

Report as of FY2006 for 2006AL49B: "The Fate, Transport, and Effects of Veterinary Antimicrobial Mixtures in the Environment"

Publications

- Conference Proceedings:
 - Sanders, S., P. Srivastava, J. Basile, J. Dane, and Y. Feng. 2007. The Fate and Transport of Veterinary Antimicrobials in the Environment. ASABE Paper No. 072278. ASABE Annual International Conference, Minneapolis, MN. June 17-21, 2007.
 - Sanders, S., P. Srivastava, J. Basile, Y. Feng, J. Dane, and M. Barnett. 2007. The Fate and Transport of Veterinary Antimicrobials, Sulfadimethoxine and Ormetoprim, in the Environment. AWRA 2007 Summer Specialty Conference, Emerging Contaminants in the Environment: Issues, Investigations, and Solutions. Vail, CO., June 25-27, 2007.
 - Sanders, S., P. Srivastava, J. Basile, J. Dane, and Y. Feng. 2006. The Fate and Transport of Veterinary Antimicrobials in the Environment. ASABE Paper #062135. ASABE Annual International Conference, Portland, OR., July 9-12, 2006.
- Articles in Refereed Scientific Journals:
 - Sanders, S., P. Srivastava, Y. Feng, J. Dane, J. Basile, and M. Barnett. 2007. Sorption of the Veterinary Antimicrobials, Sulfadimethoxine and Ormetoprim, in Soil. *J. Environ Qual.* (in review)
- Dissertations:
 - Sanders, S. 2007. The Fate and Transport of the Antimicrobials Sulfadimethoxine and Ormetoprim in the Environment. M. S. Thesis. Auburn University, Auburn, AL.

Report Follows

Statement of Problem and Research Objectives

Pharmaceuticals and personal care products (PPCPs) have recently been classified as “emerging” environmental contaminants. Among the emerging PPCPs, occurrences of antibiotics in the environment have raised particular concerns because their bioactivity often remains after excretion in human and animal wastes and because they have been linked to the development of antibiotic resistant genes in nature. Antibiotics belong to a class of bacterial static and bactericidal compounds collectively referred to as antimicrobials. Antimicrobials are used for therapeutic purposes in human and veterinary medicine, and also for nontherapeutic purposes such as growth promotion in farm animals.

Sulfadimethoxine (SDM) and Ormetoprim (OMP) are two veterinary antimicrobials belonging to the antimicrobial classes of sulfonamides and diaminopyrimidines, respectively. The drug combination of SDM and OMP is approved for use in dogs, chickens, partridges, ducks, catfish, salomids, and turkeys. Of particular interests are its usages in catfish and chickens because of the potentially high risks of environmental contamination. The combination administered to poultry (Rofenaid® 40) and catfish (Romet® 30) primarily enters the environment through land-applied poultry waste and via fish feed and fish excrements.

The poultry and aquaculture industries are important food supplier and generate a great percentage of economic gains in many southern states. These important industries must rely on the use of antimicrobials to maintain healthy animals; however the environmental risks of using these antimicrobials should be fully understood. In order to quantify the ecological risks posed by antimicrobials and to develop management practices to reduce these risks, sound estimates are needed of predicted environmental concentrations (PECs). Calculating accurate PECs requires a thorough understanding of fate and transport processes. The fate of SDM and OMP is often the soil or sediment. Therefore, understanding the SDM and OMP fate and transport in soil is important for the determination of their potential mobility to surface and ground water.

The goal of this research was therefore to study the fate and transport of SDM and OMP in soils and sand representative of poultry and aquaculture in the southeastern United States. Specific objectives were to:

1. Determine the sorption of OMP and SDM and calculate their respective sorption coefficients using batch sorption equilibrium experiments; and
2. Compare the sorption of OMP and SDM when administered in combination as co-solutes as well as when administered individually as single solutes.

Methodology

Two soils representative of agriculture and aquaculture in the southeastern United States were chosen for this experiment. A Coastal Plain soil (Soil 1) was collected from Geneva County, AL and a Tennessee Valley soil (Soil 2) was collected from Sevier County, TN. Because southeastern U.S. soils are generally sandy, pure sand, Ottawa 4.0 was also used to understand the sorption by sand alone (i.e., no clay minerals or organic matter). Antimicrobial sorption will at a minimum be affected by clay and organic matter content, cation exchange capacity and pH; therefore, the selected soils represent several of the physical and chemical characteristics commonly found in the southeastern U.S. Major soil physical and chemical properties (Table 1) were determined at the Soil Testing Laboratory at Auburn University. Both soils were air-dried, ground, and sieved (≤ 2 mm diameter). The sand was muffled at 550°C for four hours to eliminate any possible organic matter. Following this, the sand and soils were irradiated using a ^{60}Co source at 5MRads to eliminate microorganisms that could potentially biodegrade the antimicrobials. The antimicrobials, Ormetoprim (OMP; 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl) pyrimidine) and Sulfadimethoxine (SDM; N'-(2,6 Dimethoxy-4-pyrimidinyl) sulfanilamide), were obtained from Chem Service, Inc., West Chester, PA and Sigma Aldrich, St. Louis, MO, respectively. For the development of the stock solutions, both antimicrobials were initially dissolved in methanol such that the final working solutions contained less than 0.2% methanol.

Table 1. Physical and chemical properties of the selected soils and sand.

Soil Acronym	Description	Order	pH	%Organic Matter	%Sand	%Silt	%Clay	eCEC* (cmol _c /kg)
Soil 1	Plintic Kandiodults	Ultisol	5.03	1.5	81.5	13.5	5	3.19
Soil 2	Typic Eutorchrepts	Inceptisol	4.66	2.07	52	38	10	6.64
Sand	Ottawa 4.0	---	4.92	0	100	0	0	0.33

* eCEC: effective cation exchange capacity

In order to determine the time for SDM and OMP to reach sorption equilibrium ($dC/dt = 0$) in Sand, Soil 1, and Soil 2, batch kinetic experiments were conducted at the natural, unaltered pH of the media. Preliminary soil/solution ratio experiments were performed and the optimum ratio for all combinations was determined to be 1:20 (soil:solution). Antimicrobials were administered to a working solution of 0.01 M CaCl₂ in deionized water. A 100 µg·L⁻¹ concentration was chosen for these experiments because it is approximately the midpoint concentration for batch equilibrium experiments. One gram of each soil or sand was weighed and placed into a 50 mL polypropylene centrifuge tube, the sorbate (OMP or SDM, 20 mL) was added, and the suspension was placed on a reciprocating shaker for a specified time at 25°C. Duplicate samples were removed from the shaker after 1, 2, 5, 8, 16, 24, 39, 48, and 68 hours, respectively. Additionally, duplicate controls were run for each time slot (no sand or soil; solution only). After the specified times, samples were centrifuged at 1200 g for 45 minutes. The supernatant was determined to be clear, immediately removed from the soil (sorbent), filtered with a 0.45 µm PTFE membrane filter, placed in 750 µL polypropylene autosampler vials, and acidified with formic acid for sample preservation so that the final amount of acid was less than 1%. The results for the kinetic experiments suggested that apparent equilibrium was reached for both SDM and OMP in all three media after 24 hours. However, 48 hours was chosen as the equilibrium time for both antimicrobials.

Batch sorption equilibrium experiments were used to determine the sorption of SDM and OMP individually as single solutes and in combination as co-solutes in the selected soils and sand. In other words, three separate experiments were performed in each soil and sand, one with OMP only, one with SDM only, and one with OMP and SDM administered in combination. Concentrations of 5, 10, 50, 100, 250, and 500 µg·L⁻¹ were employed for sorption isotherms because they equated to the range of antimicrobial concentrations found in the environment. Each soil or sand (1 g) was placed into a 50 mL polypropylene centrifuge tube, solution was added (20 mL), and the suspension was equilibrated on a reciprocating shaker in the dark at 25°C. The above procedure was performed in triplicate for each of the six concentration levels. Additionally, duplicate controls for each concentration (no sand or soil; solution only) were run to verify the initial concentration, while a blank (sand or soil, CaCl₂ solution only) was analyzed to verify the lack of laboratory contamination. After equilibration, the samples were centrifuged and prepared for analysis using the procedure described above.

Results

OMP and SDM sorbed differently in Sand, Soil 1, and Soil 2 (Table 2). All distribution coefficients and Freundlich sorption coefficients were similar to those found for other mobile antimicrobials. OMP linear sorption coefficients, K_d , for the single solute and co-solute experiments ranged from 1.3 to 89.7 L·kg⁻¹. The SDM K_d value in Soil 1 was 25.8 L·kg⁻¹. Research performed on other sulfonamides have determined low sorption coefficients, K_d , in the range of 0.62-10 L·kg⁻¹, indicating a relatively high mobility of these compounds.

The carbon normalized sorption coefficient, K_{oc} , was determined for each antimicrobial in the single solute and co-solute systems for Soil 1 and Soil 2 (Table 2). The Tennessee soil sorbed more antimicrobial than the Alabama soil, even when normalized to the organic carbon fraction. The K_{oc} were nearly twice as much in Soil 2 than in Soil 1 for OMP single solute, OMP

co-solute, and SDM single solute. The SDM co-solute K_{oc} , however, only exhibited a 28% increase in Soil 2 compared to Soil 1. The large increase in K_{oc} values between soils either indicates that factors other than soil organic carbon are contributing to the antimicrobial sorption or that the carbon of one soil was more reactive than that of the other soil. Soil 2 has a higher cation exchange capacity, higher clay content, and lower pH than Soil 1. At the lower pH, more of SDM and OMP would be in the cationic species and would likely have a stronger attraction to the negatively charged soil surface.

Table 2. Sorption model coefficients for ormetoprim (OMP) and sulfadimethoxine (SDM) administered as single solutes and co-solutes in two soils and sand. OMP and SDM administered concentration range, 5-500 $\mu\text{g}\cdot\text{L}^{-1}$. Performed at the natural, unaltered soil pH.

Sorbent	Solute	Linear parameters			Freundlich parameters		
		K_d^a	R^2	K_{oc}^a	K_{Fr}^b	n	R^2
Sand	OMP single solute	1.3 (1.0-1.6) ^c	0.60	--	16.0 (11.3-22.8)	0.58 (0.50-0.67)	0.94
	OMP co-solute	4.96 (4.2-5.7)	0.85	--	12.2 (9.5-15.7)	0.86 (0.79-0.92)	0.98
	SDM single solute	0.4 (0.4-0.5)	0.95	--	3.1 (2.1-4.6)	0.64 (0.52-0.75)	0.98
	SDM co-solute	2.5 (1.2-3.8)	0.72	--	79.8 (31.7-201.0)	0.44 (0.24-0.64)	0.65
Soil 1	OMP single solute	21.9 (19.7-24.1)	0.93	2517.24	47.1 (34.0-65.4)	0.90 (0.80-0.99)	0.96
	OMP co-solute	30.9 (28.4-33.4)	0.95	3551.72	50 (38.4-65.1)	0.93 (0.85-1.01)	0.98
	SDM single solute	10.4 (9.2-11.6)	0.94	1195.40	2.1 (0.9-5.1)	1.32 (1.09-1.55)	0.92
	SDM co-solute	12.5 (10.0-15.0)	0.80	1436.78	134.9 (82.9-219.8)	0.54 (0.41-0.67)	0.89
Soil 2	OMP single solute	58.3 (53.9-62.8)	0.96	4855.91	84.9 (57.8-124.6)	0.97 (0.84-1.10)	0.94
	OMP co-solute	89.7 (84.6-94.9)	0.98	7471.26	115.1 (93.8-141.2)	0.98 (0.90-1.05)	0.98
	SDM single solute	25.8 (23.1-28.5)	0.94	2148.93	14.5 (10.3-20.4)	1.10 (1.01-1.20)	0.98
	SDM co-solute	22.1 (19.7-24.6)	0.94	1840.75	106.8 (80.1-142.5)	0.71 (0.63-0.79)	0.97

^a K_d , K_{oc} in units $\text{L}\cdot\text{kg}^{-1}$; ^b K_{Fr} in units $\text{L}\cdot\text{kg}^{-1}$ if $n=1$, else in units $\mu\text{g}^{1-n}\cdot\text{L}^n\cdot\text{kg}^{-1}$; ^cConfidence Intervals (95%)

Each, an increased cation exchange capacity and clay content, and a reduced pH could enhance sorption and appear to be significant based on the dramatic differences in the K_{oc} values of the two soils. Additionally, Sand had a relatively low sorption capacity for either antimicrobial, which indicates that the sand in the soils likely does not play a large role in sorption. The predicted carbon normalized sorption coefficients, K'_{oc} , were calculated based on antimicrobial octanol/water partition coefficients, K_{ow} . The K'_{oc} based on K_{ow} underestimated K_{oc} for both OMP and SDM. The K'_{oc} for OMP was calculated to be $7.42 \text{ L}\cdot\text{kg}^{-1}$, which is much less than the resulting K_{oc} values for OMP (Table 2). The SDM K'_{oc} of $18.45 \text{ L}\cdot\text{kg}^{-1}$ also underestimated the actual K_{oc} .

OMP overall was best fit by the Freundlich equation with all correlation coefficients (R^2) ≥ 0.94 (Table 2); however, the linear isotherm also yielded $R^2 \geq 0.93$ for both Soil 1 and Soil 2 but not for Sand, which did not yield a good linear fit for OMP. This was contributed to the low sorption potential of sand with OMP, in which the sand approached a limited number of sorption sites; however, it was not fit well with the Langmuir isotherm (data not shown). SDM also fit

reasonably well with the Freundlich equation ($R^2 > 0.92$) (Table 2), but was perhaps best fit with a linear isotherm for Soil 1 and Soil 2. The Freundlich models for Soil 1 and Soil 2 with SDM yielded slightly unfavorable sorption ($n > 1$) indicating that these two soils have very little sorption capacity for SDM at low concentrations, but have an increased capacity as the SDM concentration increases. Both SDM and OMP were found to fit better with the Freundlich or linear relationship than by the Langmuir equation. This is likely because the concentrations used in these experiments were limited to the relatively low concentrations found in the environment. Perhaps at higher concentrations, the soils or sand would reach a maximum number of sorption sites for the OMP and SDM.

The co-solute batch sorption equilibrium experiments involved the same approach as the single solute systems except in co-solute batch experiments both antimicrobials were administered together. Model results from these experiments are also presented in Table 2. SDM and OMP followed the same sequence of sorption for co-solute as for single solute systems, viz., Sand < Soil 1 < Soil 2. This was necessarily expected because it follows the sequence of increasing soil organic matter (SOM), cation exchange capacity, clay content (more surface area for sorption), and decreasing pH (Table 1). As stated earlier, the decreasing pH would cause cationic speciation of SDM and OMP, where there would be more affinity for the negatively charged soil surface.

The co-solute sorption of OMP was fit well by both the linear and Freundlich equation (Table 2). However, in the co-solute sorption, the Freundlich nonlinearity coefficient, n , is closer to 1 than in the single solute sorption, indicating that sorption for OMP is more linear in the co-solute system. Additionally, more OMP sorption occurs when in combination with SDM than when administered as a single solute. SDM co-solute sorption is fit best by the Freundlich isotherm and is more nonlinear than the single solute isotherm. The SDM co-solute sorption in the two soils exhibited favorable sorption ($n < 1$) whereas the SDM single solute experiment exhibited unfavorable sorption ($n > 1$).

Sorption of OMP and SDM in this study followed the sorption trend of Sand < Soil 1 < Soil 2. This was expected due to the increasing SOM. The relatively low sorption coefficients for SDM and OMP found in this study suggests that both of these antimicrobials will be relatively mobile in soils and have potential to reach surface and groundwaters. The presence of both SDM and OMP in combination illustrated an enhanced sorption of OMP and a more favorable sorption of SDM ($n < 1$). However, in the low environmentally relevant concentrations, there does not appear to be much difference in the single solute and co-solute experiments. The results of this study further suggest that more attention be given to predicting antimicrobial sorption than simply relying on the organic carbon sorption predictions alone. For OMP these predictions were shown to underestimate the carbon normalized sorption coefficient. This study reveals that OMP sorbed more strongly in the soils and sand than SDM, but both antimicrobials are likely to be relatively mobile and may be found in nearby water sources.