $\mu\text{g}$ )								
Chortetracycline	0.05 (0.02) a	0.49 (0.21) b	12.46 (4.25) c	2.04 (1.28) a	6.82 (6.82) A	23.36 (8.73) b	0.49 (0.21) a	2.04 (1.28) b	24.64 (14.87) c
Oxytetracycline	0.01 (0.00) a	0.47 (0.18) b	2.84 (0.46) c	3.76 (1.79) b	13.28 (1.27) A	52.72 (16.04) c	0.47 (0.18) a	3.76 (1.79) b	43.94 (27.01) c
Ivermectin	0.04 (0.01) a	1.02 (0.49) b	0.29 (0.08) c	4.24 (1.65) a	3.60 (0.82) A	9.37 (4.81) a	1.02 (0.49) a	4.24 (1.65) b	15.99 (11.61) c

Significance  $p < 0.05$ Different letters indicate statistical differences ( $p < 0.05$ ) between treatment levels, as determined by negative binomial regression.

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