RESEARCH NOTE

Presence of Macrolide-Lincosamide-Streptogramin B and Tetracycline Antimicrobials in Swine Waste Treatment Processes and Amended Soil

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ABSTRACT: Little is known about the fate of antimicrobials during common agricultural waste handling procedures. To better define the potential scope of this problem, concentrations of antimicrobials throughout the waste treatment process were estimated based on known antimicrobial usage, and the resulting predictions of high antimicrobial concentrations indicated the need for further investigation. Samples from building pits, a solids settling basin, a holding pond, and soil amended with waste treatment byproducts were therefore analyzed for traditional chemical parameters and macrolide, lincosamide, and tetracycline antimicrobials. Substantial improvements in water quality were observed during the treatment process. While the macrolide tylosin was not detected, chlortetracycline, oxytetracycline, and lincomycin were found at high concentrations throughout the waste treatment process. Oxytetracycline and lincomycin were also detected in soil from a field amended with waste treatment byproducts. *Water Environ. Res.,* **77,** 57 (2005).

KEYWORDS: antimicrobials, animal production, macrolide-lincosamide-streptogramin B, tetracyclines, swine waste.

Introduction

Antimicrobials are emerging environmental contaminants (Halling-Sorensen et al., 1997; Kolpin et al., 2002). One probable source of environmental antimicrobial contamination is animal production. During animal production, antimicrobials are used for therapeutic purposes and for growth promotion and prophylaxis. The antimicrobials and their derivatives are largely excreted by the animals (Halling-Sorensen et al., 2001) and animal waste and byproducts of animal waste treatment are therefore likely to contain substantial levels of antimicrobials. Recent studies have detected antimicrobials in manure-amended soils (De Liguoro et al., 2003; Hamscher et al., 2002) and in surface and groundwaters proximal to animal production facilities, supporting the hypothesis that agricultural use of antimicrobials is contributing to this contamination (Campagnolo et al., 2002; Kolpin et al., 2002; Yang and Carlson, 2003). Further research is needed to clarify the contributions of agricultural antimicrobial use to environmental contamination and to evaluate the probable consequences of such contamination, both with respect to the ecology of these environments and with respect to the transmission of antimicrobial resistance.

The determination of antimicrobial concentrations in environmental and agricultural samples is complicated by the presence of multiple antimicrobials, by the relatively low concentrations present, and by their adsorption to soil, manure, and other materials. Liquid chromatography-mass spectrometry (LC-MS) methods are frequently used because they provide good specificity and sensitivity (detection limits are in the range of 0.05 μg/L) (Kanfer et al., 1998; Oka et al., 2000). Because of the strong adsorption of some antimicrobials, solid phase extraction (SPE) generally precedes chromatographic analysis for soil and manure samples. Extraction recoveries vary depending on the SPE technique used and the antimicrobial(s) present. For example, recoveries of 70 to 90% have been reported for SPE of tetracyclines in swine lagoon samples, manure slurry, and soil (Hamscher et al., 2002; Zhu et al., 2001). Tylosin recoveries from soil amended with manure were lower, at approximately 60 to 67% (Hamscher et al., 2002), presumably because of the stronger sorption of macrolides to the soil matrix.

In swine grower and finisher production, bacitracin, chlortetracycline, and tylosin are the most commonly used antimicrobials (USDA, 2002). Bacitracin is limited to topical use in human medicine and results in no known cross-resistance (Phillips, 1999). Chlortetracycline and tylosin, on the other hand, both result in cross-resistance to antimicrobials currently used in human medicine. Chlortetracycline and other tetracycline antimicrobials are polyketides that act by inhibiting bacterial protein synthesis. Tylosin and other macrolide antimicrobials consist of macrocyclic lactone rings with sugars linked by glycosidic bonds. Macrolides also act by inhibiting bacterial protein synthesis. Cross-resistance is often observed between macrolide antimicrobials and the structurally unrelated lincosamides and streptogramin B antimicrobials, because these compounds act by binding to overlapping target sites in microorganisms. Therefore, for issues of antimicrobial resistance, the macrolide-lincosamide-streptogramin B (MLS_B) antimicrobials are often considered together.

The purpose of this work was to evaluate the effects of swine waste handling and treatment practices on the levels of antimicrobials in various treatment units and soils to which waste byproducts were applied. Information on antimicrobial usage and waste handling procedures at a swine farm was combined with waste treatment process performance data and concentrations of tetracycline, macrolide, and lincosamide antimicrobials in building, holding pond, and soil samples. This comprehensive analysis of one farm provides insight into the fate of tetracyclines and MLS_B antimicrobials during waste treatment and provides an initial step toward understanding the broader consequences of agricultural antimicrobial use.

Methodology

Farm Description and Sampling Procedures. The farm is an all in–all out (all animals enter and leave a building at the same time) nursery to finish operation of approximately 2400 hog heads with typical waste handling and treatment facilities. This study focused on the finisher stage of the facility, which is the part of the farm that houses animals from 10 to 28 weeks of age. Samples were collected on December 12, 2001, from one location in the shallow pits underneath slotted floors in each of two finisher buildings, two locations in one solids settling basin, two locations in the holding pond, and five locations in manure fertilized land. Solids settling basin and holding pond samples were taken next to and opposite the influent pipe and were composite samples over the entire depth of the units. All samples, except for the soil samples, were stored in new, high-density polyethylene bottles (ColeParmer, Vernon Hills, Illinois), which were washed with a hydrochloric acid (HCl) solution when phosphate analyses were to be performed (APHA, 2000). Soil cores were taken from the top 200 mm because holding pond contents and sludge from the solids settling basins were applied without incorporation to the soil. The five soil samples were taken from the four corners and the center of the field. For each sample, soil cores were obtained from the chosen site and from locations approximately one meter North, South, East, and West of this site. Five soil cores, therefore, represented a single sample and were stored together in a soil-sampling bag (Bageroft Packaging L.L.C., Chicago, Illinois). The soil sampler was sterilized by washing with ethanol and then flaming it between samples. The samples were transported on ice and processed as described below.

Characterization of Waste Handling and Treatment Performance. Suspended solids (SS), volatile suspended solids (VSS), total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD), total five-day biochemical oxygen demand (BOD_5), and nutrient concentrations (total nitrogen [total-N], ammonium + ammonia [NH_3-N], nitrate [NO_3-N], total phosphorus [total-P], and ortho-phosphate [PO_4^{3-}-P) were determined according to standard methods (APHA, 2000). Colorimetric assays were performed using a DR 4000 spectrophotometer (Model 4000, Hach Company, Loveland, Colorado), except for the NH_3-N analyses, for which a microplate reader (340 ATCC, SLT Lab Instruments, Grodig/Salzburg, Austria) was used. For SCOD, NO_3-N, and PO_4^{3-}-P analyses, samples were filtered through 0.45-μm filters and then stored at 4°C until analysis was performed within 48 hours of collection; all other analyses were performed within 24 hours.

Antimicrobial Quantification. Samples for antimicrobial analysis were frozen immediately upon arrival to the laboratory. These frozen samples were subsequently combined to form composite samples from the buildings, the holding pond, and the soil amended with holding pond contents and solids from the solids settling basins, and were shipped on ice to the Institute of Agriculture and Natural Resources at the University of Nebraska (Lincoln, Nebraska) for analysis. The liquid samples were diluted in 0.5-M potassium phosphate/citric acid buffer pH 2.5 (tetracyclines) or a neutral phosphate solution (macrolides) and extracted using Oasis HLB cartridges (Waters Corporation, Milford, Massachusetts). Soil samples were extracted twice with 1-M citric acid/sodium citrate pH 4 and twice with a mixture of acetone and formic acid pH 4. Most of the acetone was evaporated before cleanup with HLB cartridges. Following SPE, concentrations of tetracycline, macrolide, and lincosamide antimicrobials were determined using a triple quadrupole liquid chromatography tandem mass spectrometer as previously described (Snow et al., 2003; Zhu et al., 2001).

Results and Discussion

Waste Handling and Treatment Procedures and Process Performance. The waste collection facilities at the selected farm consist of shallow pits underneath slotted floors in the finisher buildings. The waste from the pits is allowed to flow by gravity to the solids settling basins approximately on a weekly basis (pull-plug system). Water from the holding pond is used to recharge the pits (to a depth of approximately 150 mm). One of the solids settling basins also receives waste from the nursery building, which is recharged with fresh well water after drainage of the waste. The effluents of the solids settling basins flow into the holding pond, which is otherwise undisturbed. Each fall, the solids from the solids settling basins and the entire holding pond contents are land-applied without incorporation to the soil. Although the final basin functions as a holding pond because its entire contents are removed annually, the water quality improvement accomplished in this process (described below) suggests that its performance is similar to that of a lagoon.

Analysis of the waste handling and treatment system was difficult because (1) none of the units were substantially mixed, (2) the waste drainage schedule for the buildings was not fixed, (3) waters with different characteristics were used to recharge the building pits (holding pond water, which, in itself, had variable characteristics, was used to recharge the finisher buildings, while well water was used for the nursery building), (4) the flow from the solids settling basins to the holding pond was regulated by the depth of the solids settling basin contents, which was subject to variations in the building drainage schedule, rainfall events, and timing and amount of land application of solids. Nevertheless, chemical characterizations of the samples collected from finisher buildings, one of the solids settling basins, and the holding pond provide useful information on the degree of treatment taking place in the different unit processes (Table 1). As expected, the concentrations determined for the waste collected in the building pits were very high. The content of the settling basin was low at the time of sampling, resulting in a high level of SS and making it difficult to evaluate the settling performance of this process. Nevertheless, lower concentrations were observed in the solids settling basin than in the building samples, particularly for VSS, SCOD, BOD_5, total-N, and NH_3-N, suggesting that the amount of biological degradation in the solids settling basin was substantial. The high total-P level in the solids settling basin indicates that phosphorus accumulated in the solids settling basin (e.g., as struvite). The two samples collected from the holding pond had substantially different characteristics, suggesting that the holding pond was mixed only to a limited extent, which precluded averaging these data.

However, comparison of the results obtained for the holding pond samples to those determined for the solids settling basin indicate that biologically mediated hydrolysis and degradation of organic

Table 1—Chemical analysis results of swine waste treatment samples.*

Location	pH	SS (%)	VSS (%)	TCOD (mg O_2/L)	SCOD (mg O_2/L)	BOD_5 (mg O_2/L)	total-N (mg N/L)	NH_3N (mg N/L)	NO_3^--N (mg N/L)	total-P (mg P/L)	PO_4^{3-}-P (mg/ P/L)
Finisher buildings	7.7	6.4	5.3	68 053	17 506	23 100	6675	4135	16.8	2071	471
Solids settling basin 1	7.8	6	3.6	65 341	5541	6070	4910	3571	5.8	3460	354
Lagoon (low solids)	8.1	1.1	0.4	4722	1192	582	1510	414	0.7	218	90
Lagoon (high solids)	7.9	4.3	2.2	38 181	1743	1035	2450	1217	3.6	2600	189

* Values are reported as averages for the samples from each of two finisher buildings and two samples from solids settling basin 1.

compounds was also substantial in the holding pond. Based on the high levels of antimicrobials in these units, as described below, this activity probably occurred because of the presence of antimicrobial-resistant microorganisms. This hypothesis is supported by findings in a related study in which high levels of tetracycline and MLS_B-resistant bacteria were observed in these samples (Jindal et al., 2004). At this point, it is not possible to evaluate the effect of the antimicrobials on the treatment process performance and it is thus unclear whether the performance would have been better in the absence of antimicrobials.

Antimicrobial Usage and Estimated Concentrations in Waste Handling and Treatment Processes and in Soil. The antimicrobials used included representatives of several major classes as detailed in Table 2. Members of the tetracycline and MLS_B classes of antimicrobials were used at subtherapeutic (chlortetracycline, tilmicosin, tylosin, and virginiamycin) and therapeutic levels (oxytetracycline and lincomycin). To better understand the implications of this usage information for the waste handling and treatment processes, maximum anticipated concentrations of antimicrobials in the building pits, holding pond, and soil were estimated (Table 3). The mass of antimicrobial entering the building pits was estimated based on the antimicrobial usage reported by the farmer, manufacturer information, and values reported in the literature for unmetabolized drug excretion (Dohono et al., 1993; Halling-Sorensen et al., 2001). For therapeutic antimicrobials, recommended dosages and two doses per production cycle were assumed because no record of this information was maintained by the farmer. Manure production volume was estimated based on numbers and ages of pigs and standard manure production rates (Fulhage and Hoehne, 2001). For soil concentrations, the result of a single manure application over a known area (0.162 km^2) and up to a depth of 200 mm was calculated. To estimate maximum anticipated concentrations, antimicrobial degradation and other removal processes were assumed not to take place.

Quantification of Antimicrobials in Swine Waste Treatment Processes. The concentrations of antimicrobials in the finisher building, holding pond, and soil samples were quantified by LC-MS and compared to the above estimates (Table 3). Even after assuming a conservative extraction efficiency of 60%, the LC-MS results were lower than the predicted maximum concentrations (except for lincomycin), suggesting that degradation took place. The LC-MS results indicate substantial variation in concentrations among antimicrobials, which is not surprising given their different chemical properties. Tylosin was not detected in any of the samples despite its use as a subtherapeutic antimicrobial. This result is consistent with previous reports of rapid tylosin biodegradation in laboratory systems (Angenent et al., 2001; Gavalchin and Katz, 1994; Loke et al., 2000) and the absence of tylosin in manure and soil samples (Campagnolo et al., 2002; De Liguoro et al., 2003; Hamscher et al., 2002; Zhu et al., 2001). Potential tylosin degradation products were not analyzed. Chlortetracycline and oxytetracycline were found at milligram-per-liter concentrations in

Table 2—Subtherapeutic and therapeutic antimicrobial usage.

Additive[a]	Antimicrobial	Class	Additive in feed (g/kg [lb/ton])	Antimicrobial per bodyweight (mg/kg)	Age (weeks)
Subtherapeutic					
Mecadox	Carbadox	Quinoxaline	10 (20)	8.08	3 to 7
Pulmotil	Tilmicosin	Macrolide	7.5 (15)	43.91	8 to 10
Tylan	Tylosin	Macrolide	1.25 (2.5)	1.90	11 to 13
CSP 250	Chlortetracycline	Tetracycline	5 (10)	7.56	14 to 15
	Sulfathiazole	Sulfonamide	5 (10)	7.56	14 to 15
	Penicillin	β-Lactam	5 (10)	3.78	14 to 15
Stafac	Virginiamycin	Streptogramin B	0.25 (0.5)	1.55	16 to 28
Therapeutic			Additive in water (mg/L [mg/gal])		
Terramycin 343	Oxytetracycline	Tetracycline	90 (340)	22.03	11 to 28[b]
Lincomix	Lincomycin	Lincosamide	66 (250)	8.37	3 to 10[b]
Sulfatrimethoprim		Sulfonamide and Diaminopyrimidine	NA	NA	11 to 28[b]

[a] Tylan and Pulmotil are trademarks of ElancoAnimal Health (Indianapolis, Indiana). CSP is a trademark of Boehringer-Ingelheim Vetmedica (St. Joseph, Missouri). Mecadox and Stafac are trademarks of Phibro Animal Health (Ford Lee, New Jersey). Terramycin 343 and Lincomix are trademarks of Pfizer Animal Health (New York).

[b] Administered for periods of four to five days until symptoms disappear.

Table 3—Antimicrobial concentrations in swine waste treatment samples.

Antimicrobial	Estimated			Measured		
	Building[a] μg/L	Holding pond[b] μg/L	Soil[c] ng/g	Building μg/L	Holding pond μg/L	Soil ng/g
Tetracycline	d	d	d (14)	166	214	9
Oxytetracycline	229 545	13 056	837 (152)	4260	2367	254
Chlortetracycline	72 752	7845	503 (253)	3482	3941	4
Tilmicosin	d	3014	193 (4)	47	67	<10
Tylosin	11 822	4526	290 (0)	<2.0	<2.0	<2.0
Lincomycin	d	320	15 (270)	5021	4216	9

[a] Estimated building concentrations do not include dilution by holding pond water and were calculated for the period that the antimicrobial was in use.
[b] Estimated holding pond concentrations were calculated including the entire 26-week production cycle.
[c] Soil concentrations were calculated using estimated holding pond concentrations. The results of similar calculations using actual holding pond concentrations are shown in parentheses.
[d] Antimicrobial was not in use at this location.

building and holding pond samples. Other studies have reported a range of 0.095 to 12 mg/L for concentrations of tetracyclines in lagoon samples, which is consistent with our results (Campagnolo et al., 2002; Zhu et al., 2001). The degradation products tetracycline, anhydrotetracycline, anhydrochlortetracycline, and β-Apo-oxytetracycline were also found in the building and holding pond samples, although combined these degradation products comprised less than 5% of the total tetracyclines detected. The concentrations of oxytetracycline and chlortetracycline observed in building and holding pond samples are at or above the known minimum inhibitory concentrations (MICs) for these antimicrobials (oxytetracycline 0.25 to 8 mg/L [Richez, 1994] and chlortetracycline 0.19 to 4.8 μg/L [Gustafson, 1995; Rogalski, 1985]) and would therefore be expected to inhibit the growth of sensitive microorganisms. Some of the results reported in Table 3 require further explanation. First, tilmicosin and lincomycin were detected in the finisher buildings, despite their application in the nursery building. One potential explanation for this apparent discrepancy involves the use of holding pond water to recharge the finisher building pits, because the holding pond also received waste from the nursery building. These antimicrobials could also have been transferred to the finisher building inside the animals; indirect support for this hypothesis is provided by the fact that another antimicrobial, chlortetracycline, was found in fresh fecal samples for at least 30 days following treatment (Hansen et al., 2002). Second, when compared to our estimate, the LC-MS determined levels of lincomycin were unexpectedly high. The holding pond estimate of 320 μg/L was based on two four-day doses of lincomycin for the nursery building. However, this nursery building only houses one-fourth of the total pigs moving into the finisher buildings. If, as proposed above, lincomycin was transferred to the finisher building inside the animals, then it would be more appropriate to estimate lincomycin concentrations using the total number of pigs in the finisher buildings. With this revision, the estimated holding pond concentration of lincomycin becomes 1280 μg/L. The remaining discrepancy could be because of uncertainties about the timing, number, and length of therapeutic treatments that were given during this production cycle. Because the recommended dosages are weight based, the timing of therapeutic treatments has particular importance for these calculations. If lincomycin would have been used to treat pigs in the finisher buildings, only one additional four-day treatment would be required to bring the estimated and observed concentrations into agreement. In any case, the observed lincomycin concentrations are strikingly high, 20- to 1000-fold higher than a previous report quantifying lincomycin in lagoon samples (Campagnolo et al., 2002), and approximately the reported MIC of 0.2 to 16 mg/L for lincomycin (Kotarski, 1995). These results suggest a long half-life and significant accumulation of lincomycin in conventional swine waste treatment systems.

The process of land application involves a substantial dilution of the wastes and therefore results in lower (predicted and observed) antimicrobial concentrations in the soil. For oxytetracycline, the observed soil concentration was similar to the predicted concentration, in agreement with recent work that reported oxytetracycline to bind strongly to soil and to be relatively stable (Halling-Sorensen et al., 2003; Loke et al., 2002). However, despite their structural similarities, the same was not observed for chlortetracycline, suggesting that chlortetracycline was mobile or subject to degradation.

Oxytetracycline and chlortetracycline had similar extraction efficiencies in lagoon water (Zhu et al., 2001), but no previous work was found that permitted a comparison of mobility or degradation rates for oxytetracycline and chlortetracycline. The measured concentration of lincomycin in the soil was also lower than the concentration estimated using the actual level observed in the holding pond. Lincomycin has been reported to be relatively mobile (Hornish et al., 1985), and lincomycin bioactivity was decreased with a half-life of 20 days in mixtures of urine, feces, and soil (Pharmacia and Upjohn, 2001), so one or both of these removal mechanisms may be substantial for this antimicrobial. The antimicrobial concentrations in the soil samples were below reported MICs. It is therefore possible that the observed antimicrobial concentrations do not cause a significant change in microbial activity in the soil. However, selection for resistance may occur at concentrations below the MICs. Antimicrobial resistant bacteria may also be introduced to the soil through application of the solids from solids settling basins and holding pond contents. Therefore, data on MLS_B and tetracycline resistance in these and other samples are required to fully evaluate the possible effects of applying waste products containing antimicrobials and antimicrobial-resistant bacteria to soil.

Conclusion

This work provided a preliminary investigation of the levels of macrolide, lincosamide, and tetracycline antimicrobials during swine waste collection and treatment and after land application of treated wastes. Despite routine use, the macrolide tylosin was not detected in any of the samples analyzed. However, chlortetracycline, oxytetracycline, and lincomycin were found at high concentrations (mg/L) throughout the treatment process, and oxytetracycline and lincomycin were also detected in soil samples from a field previously amended with waste treatment byproducts. Taken together, these results indicate that some antimicrobials can accumulate to high levels in conventional swine waste treatment systems. The effects of these antimicrobials on waste treatment process performance, soil microbial ecology and productivity, and the spread of antimicrobial resistance deserve further attention.

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References

American Public Health Association; American Water Works Association; Water Environment Federation (2000) *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; Washington, D.C.

Angenent, L. T.; Mau, M.; Jindal, A.; George, U.; Zahn, J. A.; Raskin, L. (2001) Monitoring Antibiotic Resistance in Biological Waste Treatment Systems. Proceedings of the 74th Annual Water Environment Federation Technical Exposition and Conference, Atlanta, Georgia, October 13–17; Water Environment Federation: Alexandria, Virginia.

Campagnolo, E. R.; Johnson, K. R.; Karpati, A.; Rubin, C. S.; Kolpin, D. W.; Meyer, M. T.; Esteban, J. E.; Currier, R. W.; Smith, K.; Thu, K. M.; Mcgeehin, M. (2002) Antimicrobial Residues in Animal Waste and Water Resources Proximal to Large-Scale Swine and Poultry Feeding Operations. *Sci. Total Environ.,* **299,** 89–95.

De Liguoro, M.; Cibin, V.; Capolongo, F.; Halling-Sorensen, B.; Montesissa, C. (2003) Use of Oxytetracycline and Tylosin in Intensive Calf Farming: Evaluation of Transfer to Manure and Soil. *Chemosphere,* **52,** 203–12.

Dohono, A. L.; Darby, J. M.; Helton, S. L.; Sweeney, D. J.; Occolowitz, J. L.; Dorman, D. E. (1993) Tilmicosin Metabolism Study in Tissues and Excreta of Pigs Fed 400 ppm 14C-tilmicosin. Lilly Research Laboratories: Basingstoke, United Kingdom; report submitted to the World Health Organization: Geneva, Switzerland.

Fulhage, C.; Hoehne, J. (2001) Sizing Manure Storage, Typical Nutrient Characteristics. Livestock Poultry Environmental Stewardship Curriculum, MidWest Plan Service (MWPS), Iowa State University: Ames, Iowa.

Gavalchin, J.; Katz, S. E. (1994) The Persistence of Fecal-Borne Antibiotics in Soil. *J. AOAC Int.,* **77,** 481–485.

Gustafson, R. H. (1995) MIC Tests of Veterinary Clinical Isolates, Tetracycline. American Cyanamid Company: Princeton, New Jersey; report submitted to the World Health Organization: Geneva, Switzerland.

Halling-Sorensen, B.; Jensen, J.; Tjornelund, J.; Montforts, M. H. M. M. (2001) Worst-Case Estimations of Predicted Environmental Soil Concentrations (PEC) of Selected Veterinary Antibiotics and Residues used in Danish Agriculture. *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks.* K. Kummerer, Ed. Springer-Verlag: Berlin, 143–157.

Halling-Sorensen, B.; Lykkeberg, A.; Ingerslev, F.; Blackwell, P.; Tjornelund, J. (2003) Characterisation of the Abiotic Degradation Pathways of Oxytetracyclines in Soil Interstitial Water Using LC-MS-MS. *Chemosphere,* **50,** 1331–42.

Halling-Sorensen, B.; Nors Nielsen, S.; Lanzky, P. F.; Ingerslev, F.; Holten Lutzheft, H. C.; Jorgensen, S. E. (1997). Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment—A Review. *Chemosphere,* **36,** 357–393.

Hamscher, G.; Sczesny, S.; Hoper, H.; Nau, H. (2002) Determination of Persistent Tetracycline Residues in Soil Fertilized with Liquid Manure by High-Performance Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry. *Anal Chem.,* **74,** 1509–18.

Hansen, L. H.; Aarestrup, F.; Sorensen, S. J. (2002) Quantification of Bioavailable Chlortetracycline in Pig Feces Using a Bacterial Whole-Cell Biosensor. *Vet. Microbiol.,* **87,** 51–7.

Hornish, R. E.; Gosline, R. E.; Arnold, T. S.; Butine, T. J.; Smith, L. J.; Subacz, C. J. (1985) Pharmacokinetics and Oral Absorption of Lincomycin (U-10, 149) in Swine. Agricultural Research and Development Laboratories, The Upjohn Company: Kalamazoo, Michigan; report submitted to the World Health Organization: Geneva, Switzerland.

Jindal, A.; Kocherginskaya, S.; Robert, M.; Mackie, R. I.; Raskin, L.; Zilles, J. L. (2004) Antimicrobial Resistance in Swine Waste Treatment Systems. (not yet published).

Kanfer, I.; Skinner, M. F.; Walker, R. B. (1998) Analysis of Macrolide Antibiotics. *J. Chromatogr. A,* **812,** 255–86.

Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. (2002) Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000: A National Reconnaissance. *Environ. Sci. Technol.,* **36,** 1202–11.

Kotarski, S. F. (1995) Safety Evaluation of the Lincosamindes: Gut Flora Effects. The Upjohn Company: Kalamazoo, Michigan; report submitted to the World Health Organization: Geneva, Switzerland.

Loke, M.-L.; Ingerslev, F.; Halling-Sorensen, B.; Tjornelund, J. (2000) Stability of Tylosin A in Manure Containing Test Systems Determined by High Performance Liquid Chromatography. *Chemosphere,* **40,** 759–765.

Loke, M. L.; Tjornelund, J.; Halling-Sorensen, B. (2002) Determination of the Distribution Coefficient (log Kd) of Oxytetracycline, Tylosin A, Olaquindox and Metronidazole in Manure. *Chemosphere,* **48,** 351–61.

Oka, H.; Ito, Y.; Matsumoto, H. (2000) Chromatographic Analysis of Tetracycline Antibiotics in Foods. *J. Chromatogr. A,* **882,** 109–33.

Pharmacia and Upjohn (2001) Lincomix Soluble Powder Material Safety Data Sheet. Pharmacia and Upjohn: Kalamazoo, Michigan.

Phillips, I. (1999) The Use of Bacitracin as a Growth Promoter in Animals Produces no Risk to Human Health. *J. Antimicrobiol. Chemother.,* **44,** 725–8.

Richez, P. (1994) Antibacterial Activity of Tetracycline and Oxytetracycline Against Human Gut Flora. Datavet Veterinary Drug Consultants, Intervet International: Boxmeer, Netherlands; report submitted to the World Health Organization: Geneva, Switzerland.

Rogalski, W. (1985) Chemical Modifications of the Tetracyclines. *The Tetracyclines. Hlavka and Boothe.* Springer Verlag: Berlin, 233.

Snow, D. D.; Cassada, D. A.; Monson, S. J.; Zhu, J.; Spalding, R. F. (2003) Trace Analysis of Tetracycline and Macrolide Antibiotics Using Phase Extractioin and Liquid Chromatography-Tandem Mass Spectrometry. *Liquid Chromatography/Mass Spectrometry, MS/MS and Time of Flight MS: Analysis of Emerging Contaminants.* I. Ferrer and E. M. Thurman (Eds.); American Chemical Society: Washington, D.C.

USDA (2002) Part II: Reference of Swine Health and Health Management in the United States, N355.0202. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System: Fort Collins, Colorado.

Yang, S.; Carlson, K. (2003) Evolution of Antibiotic Occurrence in a River Through Pristine, Urban and Agricultural Landscapes. *Water Res.,* **37,** 4645–56.

Zhu, J.; Snow, D. D.; Cassada, D. A.; Monson, S. J.; Spalding, R. F. (2001) Analysis of Oxytetracycline, Tetracycline, and Chlortetracycline in Water Using Solid-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry. *J. Chromatogr. A,* **928,** 177–86.