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# Detection, Occurrence and Fate of Emerging Contaminants in Agricultural Environments

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## Detection, Occurrence and Fate of Emerging Contaminants in Agricultural Environments

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### Abstract

A total of 43 papers published in 2014 were reviewed ranging from detailed descriptions of analytical methods, to fate and occurrence studies, to measuring and predicting biological effects for a wide variety of emerging contaminants likely to occur in agricultural environments. New methods and studies on veterinary pharmaceuticals, natural and synthetic steroids, and antibiotic resistance genes in agricultural environments continue to expand our knowledge base on the occurrence and potential impacts of these compounds. This review is divided into the following sections: Introduction, Analytical Methods, Occurrence and Fate, Antibiotic Resistance Genes, and Risk Assessment.

### Keywords

pharmaceuticals; steroid hormones; anthelmintics; analytical methods; water and wastewater; agricultural environments

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## Introduction

Water resources in agricultural environments are impacted by a wide variety of contaminants including nutrients, sediments, and pesticides. These groups of contaminants typically occur at easily measured concentrations in surface run-off in agricultural water sheds. Nutrients, especially nitrogen, and pesticides have also been shown to impact ground water quality in areas susceptible to contamination. Less well-known are the impacts of newer classes of contaminants such as pharmaceuticals, steroids, antibiotics and antibiotic-resistance genes of bacteria. These “emerging” contaminants clearly have potential to enter the environment and cause known or suspected adverse ecological or human health effects. Release of these contaminants to the environment has very likely occurred for quite some time, but methods for their detection at environmentally-relevant concentrations have only recently become available.

Evaluating the environmental fate and effects of emerging contaminants includes compounds such as surfactants, antibiotics and other pharmaceuticals, steroid hormones and other endocrine-disrupting compounds (EDCs), fire retardants, sunscreens, disinfection byproducts, new pesticides and pesticide metabolites, and naturally-occurring algal toxins. Detection of these contaminants in environmental matrices (water, wastewater, soils, and sediments) is particularly challenging because of the low detection limits required, the complex nature of the samples, and difficulty in separating these compounds from interferences. New extraction and clean-up techniques, coupled with improvements in instrumental technologies provide the needed sensitivity and specificity for accurate measurement.

The objective of this paper is to review the literature published in 2014 evaluating the detection, fate, and occurrence of emerging contaminants, with a particular focus on those contaminants likely to be found in agricultural systems. Relevant contaminants are antibiotics and other pharmaceuticals associated with wastewater, EDCs (particularly hormones and anabolic steroids), and antibiotic resistance genes (ARGs) in bacteria. Studies on pesticides and flame retardants are not reviewed unless they were evaluated in the same study.

## Analytical Methods

A high throughput, multi-residue method for the analysis of antibiotics in fresh water and seawater has been described by Bayen et al. (2014). The method used LC-MS/MS coupled with direct sample injection providing trace level analysis of seven antibiotics (lincomycin, tylosin, chloramphenicol, sulfamerazine, sulfamethoxazole, sulfadiazine, and sulfamethazine). Recoveries averaged  $95 \pm 14\%$  and  $96 \pm 28\%$  for fresh water and seawater, respectively. Method detection limits ranged between 0.037 ng/L and 226 ng/L in fresh water and between 0.016 ng/L to 26 ng/L in seawater. The authors indicate that the direct injection method is sensitive enough for most environmental screening applications where fast analyses are required.

Hu et al. (2014) developed a multi-residue method for electrospray ionization LC-MS/MS determination of 26 veterinary antibiotics in swine wastewater effluent. Samples were obtained from groundwater, fish pond water, biogas digester water, and lagoon wastewater locations. The target antibiotics were concentrated before analysis by utilizing a lyophilization/reconstitution procedure to minimize degradation. Detection limits ranged from 0.1 ng/L for roxithromycin to 6.5 ng/L for difluoxacin. Recoveries exceeded 70% for all analytes except for sulfaquinoxaline. Results obtained from the application of the method to the pig farm samples are presented.

Piatkowska et al. (2014) compared various sample extraction and clean-up techniques for the simultaneous analyses of multi-class veterinary pharmaceuticals in food products and environmental samples. The classes of antibiotics included: sulphonamides, fluoroquinolones, tetracyclines, macrolides, penicilines, cephalosporins, nitroimidazoles, benzimidazoles, coccidiostats, amphenicoles, and insecticides as well as tiamulin and lincomycin. While other techniques were found to be better for some analytes, the authors determined that extraction with 0.1% formic acid in acetonitrile:water (8:2) followed by HybridSPE™ clean-up gave the most consistent quantitative recoveries over the range of analytes studied. The authors also compared the time invested in each of the extraction and clean-up steps.

Anthelmintics are another group of veterinary pharmaceutical compounds used for treating animal diseases caused by parasitic worms (helminthes). They can be found in animal tissue as well as in the environment. While there are a number of reports measuring residues of anthelmintics in animal tissue, a few investigators are beginning to develop methods to evaluate their occurrence and fate in the environment.

Zrncic et al. (2014) describe a method using high performance liquid chromatography coupled to quadrupole linear ion trap mass spectrometry to investigate the occurrence of 10 anthelmintic compounds such as moxidectin, flubendazole, fenbendazole, mebendazole, oxibendazole, albendazole, triclabendazole, febantel, praziquantel, and levamisole in 11 river samples from the Llobregat River and two tributaries (River Anoia and Cardener) located in Catalonia, North East Spain. Among the selected anthelmintic compounds, levamisole (54.5%) and febantel (45.5%) were the most commonly detected, while no detection of mebendazole and praziquantel occurred. Samples collected from the tributaries showed the occurrence of febantel and levamisole, while febantel, fenbendazole, levamisole, moxidectin, oxibendazole, and triclabendazole were detected in samples collected from the Llobregat River. None of the anthelmintic compounds were detected at the source, while most of the compounds were detected at the discharge of the Llobregat River to the Mediterranean Sea close to Barcelona city. At this location, the highest cumulative anthelmintics concentration (43.27 ng/L) and the highest concentration of a single anthelmintic compounds, levamisole (39.4 ng/L) was observed.

Cerqueira et al. (2014) developed a new version of the QuEChERS™ method to evaluate the occurrence of pharmaceutical compounds and personal care products in drinking-water sludge using ultra high pressure liquid chromatography-tandem mass spectrometry. Samples of sludge were collected after the flocculation step with aluminum sulfate at the CORSAN

water facility in Rio Grande, Rio Grande do Sul State, Brazil and used as a soil conditioner. Among the 21 pharmaceutical compounds investigated, one anthelmintic compound, mebendazole, was also included. No detection of mebendazole occurred throughout the study.

### Sample Preservation

The stability of 56 antibiotic compounds in purified water samples was studied by Llorca et al. (2014) using three different sample preservation methods: 1) at  $-20^{\circ}\text{C}$ ; 2) at  $-20^{\circ}\text{C}$  with 0.1% EDTA, and 3) through solid phase extraction (SPE) pre-concentration. The results showed that only 33 analytes were stable ( $>80\%$  recovered) for 1 week and after 12 weeks, only 22 analytes were stable. The type of antibiotic influenced the stability profile as the penicillins, nitroimidazoles, lincosamides, cephalosporines, and sulfonamides were more stable than the tetracyclines, macrolides, quinolones, and fluoroquinolones. The authors concluded that the best option was to analyze shortly after sample collection, typically within the first week, but the samples could be preserved longer, depending on the class of analyte and preservation method.

## Occurrence and Fate

### Veterinary Pharmaceuticals

Petrovic et al. (2014) investigated the occurrence of 81 pharmaceuticals compounds in waste, surface, underground, and drinking water samples collected in the northern part of Serbia. This method included three anthelmintics - albendazole, levamisole, and thiabendazole. Albendazole and levamisole occurred in 14% and 3% of all samples, respectively. Albendazole was detected in underground (33.3%) and drinking (60%) water samples, while levamisole was detected only in surface water (9%). Albendazole ranged up to 1.9 ng/L in groundwater, and up to 2.8 ng/L in drinking water. Levamisole was detected only once at 1.5 ng/L.

Robles-Molina et al. (2014) investigated the occurrence of 373 compounds, including pesticides, PAHs, nitrosamines, pharmaceutically active compounds in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain during a period between 2009 and 2011. This area was primarily impacted by agriculture, however industrial and municipal wastewater discharges may also occur. The methods include three anthelmintics compounds: albendazole, levamisole, and thiabendazole. Albendazole was not detected in any of the sampling locations during the study, while thiabendazole was detected during the entire study regardless of the season and the location. Levamisole was not detected during summer 2009, but it was detected during the remaining sampling campaigns. Thiabendazole and levamisole ranged between 0.2 and 9.2 ng/L, and between below the detection limit and 9 ng/L, respectively.

Xu et al. (2014) studied the occurrence of antibiotics in sediment, lake water and pore water from Taihu Lake (China) using HPLC-MS/MS in positive electrospray mode. Analytes studied were from the sulphonamide, fluoroquinolone, and tetracycline classes of compounds as well as trimethoprim. Maximum total concentrations of 731  $\mu\text{g}/\text{kg}$ , 1210 ng/L, and 216

ng/L were found in the sediment, lake water, and pore water, respectively. Analyte recoveries of antibiotics ranged from 63% to 124% in sediment and 83% to 114% in water. Detection limits for the antibiotics ranged from 0.3 µg/kg to 3.9 µg/kg in sediment and 0.1 ng/L to 3.6 ng/L for water.

Cheng et al. (2014) studied the seasonal variations of two tetracycline and two fluoroquinolone antibiotics in surface water, overlying water, pore water, and sediment samples from Baiyangdian Lake (China). The target antibiotics were extracted and then analyzed by electrospray ionization LC-MS/MS. The results indicated that the concentrations of the antibiotics varied significantly depending on the time and location of sampling. The authors also found that the fluoroquinolones had a higher tendency to partition into the adsorbed phase compared to tetracyclines. Additionally, the results of the seasonal vertical concentration profiles indicated that previous sedimentary accumulations of the antibiotics could supply a source of contamination back into the lake under certain environmental conditions.

Moreno-Gonzalez et al. (2014) investigated the spatial and seasonal distribution of 69 pharmaceutical compounds from 13 different therapeutic groups along the El Albuñón watercourse and its main tributary. The investigated area, located in the South East of Spain, receives limited precipitation and is highly impacted by intensive agriculture and tourism. Among the 69 compounds investigated, two anthelmintic compounds, levamisole and thiabendazole, were selected. Levamisole was often detected at concentrations higher than the analytical detection limit (17% to 71.4%) during the study, while thiabendazole was detected at concentrations higher than the analytical detection limit during the entire study regardless of the season. Levamisole and thiabendazole ranged up to 14.3 ng/L and 9.2 ng/L, respectively.

Chen and Zhou (2014) reported the occurrence and behavior of five classes of antibiotics in river water and sediment downstream from agricultural areas. Sulfonamides occurred in the highest concentrations (up to 859 ng/L) in water, while tetracyclines and macrolide antibiotic concentrations were higher in associated river sediment, averaging 12–18 ng/g dry weight. Partitioning and persistence were related to chemical properties.

Cruz-Morato et al. (2014) investigated the ability of wastewater bioreactor treatment to remove 51 pharmaceutically active compounds. Among these compounds, three anthelmintic compounds, albendazole, levamisole, and thiabendazole, were also investigated. Albendazole, 31 ng/L, was the only anthelmintic detected. However, complete removal was achieved during the wastewater treatment by *T. versicolor*.

## **Steroid Hormones**

Tile drainage systems have been extensively used to improve productivity of row-crop agriculture. Gall et al. (2014) describe the potential for tile drainage to increase losses of steroid hormones from fields fertilized with livestock manure. Using results from an experimental farm, they estimate and relate estrogen and androgen loading through tile drainage beneath fields receiving swine and dairy manure. Episodic spring rainfall events produced the highest loading rates for all groups of compounds.

A similar view for episodic release and transport of steroid hormones and pharmaceuticals was presented by Kolok et al. (2014). It is likely that soils and river sediments serve as a source and sink for many biologically active compounds including steroid hormones and veterinary pharmaceuticals. Because of the nature of the chemicals and the release mechanisms, measureable concentrations of many contaminants may only be found in the aqueous phase for a short time after precipitation.

Jones et al. (2014) evaluated leaching rates and mass balance of metabolites of the anabolic steroid trenbolone acetate using experimental manure mesocosms. Two metabolites were monitored in manure contained in glass reservoirs filled to overflowing with irrigation water. Overflow water was sampled and analyzed for the metabolites over time.

Cavallin et al. (2014) suggest a method for combining steroid hormone concentrations in run-off with bioassays for integrated environmental risk assessment. The study used samples collected from multiple watersheds, measured estrogenicity and androgenicity using cell bioassays, and 48-hour fish bioassays. Low concentrations of natural and synthetic estrogens and androgens were detected in surface run-off using previously published methods and compared to both types of bioassay results. Estrogenic activity was indicated through the cell bioassays, but fish exposures did not exhibit dose-dependent biological effects.

Sangster et al. (2014) evaluated the fate of 17 $\beta$ -estradiol and 17 $\beta$ -trenbolone in silty loam and sandy sediments, respectively, by measuring the concentrations and (anti)estrogenicity of parent compounds and metabolites. The bioavailability of these compounds and their metabolites were indicated by the changes in hepatic vitellogenin mRNA expression of fathead minnows. The results showed that both 17 $\beta$ -estradiol and 17 $\beta$ -trenbolone were biotransformed to more persistent metabolites, estrone and trendione, and the parent compounds and metabolites were predominantly associated with sediments. Rapid degradation of 17 $\beta$ -estradiol in either aqueous or sorbed phases resulted in no significant changes in hepatic vitellogenin expression in male minnows. Significant reductions of vitellogenin were observed in females exposed to 17 $\beta$ -trenbolone contaminated sediment, which indicated that sediment-associated steroid hormones were bioavailable to fish. Different concentration and vitellogenin expression profiles in two sediments suggested that sediment properties affect the fate and bioavailability of these compounds.

Qi et al. (2014) investigated the sorption and desorption of testosterone in five soil fractions of different particle sizes. An agricultural soil was fractionate into sand, medium silt, fine silt, and two clays using wet sieving and repeated sedimentation methods. Sorption capacity for testosterone ranks as sand < silt < clay, and small particles have both greater sorption rate and lower desorption rates. Specific surface area and surface functional groups determined the sorption kinetics and capacities of soil fractions. Hydrogen bonds were considered as a main sorption mechanism for testosterone in silt and clay fractions, but not in sand. Highest desorption hysteresis was observed in clay, which helped explain why clays had the least desorption potential.

A review of antibiotic soil sorption was conducted by Wegst-Uhrich et al. (2014) who also presented new case studies on properties of sulfonamides and macrolides. Their review

focuses on the factors that affect the soil-water distribution coefficient ( $K_d$ ) which includes environmental factors like pH and organic matter content as well as experimental design factors including concentration range, analytical method, and transformations. Their case study found that both tylosin and sulfamethazine sorption increased as pH was reduced which indicates that electrostatic forces are the dominant sorption mechanism. Ionic strength and organic matter played a less significant role in soil sorption. The authors conclude by highlighting the importance that microbial activity plays in the soil environment and that sorption studies should also include transformation products formed through biotic and abiotic processes.

Sura et al. (2014) examined the effects of stockpiling on the dissipation of chlortetracycline, tylosin, and sulfamethazine in cattle manure during the winter season. Beef cattle were administered chlortetracycline alone, a mixture of chlortetracycline and sulfamethazine, tylosin alone, or no antibiotics. Dissipation kinetics of the three antibiotics can be explained by exponential decay. It took 20.8 days for sulfamethazine, 4.7 days for tylosin, 1.8 days for chlortetracycline alone or 6.0 days when mixed with sulfamethazine to reach 50% dissipation. The presence of antibiotics appeared to have no inhibitory effects on microbial activities, as evidenced by the temperature and mass losses of carbon and nitrogen in the stockpiles. By comparing the dissipation rates of antibiotics measured in this study to those measured in a previous study, the authors concluded that stockpiling can be as effective as windrow composting in mitigating the antibiotics in manure.

### Predicting Occurrence and Effects

In lieu of traditional fate and transport modeling based on estimated usage patterns, Boxall et al. (2014) employed inverse modeling to predict overall removal rates of pharmaceuticals in the environment. The combination of a hydraulic model coupled with prescription and monitoring data from representative sites allowed the researchers to estimate exposure across the entire UK landscape. Agreement between surface water monitoring studies validated the model. Of the 12 study compounds modelled, ibuprofen and diclofenac were found to pose an unacceptable risk in 49.5 % and 4.5 % of river reaches respectively.

In contrast, Celle-Jeanton et al. (2014) compared predicted environmental concentrations (PECs), generated with two previously developed models, with measured environmental concentrations (MECs) in France. Their analysis showed that for many compounds, model generated PECs do not agree with MECs on an annual or temporal basis.

Chitescu et al. (2014) developed a “supply chain” model for pharmaceutical emission to the environment, dissipation through environmental processes, uptake into plants, subsequent bioaccumulation in livestock and transfer to animal products consumed in the human food chain. The model was used to conclude risks associated with uses of oxytetracycline, sulfamethoxazole, and ketoconazole were likely to be minor. Their model provides the framework for the assessment of any number of pharmaceutical contaminants in many different agriculture processes.

Houtman et al. (2014) used results from 2 years of monitoring of surface and artificially-recharged water to estimate the drinking water exposure to 42 pharmaceutical compounds,

including those with both human and agricultural uses. Pharmaceutical concentrations averaged up to 25 ng/L, though most averages were below 5 ng/L. Lifetime exposure from drinking water was estimated using highest concentrations over the monitoring period, and calculated assuming a consumption rate of 2 L/day. Estimated consumption rates were on the order of 1 mg or less over a 70 year time frame.

Liu et al. (2014a) investigated the distribution, bioconcentration, metabolism, and biomarker response of the macrolide antibiotic roxithromycin in fish. Roxithromycin was found to concentrate in the liver and primarily excreted in the bile. Increased activity in the brain and liver provide evidence of biomarker response to roxithromycin. Tetracycline bioaccumulation in the crustacean *Daphnia magna* was studied by Kim et al. (2014). While they found that aqueous uptake was more efficient than through food, biomagnification factor ( $0.19 \pm 0.04$ ) shows that magnification of tetracycline through the food chain will not occur.

Identifying the mode of uptake and translocation potential of tetracycline in *Iberis sempervirens*, a ground cover plant, was the principal objective of Di Marco et al. (2014). They found the internalized tetracycline was found in intercellular spaces and rarely found in cells. Their study also demonstrated toxic effects of the antibiotic including reduced plant growth and inhibited photosynthetic activity. They also identified increased enzymatic activity of plant cells in response to oxidative pressures resulting from tetracycline uptake.

### Effect of Mixtures

Another trend in the 2014 literature is an increased focus on the effects of antibiotic mixtures in the environment. Typically, toxicological studies have focused on the exposure of a single compound. However, in the environment, pharmaceuticals are almost always found in mixtures of multitudes of compounds.

Liu et al. (2014b) studied the combined effects of spiramycin and amoxicillin on the cyanobacteria *Microcystis aeruginosa*. They revealed that the combination of the two compounds could be both synergistic (greater than the added effects of each of the compounds alone) or antagonistic depending on the concentration ratio. At environmentally relevant concentrations the combined effect on the cyanobacteria stimulated algal growth. As the ratio of spiramycin was increased, inhibition of algal growth followed.

Aquatic toxicity of ten veterinary antibiotics and a few four of their binary combinations to *Daphnia magna* and *Daphnia curvirostris* was studied by Bona et al. (2014). Prolonged exposure led to mortality or reproduction inhibition. They also found that for the paired compounds no toxic synergism was observed and the additive effects of each compound represented the worst case.

Sulfonamide antibiotics were the focus of Majewsky et al. (2014). This work identified 11 transformation products of the antibiotic sulfamethoxazole and tested their toxicity individually and as a mixture. Individual exposures showed that the mechanism of transformation played an important role in their bioactivity with some products showing a greater inhibitory effect than the parent compound. The combined effects of the

sulfamethoxazole degradation products were found to be additive. The authors conclude that environmental risk of sulfonamides should consider the total amount of all sulfonamides present as well as the amount of their transformation products.

The impact of photolysis degradation and mixture toxicity of pharmaceuticals was examined by Wang and Lin (2014). They compared toxicity of individual compounds and mixtures to the same compounds after irradiation. Results showed that photolysis results in the rapid transformation of pharmaceuticals but rarely results in total mineralization. Even though photo-transformed species may be benign individually, in mixtures synergistic toxic effects were observed.

The studies conducted by Majewsky et al. (2014) et al. and Wang and Lin (2014) represent the first investigations of the toxic effects of mixtures of degradation products. Their results provide further evidence that the risk associated with pharmaceuticals is not well represented by the analysis of single compounds.

## Antibiotic Resistance Genes

In December of 2013, the Food and Drug Administration announced the implementation of a voluntary plan to phase out the agricultural use of antibiotics that have significant “medical importance” to humans in an effort to combat the growing concern over antibiotic resistance proliferation. The result of this initiative is seen in the 2014 literature with a number of studies that investigate the value and necessity agricultural use of antibiotics and their associated risks.

The increasing incidence of antibiotic resistance in bacteria agricultural environments is thought to be due in part through the use and occurrence of these compounds. The biological effects can be studied through measurement of a variety of methods including quantification of antibiotic resistance genes (ARGs).

Expanding upon previous research concerning ARGs in livestock manure, Wichmann et al. (2014) identified and examined the resistome (ARGs and their originators) in dairy cow manure. Specifically, they focused on resistance genes corresponding to antibiotics commonly used on dairy cows: chloramphenicol, kanamycin, tetracycline, and  $\beta$ -lactam. Utilizing functional metagenomics, the authors created gene libraries for each manure sample and identified resistance gene determinants originating from multiple bacterial phyla, including “novel chloramphenicol resistance determinants that are only distantly related to previously known genes.” Furthermore, this research effort deduced that some microbes in cow manure might harbor multiple ARGs which can be horizontally transferred to soil microbes through direct contact. Ultimately, the efforts presented in this paper have made great strides in generating a comprehensive picture of the resistome in cow manure.

Su et al. (2014) used functional metagenomics to understand the diversity and distribution of previously unknown ARGs in soil and sediment samples. One soil sample was collected from a field with a history of manure application, two sediment samples from rice paddies, and one sediment sample from a shrimp pond. Purified sample DNA was digested and cloned into the *E. coli* pUC19 vector. One metagenomics library was created for each

sample, containing over 100 genomes per library. Overall 45 clones were identified, conferring resistance to 13 different antibiotics (e.g., rifampicin, chloramphenicol, aminoglycoside, or tetracycline). Two novel cluster genes coding acetyltransferase, which can degrade antibiotic aminoglycosides, were discovered. Additionally, two transporter proteins conferring chloramphenicol resistance were identified. Analysis of the amino acid sequences showed that the two proteins were substantially different from previously studied chloramphenicol/florfenicol exporter proteins. The discovery of previously unknown genes proves that functional metagenomics could unlock new information about ARGs in the environment.

Using a mass balance approach, Fahrenfeld et al. (2014) determined the effects of two manure types, manure slurry and dry-stack manure, on the presence and persistence of ARGs in soil receiving manure applications. Field tests were conducted under varying environmental conditions. Throughout the study, application of manure was related to higher loading rates of heavy metals and ARGs when compared to the application of dry-stack manure under similar conditions. Of the specific genes analyzed, *tet(O)* and *tet(W)* did not increase in amended soils over the course of the study when compared to the unamended soils. Similarly, while manure application initially increased the level of *ermF*, *sul1*, and *sul2* in soil, all these ARGs dropped to background levels within two months of initial application. The authors concluded that antibiotic use was not the only factor responsible for determining the fate of ARGs in soil environments. Rather, DNA degradation and bacterial host death may also play a crucial role in determining the extent of ARG attenuation in soil.

Berglund et al. (2014) examined the potential of constructed wetlands in removing ARGs and antibiotics from wastewater. Four wetlands were spiked with antibiotics commonly found in wastewater at environmentally relevant concentrations. The authors monitored the concentrations of 12 antibiotics, their corresponding ARGs, and cDNA reverse transcribed from sediment RNA, as well as the overall bacterial diversity over the course of 25 days. Microbial diversity remained fairly constant in all wetlands while the concentrations of antibiotics in the wetlands dropped to levels comparable to those in wastewater treatment plant effluents by the end of the study. Additionally, *sul1* cDNA was the only ARG associated cDNA that was detected in the wetland throughout the study. For all the antibiotics tested, clarithromycin had the lowest removal rate of 59%. The removal rates for norfloxacin, ciprofloxacin, doxycycline and azithromycin even reached 100%. The authors suggested that wetlands can be a low risk option to remove antibiotics from wastewater. The authors cautioned that higher levels of antibiotics could potentially inhibit the microbes in wetlands while creating an ARG reservoir.

Hsu et al. (2014) studied the concentrations of antibiotics and ARGs in a river upstream and downstream from a swine production facility in Taiwan as well as well water and wastewater from an onsite wastewater treatment facility located on the premises. With eight sulfonamide compounds tested, only two were detected in measurable levels downstream of the treatment plant: sulfadimethazine (SMT) and sulfadimethoxine (SDM). While both SMT and SDM were diluted/treated to unquantifiable levels at the exit of the wastewater treatment facility, SMT concentration later peaked downstream. The authors also studied the class 1 integrons associated with the transfer of the sulfonamide resistance gene *sul1*. They concluded that

horizontal gene transfer could have led to the prevalence of sulfonamide resistant microbes, changing the makeup of the microbial communities further downstream in the river. The authors pointed out that other anthropogenic and natural triggers could also cause the increase of sulfonamide antibiotic resistant bacteria and corresponding ARGs.

Joy et al. (2014) investigated the prevalence of three antibiotics and two ARG classes during the anaerobic storage of swine manure. The concentrations of bacitracin, chlortetracycline, and tylosin were monitored to determine their degradation kinetics. Bacitracin, chlortetracycline, and tylosin concentration declines were modeled using first order decay equations. The measured half-lives ranged from 1 day for chlortetracycline, to 1.9 days for bacitracin, to 9.7 days for tylosin. The relative abundance of all *tet* genes studied decreased to about 10% of their starting levels at the end of 40 days. The relative abundance of *erm*(F) decreased 90% at the end of the storage experiment, whereas the relative abundance of *erm*(B) maintained 50% of its initial level. It is clear that the *erm*(B) gene did not drop as dramatically, leading the authors to conclude that the degradation products of tylosin and other antibiotics in the manure might have contributed to *erm*(B) persistence.

Amarakoon et al. (2014) measured transport of chlortetracycline, sulfamethazine, and tylosin in simulated run-off from surface applied and incorporated manure from a beef cattle feedlot. Soil incorporation reduced run-off concentrations for chlortetracycline and sulfamethazine, but had little effect on tylosin concentrations. Estimated losses of chlortetracycline were lower for incorporated manure, but not for sulfamethazine or tylosin. The results of the study suggest that losses of antibiotics are dependent on their chemical properties and generally can be reduced by manure incorporation.

The study conducted by Cook et al. (2014) examined the possible repercussions of applying poultry litter as a soil amendment in terms of spreading manure-borne pathogens and ARGs. After land application of poultry litter, the abundance of pathogens, fecal indicator bacteria, and ARGs in soil was determined using culture-based and qPCR methods. The concentrations of sulfonamide, streptomycin, and tetracycline resistance genes in soil increased up to 3 orders of magnitude after poultry litter application, and remained above the background level for up to 148 days. Enterococci were the only fecal indicator bacteria that were detected consistently throughout the experiment. The authors suggested enterococci may be good fecal indicator bacteria for field-applied poultry litter.

## Risk Assessment

The difficulty in developing a comprehensive cost-benefit analysis of the subtherapeutic use of antibiotics in agriculture was described by Durso and Cook (2014). They point out that defining background levels of antibiotic resistance is important in assessing the impacts of antibiotic contamination in agricultural environments. Their work highlights the importance of manure and manure handling as the primary mechanism for antibiotic distribution to the environment.

In an effort to identify the benefits of subtherapeutic antibiotic use in the hog industry, Key and McBride (2014) developed a stochastic frontier model to quantify their effects on

productivity. Using 2009 FDA survey results, they found that this practice results in a small positive effect to producers (1.0–1.3 %) but that the greatest improvement in hog productivity was a result of technological improvements. Their analysis revealed that in many cases where subtherapeutic antibiotic use is utilized, other deficiencies in technological equipment or expertise counteracts the small gains.

With the emergence of research conducted to address the occurrence of pharmaceuticals in the environment, Taylor and Senac (2014) address the wide variation in chemical and biological activity of these compounds. They argue that the terminology, human pharmaceuticals, is too broad and that its continued use to raise alarm is scientifically unjustifiable and risks the diversion of resources away from specific substances that may have considerably more significance than others.

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