ANTIBIOTIC USE IN AGRICULTURE AND ITS IMPACT ON THE TERRESTRIAL ENVIRONMENT

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Advances in Agronomy, Volume 87

0065-2113/05 $35.00
DOI: 10.1016/S0065-2113(05)87001-4

Since their discovery, antibiotics have been instrumental in treating infectious diseases that were previously known to kill humans and animals. However, their widespread use as an additive in animal feeds has raised concerns about the development of antibiotic-resistant microorganisms. Increasingly, more microorganisms are becoming resistant to multiple antibiotics. A high proportion of the antibiotics added to animal feed is excreted in urine or manure. In some cases, as much as 90% of the antibiotic administered orally may pass through the animal unchanged. Once excreted in urine and manure, these antibiotics can enter surface and/or groundwater through nonpoint source pollution from manure-applied lands. The literature shows that most of the antibiotics are strongly adsorbed in soils and are not readily degraded. An important environmental concern is the presence of antibiotics in sources of potable water. Except erythromycin and some sulfa drugs, most of the antibiotics found in surface waters have been only in minute quantities. In all cases, the amounts observed are in parts per billion ranges; 100- to 1000-fold below minimum inhibitory concentration. Tetracyclines and penicillins, two of the most commonly used antibiotics in animal agriculture, have seldom been found in sources of potable water. There has been some reported presence of resistant bacteria in surface waters. This may have been from transport of resistant bacteria via animal or insect vectors, in airborne dusts, or simply water flow from some antibiotic-rich setting such as manure lagoons. Direct toxic effects of antibiotics on plants and soil microflora and -fauna are unlikely because of the low concentrations at which antibiotics in manure are land-applied. The indirect effects of antibiotics on the food web, however, cannot be discounted at this stage. Decrease in some components of the soil microbial populations due to manure-applied antibiotics could cause loss of food sources for other soil organisms, which, in turn, could affect important soil microbial processes such as decomposition and mineralization. Also, repeated application of antibiotic-laden manure can provide an environment in which selection of antibiotic-resistant bacteria can occur. Prudent use of antibiotics to a bare minimum along with alternative methods that minimize development and proliferation of resistant bacteria need investigation.

I. INTRODUCTION

Since their discovery in the early 1900s, antibiotics have been instrumental in treating infectious diseases that were previously known to kill humans and animals (Kumar *et al.*, 2004). However, it has now become clear that the widespread use of antibiotics is not without problems (Halling-Sørensen *et al.*, 1998; Jørgensen and Halling-Sørensen, 2000; Rooklidge, 2004). The major concern is that widespread use of antibiotics may lead to the emergence of new strains of bacteria that are resistant to these antibiotics and, in turn, result in untreatable livestock diseases (Hirsh and Wiger, 1977; Solomons, 1978). A potentially more dangerous scenario is the possible transmission of such strains to humans, resulting in untreatable human diseases.

Although most antibiotics are used for the treatment of infections in humans and animals, a significant portion of these are also used in animal feed as a supplement to promote growth in food animals. The use of antibiotics for animal growth promotion is not new; these pharmaceuticals were approved in the United States and United Kingdom in 1949 and 1953, respectively (Witte, 2000). Antibiotics in animal feed helps increase the animal's ability to absorb feed and thus reach market weight earlier. In addition, supplementing antibiotics in animal feed helps counteract the effects of crowded living conditions and poor hygiene in intensive animal agriculture (EMS, 2000). At least four mechanisms have been suggested as explanations for antibiotic-mediated growth enhancement (Gaskins *et al.*, 2002): (i) inhibition of subclinical infections, (ii) reduction in growth-depressing microbial metabolites, (iii) reduction in microbial use of nutrients, and (iv) enhanced uptake of nutrients through the thinner intestinal wall of antibiotic-fed animals.

The antibiotic dose varies from 3 to 220 g Mg^{-1} of feed, depending upon the type and size of the animal and the type of antibiotic (McEwen and Fedorka-Cray, 2002). Even these low quantities of antibiotics encourage the selection of antibiotic-resistant bacteria (Khachatourians, 1998); however, feeds often contain more than the recommended amounts. In an examination of more than 3000 swine feeds in the United States, 25% contained antibiotics at concentrations higher than the recommended levels (Dewey *et al.*, 1997). Animals do not utilize all the antibiotics in feed and a large proportion of the added antibiotics are excreted in urine or manure (Levy, 1992). Once excreted, these antibiotics can enter the terrestrial environment through land application of manure (Fig. 1) and potentially alter the soil microbial ecosystem.

Land application of manure is a common practice in many parts of the United States. In the northern tier of the country, manure is applied even during winter over snow. Manure is land-applied because of its value in supplying nutrients to crops as well as a means of disposing unwanted waste. Although it is strongly recommended that manure application rates be based on the nutrient status of the soil and crop needs, this recommendation is not always followed and thus the manure applications have frequently been at higher than the recommended rate.

In 1997, the United States Department of Agriculture (USDA) estimated that livestock population of more than 8 billion animals (more than 95% of them chickens and turkeys) produced up to 1.32 billion Mg of manure in the United States (Table I). These numbers suggest that the presence and persistence of antibiotics in these large quantities of manure present a significant environmental problem both in terms of toxicity of these antibiotics to soil microflora and -fauna as well as to an increase in antimicrobial resistance in the environment. Baguer *et al.* (2000) claim that land application of

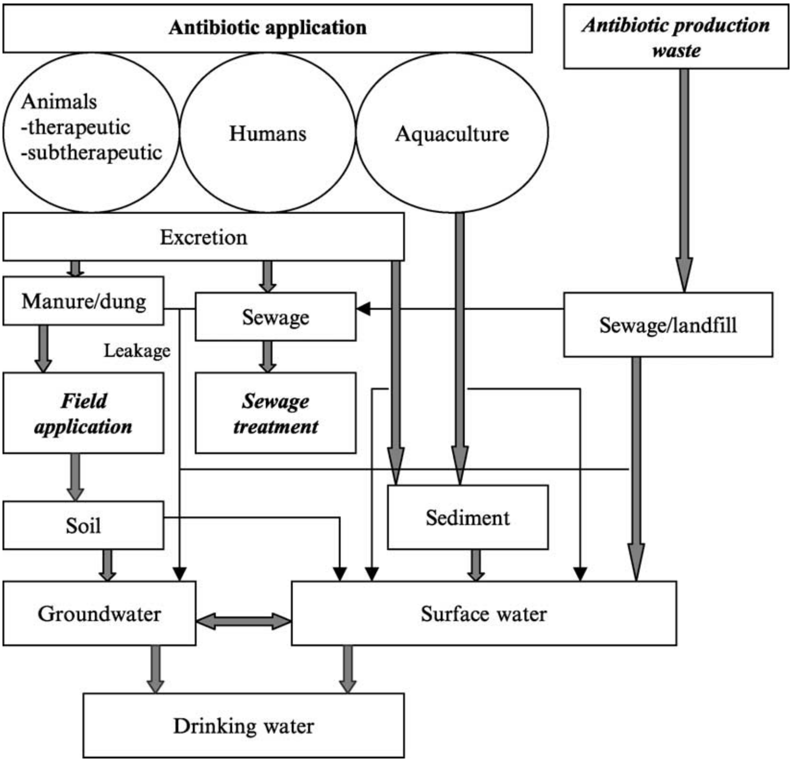

Figure 1 Antibiotic application.

Table I
Total Manure Production in the United States in 1997

Manure type	Amount (dry, Mg)
Swine	8,472,548
Poultry	16,196,865
Dairy cows	24,521,640
Feed lot beef	11,044,539
Other cattle	71,899,070
Tota1	32,134,664

Data from www.ers.usda.gov/data/manure.

antibiotic-laced manure appears to be the dominating pathway for the release of antibiotics in the terrestrial environment.

There have been reports on the presence of antibiotics in various surface bodies. A television news story reported the presence of antibiotics in a lake in Ohio (TV Network, Feb. 2000). It is unknown how these pharmaceuticals found their way to the lake. Since antibiotics are regularly fed to animals, it is likely that antibiotics present in that lake came with surface runoff from fields where manure may have been applied. Since ground and surface waters are not regularly tested for antibiotics in the United States, it is unknown to what extent this type of contamination exists in lakes, rivers, and groundwater in the United States. USGS has reported the presence of several antibiotics in 139 streams across 30 states in the United States (Kolpin *et al.*, 2002). However, the contributions of agricultural runoff versus wastewater from sewage treatment plants to the presence of antibiotics in these streams is unclear. Yang and Carlson (2003) monitored five tetracycline and six sulfonamide compounds at five sites along the Poudre River in Colorado. They found no antibiotics at the pristine site in the mountains but found all five tetracycline compounds when the river entered the agricultural landscape. Sites with urban and agricultural contributions had the highest tetracycline concentration. There was a lack of sulfonamide compounds from the agricultural landscape possibly due to natural attenuation mechanisms such as photolysis, biodegradation, hydrolysis, and adsorption. The number of antibiotic compounds found by Yang and Carlson (2003) was also higher than in the Kolpin *et al.* (2002) study. The authors speculated that the higher number and concentration in the Poudre River might be due to low flow after prolonged drought in Colorado. Kolpin *et al.* (2004) reported that in Iowa streams, antibiotics and other prescription drugs were only frequently detected during low flow conditions.

Chee-Sanford *et al.* (2001) suggested that if land application of antibiotic-laden manure continues, groundwater could become a potential source of antibiotics in the food chain. Alder *et al.* (2001) reported that concentrations of sulfonamide–sulfametazine were higher in a lake surrounded by intensive animal husbandry operations than in the effluents of wastewater treatment plants in the same area.

Until recently, research on antibiotic use has been mainly directed toward their beneficial and adverse effects on the end user, human and animal. However, there have been relatively few studies on the effect of these antibiotics on the environment. In the terrestrial environment, the antibiotics are being introduced mainly through land application of manure, sludge, and wastewater (Fig. 1). The other pathways by which antibiotics are being released in the terrestrial environment are possibly the disposal of manufacturing and hospital waste and unused and expired household products containing antibiotics in landfills. The European Agency for the Evaluation of Medicinal Products has recommended a more intensive environmental safety evaluation of veterinary medicinal products if any ingredient or metabolite is present in manure in concentrations > 0.1 mg kg^{-1} (Haller *et al.*, 2002).

The principal concern in widespread use of antibiotics in agriculture is the increasing emergence of antibiotic-resistant bacteria in both clinically relevant strains of pathogens and normal commensal microorganisms. Four possible pathways have been suggested for the spread of antibiotic-resistant bacteria in the terrestrial environment due to antibiotics use in agriculture: (i) selection of resistant microbial population (including pathogens) in the animal gut (and then to the environment) due to shedding in feces (Kelly *et al.*, 1997; Sundin *et al.*, 1995), (ii) transfer of resistance genes (borne on plasmids, integrons, and gene cassettes) from bacteria in manure to native soil and water microbial populations (Gast *et al.*, 1988; Goyal and Hoadley, 1979), (iii) accumulation of antibiotics in animal and plant tissues that are subsequently consumed by humans whose own bacteria may, in turn, become antibiotic-resistant (Kobe *et al.*, 1995; Richter *et al.*, 1996), and (iv) manure antibiotics, when land-applied, may impart resistance to native flora and fauna which, in turn, spread to the rest of the environment. Walsh (2000) cites three mechanisms for antibiotic resistance in microbes: (1) overproduction of existing protein pumps to export antibiotic drugs from the cells, (2) minor structural changes in various proteins of target cells, and (3) destruction of the antibiotics by interaction with slightly modified preexisting enzymes in the organism. Once microbes acquire antibiotic resistance, they exchange this information with other microbes through a variety of mechanisms such as transformation, conjugation, transposition, and integrons exchange (Bower and Daeschel, 1999; Davies, 1994; Heinemann, 1999; Mazel and Davies, 1999).

The goal of this chapter is to review pertinent information about antibiotic use in agriculture and the subsequent fate of the antibiotics in the terrestrial environment.

II. ANTIBIOTIC USE ESTIMATES

Production rates for specific antibiotics and other drugs are not available in the literature. Large quantities of antibiotics are used not only for treatment of diseases in humans and animals, but also for growth promotion of animals and in soaps, creams, and disinfectants (Table II). There is no precise reporting of antibiotic use in agriculture or specifically in animal production. Furthermore, there is a wide variation in the estimations by different organizations. The Institute of Medicine estimates that 50 million pounds (22,675 Mg) of antibiotics are being produced each year, out of which 60% is used in human medicine, 32% for nontherapeutic use in agriculture, and 8% for therapeutic use in agriculture (Shea, 2003). According to the Union of Concerned Scientists, 78% of the antibiotics produced in the United States are used for nontherapeutic purposes in agriculture (Shea, 2003). McManus *et al.* (2002) estimates that between 18 to 60 Mg

Table II
Antibiotic Use in the United States (Mellon *et al.*, 2001) and the European Union (Kümmerer, 2001)

	United States	European Union
Human prescriptions	1361	5000
Tropical creams, soaps, disinfectants, etc.	680	NA
Total humans	2041	5000
Nontherapeutic	12,471	3500
Therapeutic	907	1500
Total animals	13,378	5000

NA, Not available.

of antibiotics (mainly oxytetracycline and streptomycin) are being applied to plants annually in the United States. The United States Office of Technology Assessment (1995) estimated that about 8163 Mg of antibiotics are used in animal agriculture and approximately 22 Mg in fruit tree production.

A. Antibiotic Use in Food Animals

Antibiotics are routinely used in animal agriculture to treat diseases and promote growth. It is believed that subtherapeutic levels of antibiotics in feed (3–220 g Mg^{-1} feed) help animals grow faster and decrease their susceptibility to stress-related diseases (Feinman and Matheson, 1978; Gavalchin and Katz, 1994). Commonly used antibiotics in animal agriculture are listed in Table III. Specific antibiotic doses vary, depending on the type of animal and its growth stage (Tables IV and V). Frequently, combinations of two or more antibiotics are used. Some of these antibiotics have a withdrawal period but some could be fed continuously up to the point of slaughter. The modes of action of these antibiotics vary from inhibition of protein synthesis, cell wall synthesis, and even DNA replication (Table VI).

1. Extent of Antibiotic Excretion

Most antibiotics fed to animals are poorly absorbed in the animal gut and, as a result, there is substantial excretion of antibiotics in urine and feces (Boxall *et al.*, 2002). As much as 90% of some antibiotics may be excreted as the parent compound (Table VII). Excretory organs eliminate polar compounds (tetracyclines and tylosin) more efficiently than compounds that

Table III
Some of the Antibiotics Commonly Used for Therapeutic or Subtherapeutic Purposes in Animals

Purpose	Cattle	Swine	Chickens/Turkey	Fish	Sheep
Treatment of infections	Amoxicillin* Cephapirin Erythromycin* Flouroquinolone Gentamicin* Novobiocin Penicillin* Sulfonamides Tilmicosin Tylosin	Amoxicillin* Ampicillin* Chlortetracycline* Gentamicin* Lincomycin Sulfamethazine Tiamulin Tylosin	Erythromycin* Fluoroquinolones Gentamycin* Neomycin Penicillin* Spectnomycin Tetracyclines* Tylosin Virginiamycin	Ormetoprim Sulfonamide Oxytetracycline*	Chlortetracycline* Erythromycin* Neomycin Oxytetracycline* Penicillin*
Growth and feed efficiency	Bacitracin Chlortetracycline* Lasalocid Monensin Oxyteracycline* Amoxicillin* Ampicillin* Bacitracin Ceftiofur Dihydrostreptomycin Erythromycin* Furamazone Gentamycin* Neomycin Penicillin* Streptomycin* Tilmicosin	Asanilic acid Bacitracin Bambermycin Chlortetracycline* Erthythromycin* Penicillin* Tiamulin Tylosin Virginiamycin	Bambermycin Bacitracin Chlortetracycline* Penicillin* Tylosin Virginiamycin		

*Also used in humans.

Table IV
Antibiotics as Feed Additives Used in Swine for Increased Rate of Weight Gain and Improved Feed Efficiency

Antibiotic	Level (g Mg^{-1})	Comments	Withdrawal time (days)
Arsanilic acid	50–99		5
Bacitracin	11–33		None
Bacitracin zinc	11–55		None
Bambermycins	2–5		None
Carbadox	11–28		42
Chlortetracycline	11–55		None
	110	From 6–16 weeks post-weaning	7
Lincomycin	22	Growing–finishing swine	None
Oxytetracycline	11–55		5
Penicillin	11–55		None
Roxarsone	25–37	Growing–finishing swine	5
Tiamulin hydrogen fumerate	11		None
Tylosin	11–22	Finisher	None
	22–44	Grower	None
	22–110	Starter and pre-starter feeds	None
Virginiamycin	6–11		None

Data from Herrman and Sundberg, 2001.

have high lipid solubility. Lipid soluble antibiotics such as erythromycin, clindamycin, trimethoprim, and metronidazole are often not eliminated until they are metabolized to more polar compounds (Benet *et al.*, 1990). Sulphonamides, on the other hand, are excreted either as unaltered parent compound or as acetic acid conjugates (Boxall *et al.*, 2002). However, during manure storage, these conjugates may revert back to their parent compound (Boxall *et al.*, 2002; Hirsch *et al.*, 1999).

Similar to animals, humans also excrete a large proportion of antibiotics as the parent compounds (Table VIII). Hoeverstadt *et al.* (1986) found trimethoprim and doxycycline concentrations in the range of 3 to 40 mg kg^{-1} of raw sewage and erythromycin concentrations in the range of 200 to 300 mg kg^{-1} of raw sewage. The elimination of antibiotics in the sewage treatment plants is between 54 and 99% (Ternes *et al.*, 2001). The remaining antibiotics end up in sewage sludge and effluent, which, on land application, provide another pathway for antibiotic entry in the terrestrial environment.

Table V
Antibiotics as Feed Additives Used in Beef Cattle and Calves for Increased Rate of Weight Gain and Improved Feed Efficiency

Antibiotic	Level	Comments	Withdrawal time (d)
Bacitracin zinc	35–70 mg head^{-1} d^{-1}		None
Bambermycins	1–5 g Mg^{-1}		
	2–45 g Mg^{-1}	Pasture, slaughter, stocker	None
Laidlomycin	6 g Mg^{-1}	Confined cattle for slaughter	None
Lasalocid	11–33 g Mg^{-1}	Confined cattle for slaughter	None
	60–200 mg head^{-1} d^{-1}	Dairy and beef heifers	
Melengestrol	0.25–0.50 mg head^{-1} d^{-1}	Estrus suppression in heifers	None
Monensin	6–33 g Mg^{-1}		None
Oxytetracycline	75 mg head^{-1} d^{-1}	Finishing cattle	0 to 5
Virginiamycin	9–25 g Mg^{-1}		None
	Combination antibiotics		
Chlorotetracycline	350 mg head^{-1} d^{-1}	Weight gain in presence of respiratory disease	7
Sulfamethazine	350 mg head^{-1} d^{-1}		
Lasalocid	28–33 g Mg^{-1}		None
Oxytetracycline	8.3 g Mg^{-1}		
Lasalocid	100–360 mg head^{-1} d^{-1}	Heifers fed in confinement	None
Melengestrol	0.25–0.50 mg head^{-1} d^{-1}		
Lasalocid	11–33 g Mg^{-1}	Heifers	None
Melengestrol	0.125–1.0 mg head^{-1} d^{-1}		
Tylosin	90 mg head^{-1} d^{-1}		
Monensin	6–33 g Mg^{-1}		None
Tylosin	9–11 g Mg^{-1}		
Melengestrol	0.28–2.2 g Mg^{-1}		None
Tylosin	99–397 g Mg^{-1}		
Monensin	55–132 g Mg^{-1}		

Data from Herrman and Stokka, 2001.

2. Antibiotic Levels in Manure and Soil

The most common antibiotics present in swine and turkey manures are tetracyclines, tylosin, sulfamethazine, amprolium, and nicarbazine (De Liguoro *et al.*, 2003; Kumar *et al.*, 2004; Webb and Fontenot, 1975). The concentration of these antibiotics varies from traces to as high as 216 mg L^{-1}

Table VI
Mode of Action of Different Antibiotics Generally Used in Animal Agriculture

Class/Group	Antibiotic	Mode of Action
Tetracyclines	Chlortetracycline Oxytetracycline	Inhibits protein biosynthesis
Macrolide	Tylosin Erythromycin Tilmicosin	Inhibits protein biosynthesis
Aminocyclitols	Spectinomycin	Inhibits protein biosynthesis
	Gentamicin Neomycin Apramicin	Inhibits protein biosynthesis
β-lactum	Penicillin Ampicillin	Inhibits cell wall biosynthesis
Cephalosporin	Ceftiofur	Inhibits cell wall biosynthesis
Chlorampenicol derivative	Florfenicol	Inhibits protein biosynthesis
Sulphonamides	Sulphadimethoxine Sulphachloropyridizine Sulphathiazole	Inhibits folic acid biosynthesis
Trimethoprim: Sulphathiazole	Trimethoprim: Sulphathiazole	Inhibits folic acid biosynthesis
Fluoroquinolone	Enrofloxacin	Inhibits DNA replication
	Tiamulin	Inhibits protein biosynthesis
Lincosamides	Clindamycin	Inhibits protein biosynthesis
Ionophore	Monensin	Interfere with cytoplasmic membrane

Table VII
Proportion of Antibiotics Fed Excreted in Urine and Feces

Antibiotic	% Excreted in urine and feces	Reference
Tetracyclines	80	Aiello, 1998
Chlortetracycline	75	Morrison *et al.*, 1969
Lincomycin	60	Aiello, 1998
Quinacrine	10	Kulda and Naỳnkovà, 1995
Metronidazole	40	Kümmerer *et al.*, 2000
Chloroquine	70	Goldsmith, 1992
Oleandomycin, Tylosin, Erythromycin, Salinomycin, and Monensin	50–90	Bester *et al.*, 2002 and Schlüsener *et al.*, 2003 quoting Kroker, 1983

Table VIII
Human Prescription Amounts and Excretion Rates of Some Commonly Used Antibiotics

Antibiotic compound	Daily dose (mg)	Excretion of original compound (%)
Amoxicillin	750–2250	80–90
Ampicillin	3000–6000	30–60
Penicillin V	2000	~40
Penicillin G	240–720	50–70
Sulfamethoxazole	400–1600	~15
Trimethoprim	80–360	~60
Erythromycin	200–1000	>60
Roxithromycin	150–300	>60
Clarithromycin	125–250	>60
Cloramphenicol	—	5–10
Chlortetracycline	—	>70
Tetracycline	—	80–90
Minocycline	100–200	~60
Oxytetracycline	—	>80
Doxycycline	100–200	>70

Data from Hirsch *et al*., 1999.

of manure slurry (Tables IX and X). Manure samples obtained from four swine producers in Minnesota contained traces to as high as 7.73 mg L^{-1} chlortetracycline and 4.03 mg L^{-1} tylosin (Kumar *et al.*, 2004). At a manure application rate of ~50,000 liters per hectare (equivalent to 168 kg ha^{-1} N application), this will result in land application of 387 g of chlortetracycline and 202 g of tylosin per hectare.

Only a few studies have been undertaken to determine antibiotic levels in the soil after manure application. Van Gool (1993) estimated that if all growth promoters used in the Netherlands were spread over the two million hectares of Dutch arable land, an average of 130 mg antibiotics and their metabolites per m^2 plow layer of arable land would be found, an equivalent to 0.9 mg of antibiotic kg^{-1} of dry soil. Warman *et al.* (1977) reported the presence of amprolium at 0.8 mg kg^{-1} dry soil in the top 13 cm of soil 80 days after chicken manure application. De Liguoro *et al.* (2003) found oxytetracycline concentrations at 6, 7, and < 5 μg kg^{-1} of soil at 0, 30, and 60 cm depth after cattle manure application at 96 Mg ha^{-1}. These authors also reported the presence of tylosin at < 10 μg kg^{-1} of soil. Hamscher *et al.* (2002) reported tetracycline concentrations of 86, 199, and 172 μg kg^{-1} of soil at 0–10, 10–20, and 20–30 cm depths, respectively, when amended with liquid swine manure. The corresponding chlortetracycline concentration varied from 4.6–7.3 μg kg^{-1} of soil. Studies have also shown that these antibiotics generally remain stable during manure

Table IX
Concentration of Antibiotics in Different Manures

Manure type	Antibiotics	Concentration mg kg^{-1} or mg L^{-1}	Country	Reference
Hog lagoons and cattle	Tetracyclines	0.0005–200	USA	Aga *et al.*, 2003
Swine-Liquid	Chlortetracycline	3.5–5.2	USA	Kumar *et al.*, 2004
	Tylosin	3.3–7.9		
Beef cattle	Chlortetracycline	5.3	USA	Patten *et al.*, 1980
	Oxytetracycline	11.3		
Cattle				
Fresh	Chlortetracycline	14.0	USA	Elmund *et al.*, 1971
Aged	Chlortetracycline	0.34		
Cattle feces	[^{14}C]Ceftiofur	11–216	USA	Gilbertson *et al.*, 1990
Cattle	Monensin	1–5	Canada	Donoho, 1984
Poultry	Chlortetracycline	23	Canada	Warman and Thomas, 1981
Liquid	Tetracycline	20	Germany	Winckler and Grafe, 2000
	Sulfadimidine	40		
Swine slurry	Tetracycline	5–24	Germany	Hamscher *et al.*, 2002
Swine slurry	Tetracycline	0.04–0.70	Denmark	Sengeløv *et al.*, 2003
Swine-Liquid	Tetracycline	4.0	Germany	Hamscher *et al.*, 2002
	Chlortetracycline	0.1		
Cattle (matured – 5 m)	Oxytetracycline	0.82	Italy	De Liguoro *et al.*, 2003
	Tylosin	0.1		
Cattle (day 30–day 135)	Oxytetracycline	2–19	Italy	De Liguoro *et al.*, 2003
	Tylosin	0.001–0.1		
Manure from mother pigs with farrows	Sulfamethazine	3.3–8.7	Switzerland	Haller *et al.*, 2002
	Sulfathiazole	0–12.4		
	Trimethoprim	Traces		
Manure from fattening pigs	Sulfamethazine	0.13–0.23	Switzerland	Haller *et al.*, 2002
	Sulfathiazole	0.10–0.17		
Fattening calves	Sulfamethzine	3.2	Switzerland	Haller *et al.*, 2002
	Sulfathiazole	Traces		

storage until its application to agricultural fields (Boehm, 1996; Migliore *et al.*, 1995). If these antibiotics have some persistence, concentrations will build up in soil on repeated manure application and thus present a significant potential for entry of antibiotics into the rest of the terrestrial environment.

Table X
Concentration of Different Antibiotics in Broiler Litter Samples Obtained from Virginia, United States

	Level		
Antibiotic	Average	Range	No. of samples
Oxytetracycline, mg kg^{-1}	10.9	5.5–29.1	12
Chlortetracycline[a], mg kg^{-1}	12.5	0.8–26.3	26
Chlortetracycline[b], mg kg^{-1}	0.75	0.1–2.8	19
Penicillin, units g^{-1}	12.5	0–25.0	2
Neomycin, mg kg^{-1}	0	0	12
Zinc bacitracin, units g^{-1}	7.2	0.8–36.0	6
Amprolium, mg kg^{-1}	27.3	0–77.0	29
Nicarbazine, mg kg^{-1}	81.2	35.1–152.1	25

[a]Chlortetracycline used continuously in broiler diets.
[b]Chlortetracycline used intermittently in boiler diets.
Data from Webb and Fontenot, 1975.

B. Antibiotic Use in Plants

Only two antibiotics, streptomycin and oxytetracycline, are registered by the United States Environment Protection Agency (USEPA) for use in plant agriculture (Tables XI and XII). Vidaver (2002) estimates that 53,000 ha of fruit and vegetable plants are sprayed annually with antibiotics. The USEPA fact sheets (1992 and 1993) show that both streptomycin and oxytetracycline are nontoxic to birds, freshwater invertebrates, and honeybees (RED facts, 1992, 1993). However, streptomycin is slightly toxic to fish and very toxic to algae. Concentrations at which these antibiotics may be toxic to fish and algae were not provided in the USEPA fact sheets. Depending on the treatment objective and the crop, recommended streptomycin and oxytetracycline concentrations range between 50 and 200 mg L^{-1}, and 150 and 200 mg L^{-1}, respectively. For example, in peaches and nectarines, oxytetracycline is applied at a rate of 150 mg L^{-1} in 500 to 1000 gallons of water ha^{-1}. This is equivalent to an application between 285 to 570 g ha^{-1} of active antibiotic compound in peaches and nectarines. Vidaver (2002) reported that gentamicin is routinely used in Latin American countries on fruit plants. The author concludes that its use is worrisome because of the importance of gentamicin in human medicine. In the United States, gentamicin use on plants is not permitted.

Development and use of transgenic plants to produce inexpensive antibiotics may also be a cause of environmental concern because of the presence of crop residues, roots, and root exudates in the soil (Rooklidge, 2004). These materials can act as a continuous source of residual antibiotics to soil fauna and flora.

Table XI
Antibiotics Registered for Use in Food and Nonfood Plants in the United States

Crop use, crop	Disease	Registered treatment	Streptomycin Oxytetracycline
Terrestrial food and/or feed crop use			
Apple	Fire blight	Foliar	Foliar
Bean	Halo blight	Seed	—
Celery	Bacterial blight	Foliar	—
Crabapple	Fire blight	Foliar	—
Nectarine	Bacterial fruit spot	—	Foliar
Peach	Bacterial fruit spot	—	Foliar
Pear	Fire blight	Foliar	Foliar
Pepper	Bacterial spot	Foliar	—
Potato	Bacterial soft rot	Seed	—
	Black leg	Seed	—
Quince	Fire blight	Foliar	—
Tomato	Bacterial spot	Foliar and Seed	—
Nonfood crops			
Sugarbeet-seed	Bacterial rot	Seed	Seed
Tobacco	Wildfire	Foliar and Seed	—
Ornamental herbaceous plants, shrubs, and green house ornamentals			
Anthurium	Bacterial blight	Foliar	—
Cotoneaster	Fire blight	Foliar	Foliar
Chrysenthemum	Bacterial wilt	Foliar	Cutting
Crabapple	Fire blight	Foliar	—
Elm	Lethal yellows	—	Injection
Dieffenbachia	Bacterial stem rot	Foliar	—
Hawthorn	Fire blight	Foliar	—
Palm	Lethal yellows	—	Injection
Philodendron	Bacterial leaf spot	Foliar	Foliar
Pyracantha	Fire blight	Foliar	—
Quince	Fire blight	Foliar	—
Roses	Crown gal	Foliar	—

Data from Vidaver, 2002.

III. FATE OF ANTIBIOTICS IN SOIL, MANURE, AND WATER

Persistence of antibiotics in the terrestrial environment is a key factor in determining their adverse environmental impact. Antibiotic persistence in the terrestrial environment depends not only on the antibiotic properties but also on the soil properties and weather conditions. In terms of their persistence, the important antibiotic properties are photostability, binding, and adsorption to soil solids, biodegradation, and water solubility. Antibiotics vary widely in their molecular structure, molar mass, and other physicochemical

Table XII
Use of Antibiotics in Fruit Crops in the United States

	Active ingredient applied (kg)	
Crop and year	Oxytetracycline	Streptomycin
Apple		
1995	998	7530
1997	1225	11068
1999	1315	6985
Pear		
1995	4536	3810
1997	7757	6985
1999	5398	2722
Peach		
1995	680	Not registered for peach
1997	3175	
1999	3130	

Data from McManus *et al.*, 2002.

properties (Table XIII). Ionization of most antibiotics depends on the pH of medium and *pKa* values of the antibiotics whereas antimicrobial activities of antibiotics are associated with different functional groups of the molecular structure (Thiele-Bruhn, 2003). Briefly, the tetracycline group of antibiotics are amphoteric compounds stable in acids but not in bases. These compounds form chelate complexes with divalent metal ions and β-diketones, strongly bind to proteins and silanolic groups, and are susceptible to photodegradation (Oka *et al.*, 2000; Thiele-Bruhn, 2003). Sulfonamides, in general, are characterized by two *pKa* values (Ingerslev and Halling-Sørensen, 2000), behave as weak acids, and form salts in strongly acidic or basic solutions (Thiele-Bruhn, 2003). Aminoglycosides are polar compounds, highly soluble in water and susceptible to photodegradation (Thiele-Bruhn, 2003). Most macrolides are composed of lactone structure with more than 10 C-atoms and are weak bases and thus unstable in acids (Thiele-Bruhn, 2003). Penicillin belongs to the β-lactam class of antibiotics. The antibiotic effect of penicillin is connected to the β-lactam ring, which is not stable in acidic or basic conditions. (Thiele-Bruhn, 2003). Fluoroquinolones, on the other hand, are highly stable and resist hydrolysis but degrade under UV light (Thiele-Bruhn, 2003).

A. Binding of Antibiotics to Soils

Binding of chemical compounds in soil is characterized by the slope of the adsorption isotherm curve ($K_{d,\ solid}$), a relationship between adsorbed concentrations vs. solution concentration at equilibrium. $K_{d,\ solid}$ values are also

Table XIII

Representative Antibiotics and Typical Ranges of Physicochemical Properties from Selected Classes of Antibiotics Used in Animal Agriculture[a]

Antibiotics/Class	Molar mass g mol^{-1}	Water solubility mg L^{-1}	log K_{ow}	pK_a	Henry's constant PaL mol^{-1}
Tetracyclines *chlortetracyclines,* *oxytetracyclines,* *tetracyclines*	444.5–527.6	230–52000	−1.3–0.05	3.3/7.7/9.3	1.7×10^{-23}–4.8×10^{-22}
Sulfonamides *sulfanilamide,* *sulfadiazine,* *sulfadimidine,* *sulfamethoxine,* *sulfapyridine,* *sulfamethoxazole*	172.2–300.3	7.5–1500	−0.1–1.7	2–3/4.5–10.6	1.3×10^{-12}–1.8×10^{-8}
Aminoglycosides kanamycin, neomycin, streptomycin	332.4–615.6	10000–50000	−8.1–−0.8	6.9–8.5	8.5×10^{-12}–4.1×10^{-8}
β-Lactams *penicillins:* *ampicillin,* *meropenem,* *penicillin G;* *cephalosporins:* *ceftiofur, cefotiam*	334.4–470.3	22–10100	0.9–2.9	2.7	2.5×10^{-19}–1.2×10^{-12}
Macrolides erythromycin, oleandomycin, tylosin	687.9–916.1	0.45–15	1.6–3.1	7.7–8.9	7.8×10^{-36}–2.0×10^{-26}

(*Continued*)

Table XIII (*continued*)

Antibiotics/Class	Molar mass g mol^{-1}	Water solubility mg l^{-1}	log K_{ow}	pK_a	Henry's constant Pa l mol^{-1}
Fluorquinolones *ciprofloxacin, enrofloxacin, flumequin, sarafloxacin, oxolinic acid*	229.5–417.6	3.2–17790	−1.0–1.6	8.6	5.2×10^{-17}–3.2×10^{-8}
Imidazoles *fenbendazole, metronidazole, oxfendazole*	171.5–315.3	6.3–407	−0.02–3.9	2.4	2.3×10^{-13}–2.7×10^{-10}
Polypeptides *avermectin, bacitracin, virginiamycin*	499.6–1038	not completely	−1.0–3.2		negligible–2.8×10^{-23}
Polyethers *monensin, salinomycin*	670.9–751.0	2.2×10^{-6}–3.1×10^{-3}	5.4–8.5	6.4	2.1×10^{-18}–1.5×10^{-18}
Glycopeptides *vancomycin*	1450.7	>1000	octanol insoluble	5.0	negligible
Quinoxaline derivatives (*olaquindox*)	263.3	1.0×10^{6}	−2.2	10	1.1×10^{-18}

[a]From Thiele-Bruhn (2003) *J. Plant Nutr. Soil Sci.,* **166**, 145–167.

known as distribution coefficients. Antibiotic compounds with high K_d values are strongly bound to soils and are less mobile, while compounds with lower K_d values are loosely bound to soil and can be transported to either ground or surface waters. Strongly bound antibiotics, on the other hand, are more likely to be transported with sediments in surface runoff. The mobility of antibiotics further increases if these compounds are bound to dissolved organic carbon in manure or soil (Tolls, 2001).

The extent of antibiotics binding to soils depends on the antibiotic and the soil properties. These properties include antibiotic chemical structure, water solubility, soil pH, soil clay content, and soil organic matter. In acidic soils, the basic antibiotics acquire protons and become cations ($-NH_3^+$), while acidic antibiotics remain nonionized ($HOOC^-$). In basic soils, the basic antibiotics remain nonionized ($-NH_2$), while acidic antibiotics get ionized ($-OOC^-$). The amphoteric antibiotics (like tetracyclines and some sulphonamides) may exist as anions, cations, and/or zwitterions ($-OOC^-AMH^+$), depending on the pH of the medium. Cationic antibiotics bind to soil particles through ionic interaction (Arnold *et al.*, 1998), while the acidic and amphoteric antibiotics may bind to soil through nonionic interaction.

Antibiotics can be adsorbed on broken-bond surfaces, on the basal oxygen and hydroxyl planes in 1:1 type clay minerals, and also within the interlayer spaces of 2:1-type minerals (Nowara *et al.*, 1997). These authors showed that antibiotic enrofloxacin was adsorbed in between the layers of clay mineral, thus causing the interlayer spacing of 2:1 clay minerals to expand. Similarly, Gupta *et al.* (2003) showed that chlortetracycline also increased the interlayer spacing of clay minerals in soil but tylosin did not. This difference may be because tylosin molecules are larger compared to chlortetracycline and tetracycline (Table XIII). This may also be one of the reasons that $K_{d,\ solid}$ values of chlortetracycline are much greater than those for tylosin. In addition to antibiotic adsorption by clay minerals in soils, antibiotics also adsorb strongly on natural organic matter in soil, manure, and sludge (Golet *et al.*, 2003).

The earliest work on sorption and desorption of antibiotics in soils was reported by Pinck *et al.* (1961a,b, 1962) and Soulides *et al.* (1962) in a series of four articles. Based on their reaction with clay minerals, these authors divided 9 antibiotics into three primary groups: strongly basic (streptomycin, dihydrostreptomycin, neomycin, and kanamycin); amphoteric (bacitracin, aureomycin, and terramycin); and acid (penicillin) or neutral (chloromycetin and cycloheximide). The authors showed that the first two groups of antibiotics formed complexes to varying degrees with montmorillonite, illite, and kaolinite clays. However, acidic and neutral antibiotics were only adsorbed by montmorillonite and only in small quantities. Bacitracin and aureomycin were unstable whereas terramycin was stable in the presence of alkaline clays. The average amount of antibiotic adsorbed by clays varied from 9 mg g^{-1} of kaolinite clay for strongly basic antibiotics to 318 mg g^{-1} of montmorillonite clay for amphoteric antibiotics. Bioassay experiments in the previously mentioned studies showed that there was no release of strong basic antibiotics (with one exception of dihydrostreptomycin) from montmorillonite, vermiculite, or illite clays. However, there was some release of streptomycin and dihydrostreptomycin from kaolinite. All amphoteric antibiotics were released from all types of clay minerals used in the aforementioned study.

Yeager and Halley (1990) studied the sorption/desorption of efrotomycin, a growth promoter in swine, in five soils (sand, sandy loam, loam, silt loam, and clay loam). Except for sand, efrotomycin was highly adsorbed to the other four soils (60–98%). The sorption distribution coefficient for the four soils ranged from 8 to 290 L kg^{-1}. Efrotomycin sorption on sand was only 17%. In four heavier textured soils, only 50% of efrotomycin could be desorbed, even with organic solvents such as methanol.

Tolls (2001) reviewed the sorption characteristics of antibiotics by soils and soil constituents and found the $K_{d,\ solid}$ varying from 0.2 to 6000 L kg^{-1} (Table XIV). These values suggest that the antibiotics have a wide range of mobility. The author also reported that the large variation in $K_{d,\ solid}$ values did not significantly decrease when these values were normalized with organic carbon, K_{oc} (Table XIV), thus, suggesting that clay adsorption is the main mechanism for antibiotic adsorption in soils. Kumar *et al.* (2002) measured greater sorption of chlortetracycline, tetracycline, and tylosin on a Webster clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) than on a Hubbard sandy loam (sandy, mixed, frigid Entic Hapludolls) (Table XV). This difference in sorption mainly reflected the higher clay content of the Webster clay loam (34%) than the Hubbard sandy loam

Table XIV
Range of Sorption Values of Antibiotics on Various Soils and Sediments Reported in Literature

Antibiotic	$K_{d,\ solid}$ (L kg^{-1})	K_{oc}, (L kg^{-1})
Tetracycline	400–1620	—
Oxytetracycline	420–1030	27,800–93,300
Enrofloxacin	260–6310	16,500–770, 000
Oxolinic acid	0.3–116	14–4510
Efrotomycin	8–290	580–11,000
Tylosin	8.3–128	550–7990
Sulfamethazine	0.6–31	60–208
Metronidazole	0.5 –0.7	38–56
Olaquindox	0.7–1.7	46–116
Chloramphenicol	0.2–0.4	—

Data from Tolls, 2001.

Table XV
Linear Sorption Coefficients of Three Antibiotics for Two Soils

	$K_{d,\ solid}$ (L kg^{-1})		K_{OC} (L kg^{-1})	
Antibiotics	Webster	Hubbard	Webster	Hubbard
Chlortetracycline	2386	1280	100,420	107,744
Tetracycline	2370	1147	99,747	96,548
Tylosin	92	66	3872	5555

Data from Kumar *et al.*, 2002.

(10%). Relative differences in $K_{d,\ solid}$ values between Webster clay loam and Hubbard sandy loam were much greater for tetracyclines than for tylosin. This further suggests that significant amounts of tetracyclines were possibly residing in the interlayer of 2:1 clays. Lower $K_{d,\ solid}$ and K_{oc} values in Table XV suggest that tylosin will be comparatively more mobile than tetracycline and chlortetracycline.

Rabølle and Spliid (2000) reported the sorption and mobility of four antibiotics (metronidazole, olaquindox, oxytetracycline, and tylosin) in four soils (two sandy loam, a loamy sand, and a sand). The distribution coefficient in batch equilibrium studies ($K_{d,\ solid}$) varied from 0.5 to 0.7 for metronidazole, 0.7 to 1.7 for olaquindox, 8 to 128 for tylosin, and 417 to 1026 for oxytetracycline. In leaching experiments, weakly adsorbed substances such as metronidazole and olaquindox were found in the leachate of both sandy loam and sand. However, strongly adsorbed oxytetracycline and tylosin were not detected in the leachate from any of the four soils. Since the affinity of several antibiotics to soil particles is high, these results indicate a greater probability of antibiotic losses with sediments in surface runoff than through leaching from fields where antibiotic-laden manures have been applied.

B. Biodegradation of Antibiotics

Only a few studies (Gavalchin and Katz, 1994; Kümmerer *et al.*, 2000; Marengo *et al.*, 1997; Weerasinghe and Towner, 1997) have been conducted to examine the biodegradation of various antibiotics in water, soils, or manures. The combined data (Table XVI) show that while some antibiotics like bambermycin, tylosin, and erythromycin completely biodegrade within 30 days at temperatures from 20 to 30°C, only a small proportion of other antibiotics like ciprofloxacin, ofloxacin, sarafloxacin, and virginiamycin degrade even after 30 to 80 days. Clearly, some of these antibiotics are more persistent in the environment than others. Furthermore, biodegradation depends upon the temperature; lower temperatures reduce the

Table XVI
Data on Biodegradation of Antibiotics in Various Test Systems

Antibiotic	Biodegradation %	Biodegradation Days	Method/Comments	Reference
Ciprofloxacin	0	40	Closed bottle tests	Kümmerer *et al.*, 2000
Ofloxacin	0	40	OECD 301D	
Metronidazole	5	40		
Virginiamycin	12–40	64	Aerobic incubation, 6 soils, room temp.	Weerasinghe and Towner, 1997
Chlortetracycline			Soil amended with manure	Gavalchin and Katz, 1994
30°C	56	30		
20°C	12	30		
4°C	0	30		
Bacitracin			Soil amended with manure	Gavalchin and Katz, 1994
30°C	71	30		
20°C	67	30		
4°C	77	30		
Tylosin			Soil amended with manure	Gavalchin and Katz, 1994
30°C	100	30		
20°C	100	30		
4°C	60	30		
Erythromycin			Soil amended with manure	Gavalchin and Katz, 1994
30°C	100	30		
20°C	75	30		
4°C	3	30		
Bambermycin			Soil amended with manure	Gavalchin and Katz, 1994
30°C	100	30		
20°C	100	30		
4°C	10	30		
Penicillin	36	40	OECD 301 D	Al-Ahmad *et al.*, 1999
Sarafloxacin	69–82	80	Sandy loam soil	Velagaleti *et al.*, 1993
	0.66	65	Loam soil	
	0.43	65	Silty clay loam	
	0.40	65	Sandy clay loam	
Sulfachloro-pyrazine	71	90	Laying hen feces	Van Dijk and Keukens, 2000
	65	8	Broiler feces	
^{14}C-Sarafloxacin	0.5–0.6	80	Incubation at 22°C in dark, 3 different soils	Marengo *et al.*, 1997
Amprolium	30	90	Laying hen feces	Van Dijk and Keukens, 2000
	34	8	Broiler feces	

degradation rate. The slow biodegradation of antibiotics at low temperatures could be a cause for concern in the northern tier states of the United States, Canada, and other world regions where manure is often applied in late fall or during winter when temperatures are low and soils may be frozen. Under these conditions, antibiotics in manure or soil will persist longer, thus providing greater opportunities for spread in the environment through snow-melt runoff.

Several studies have reported the half-life of various antibiotics in marine sediments (Hektoen *et al.*, 1995; Samuelson 1989), water (Ingerslev *et al.*, 2001), soil–manure slurries (Gavalchin and Katz, 1994; Ingerslev and Halling-Sørensen, 2001), manure (Morrison *et al.,* 1969), and soils (Weerasinghe and Towner, 1997). Half-life varies between a few days to as high as 300 days (Table XVII). For example, the half-life of oxytetracycline in marine sediments at 5 to 7 cm depth was greater than 300 days as compared to 87 to 173 days for virginiamycin in the sandy soils. Since the half-life of many antibiotics increases at low temperatures and in the dark, this suggests that antibiotics may persist longer in deeper soil layers and in deep waters (Hektoen *et al.*, 1995).

We evaluated the use of Quantitative Structure Property Relationship as a means to predict antibiotic degradation in the environment (A. K. Singh, K. Kumar, and S. C. Gupta, unpublished data). These authors showed that the half-life of antibiotics was negatively correlated with an index of molecular volume (Fig. 2), according to the relationship:

$$\text{Log } T_{1/2} = 37.3(\pm 3.7) - 19.7(\pm 1.9)\ \text{Log}_{10}\{\text{MV}/({}^{1}\chi - {}^{1}\chi^{v})\} \tag{1}$$

where $r^2 = 0.95$, standard deviation = 0.4, n = 6 model development, n = 1 validation, P/S = 0.25, and MV is the molecular volume and represents nonsigma electronic charge. P/S gives an indication of how likely the model is to be a good predictor of independent data. A P/S value <0.4 indicates that the model is a good predictor and that the prediction is not a chance correlation.

A single index (ratio of molecular volume to nonsigma electronic charge) in equation (1) encodes several mechanisms such as antibiotic binding to soil, its desorption from the soil, and its chemical and bio-degradation. Half-lives of several antibiotics estimated using equation (1) were similar to the published data (Table XVIII).

IV. FACTORS AFFECTING ANTIBIOTIC PERSISTENCE IN THE TERRESTRIAL ENVIRONMENT

A. Temperature

In an incubation study at three temperatures (30, 20, and 4°C), Gavalchin and Katz (1994) studied the persistence of seven antibiotics (bacitracin, chlortetracycline, erythromycin, bambermycins, penicillin, streptomycin,

Table XVII
Half-Life of Antibiotics in the Environment

Antibiotics	Background matrix	Half-life (days)	Comments	Reference
Ceftiofur	Clay loam soil	22		Gilbertson *et al.*, 1990
	Sandy soil	49		
	Silty clay loam	41		
	Aqueous	100	Hydrolysis, pH 5	
	Aqueous	8	Hydrolysis, pH 7	
	Aqueous	4	Hydrolysis, pH 9	
Chloramphenicol	Sediment	<12	Aerobic	Lai *et al.*, 1995
	Sediment	<4	Anaerobic	
Oxytetracycline	Sediment	<47	Aerobic	
	Sediment	Stable	No degradation in 70 d under anaerobic conditions	
Erythromycin	Sewage	Stable	Nonbiodegradable	Richardson and Bowron, 1985
Oxytetracycline	Marine	151		Hektoen *et al.*, 1995
Oxalinic acid	Sediments	151		
Flumequine	(0–1 cm)	60		
Sarafloxacin		151		
Sulfadiazine		50		
Trimethoprim		75		
Florfenicol		1.7		
Oxytetracycline	Marine	>300		Hektoen *et al.*, 1995
Oxalinic acid	Sediments	>300		
Flumequine	(5–7 cm)	>300		
Sarafloxacin		>300		
Sulfadiazine		100		
Trimethoprim		100		
Florfenicol		7.3		

Oxytetracycline	Sediments	30–64	In aquarium	Samuelsen, 1989
	Sediments	32	Salmon farm	
	Seawater	16	Light–24 hrs a	
	at-4°C dark		Day with 40 W	
			Fluorescent tube	
	4°C light	10		
	15°C dark	7		
	15°C light	5		
Virginiamycin	Sandy silt	87	Si 48, Cl 12	Weerasinghe and
	Silty sand	116	Si 20, Cl 5	Towner, 1997
	Silty sand	173	Si 22, Cl 8	
Bacitracin	Soil–feces			Gavalchin and
	20°C	22.5		Katz, 1994
	30°C	12		
Erythromycin	20°C	11.5		
	30°C	8		
Olaquindox	Water	4–8	Simple shake flask	Ingerslev *et al.*, 2001
Metronidazole		14–104	System simulating	
Tylosin		9.5–40	Surface waters	
Oxyteracycline		42–46		
Olaquindox	Soil-manure	5.8–8.8	Aerobic batch	Ingerslev and
			study	Halling-Sørensen, 2001
Metronidazole	Slurries	13.1–26.9	With different	
			proportion of solids	
Tylosin		3.3–8.1		
Chlortetracycline	Manure, 37°C	7	*In situ* manure	Morrison *et al.*, 1969
	28°C	>20		
Oxytetracycline	Soil water	270	Interstitial water	Halling-Sørensen *et al.*, 2003a

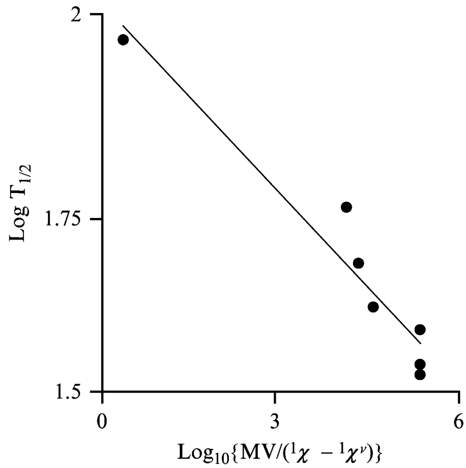

Figure 2 Correlation between Log_{10} $\{MV/({}^1\chi-{}^1\chi^v)\}$ and antimicrobials half-life ($T_{1/2}$).

Table XVIII
Predicted Half-Life (Days) of Various Antibiotics Using Quantitative Structure–Property Relationship and Published Values for Different Antibiotics

Antibiotic	Published	Predicted
Florfenicol	1.8	1.9
Flumequine	60	97
Oxolinic acid	150	157
Oxytetracycline	151	98
Sarafloxacin	250	184
Sulfadiazine	50	40
Trimethoprim	75	72

Unpublished data from A. K. Singh, K. Kumar, and S. C. Gupta.

and tylosin) commonly used in animal agriculture. After 30 days, 44% of chlortetracycline and 23% of bacitracin remained in the soil at 30 °C; 88% of the chlortetracycline, 33% of bacitracin, and 25% of erythromycin remained in the soil at 20 °C; and almost all of the chlortetracycline, erythromycin, and bambermycins, 23% of bacitracin, and 40% of the tylosin remained in the soil at 4 °C (Table XVI). This study shows that persistence of antibiotics increases with a decrease in temperature. It is likely that many of the antibiotics in fall-applied manure will remain in their original form over winter in northern latitudes where soils are seasonally frozen.

B. Soil Type

Marengo *et al.* (1997) reported the aerobic biodegradation of sarafloxacin, a fluoroquinolone antibiotic used against poultry diseases, in three soils (loam, silt loam, and sandy loam). In all three soils, sarafloxacin was mineralized to $^{14}CO_2$, but the extent of mineralization was low and varied with soil type: 0.49% in silt loam, 0.57% in loam, and 0.58% in sandy loam in 80 days. The authors speculated that these low rates of mineralization were due to strong binding of sarafloxacin to soil and thus its nonavailability to microorganisms. Aerobic incubation of ceftiofur vs. glucose (at equivalent supply of carbon) in three soils (sand, clay loam, and silty clay loam) showed the half-life of ceftiofur varied from >49, 22.2, and 41.4 days compared to 2.0, 2.8, and 7.6 days for glucose, respectively (Gilbertson *et al.*, 1990). This suggested that soil also played some role in degradation of ceftiofur.

C. Soil–Manure Ratio

Warman and Thomas (1980) studied the fate of chlortetracycline in various poultry manure:soil mixtures. After 2 h of incubation, chlortetracycline recoveries were 60% for 1:5 and 33% for 1:10 manure:soil mixture. No chlortetracycline was recovered from 1:20, 1:40, and 1:200 manure:soil mixtures. The authors concluded that a decrease in manure:soil ratio reduces the recovery of chlortetracycline, presumably due to its adsorption to soil colloids or due to its decomposition with microorganisms.

D. Animal Excreta, pH, and UV Light

Gilbertson *et al.* (1990) examined the role of animal excreta (urine and feces), soil, pH, and UV light on degradation of ceftiofur sodium, a wide-spectrum cephalosporin antibiotic. [^{14}C]-Ceftiofur quickly degraded to inactive metabolites on fortification with cattle feces. However, sterilized cattle feces inhibited the degradation of ceftiofur, thus suggesting that microorganisms or heat-labile substances were responsible for ceftiofur degradation. In the aforementioned study, hydrolysis and photolysis of the ceftiofur increased with an increase in pH and exposure to light, respectively. These authors concluded that feces play a major role in the degradation of ceftiofur followed by soil, light, and pH. It is unknown how these factors will interact under field conditions and, in turn, influence the loss of antibiotics from manure-applied fields.

For any pollutant, abiotic transformations in the environment may occur via hydrolysis and photolysis. Since most of the antibiotics are usually designed for oral intake and are resistant to hydrolysis, the research suggests that direct and indirect photolysis of antibiotics is a major mechanism for their abiotic transformation in surface waters (Andreozzi *et al.*, 2003). While the direct photolysis occurs as a result of direct absorption of solar light (Boreen *et al.*, 2003; Zepp and Cline, 1977), indirect photolysis involves natural photosensitizers like nitrates and humic acids which can either hasten or slow down the photolysis of antibiotics (Table XIX). It is well known that tetracyclines are very sensitive to photodecomposition (Oka *et al.*, 1989).

Andreozzi *et al.* (2003) studied the photodegradation of six antibiotics and found that the presence of nitrate ions in aqueous solution (5–15 mg L^{-1}) reduced the half-life of all compounds except propanolol (Table XIX). However, the presence of humic acids (concentration 5 mg L^{-1}) reduced the photodegradation of carbamazepine and diclofenac but hastened the photodegradation of sulfamethoxazole, clofibric acid, oflaxocin, and propanolol (Table XIX). In a similar study, Doll and Frimmel (2003) showed that low concentrations of natural organic matter accelerated the degradation of carbamazepine due to photochemical formation of reactive species. However, at higher concentrations of natural organic matter, the degradation rate decreased. These authors suggested that natural organic matter at higher concentration might be acting as an inner filter, radical scavenger, and/or precursor of reactive species. The previously mentioned results suggest that photodegradation of various antibiotic compounds in natural streams may be enhanced with the presence of ions like nitrates, chlorides, and organic compounds such as humic acids.

Table XIX

Ratio of Half-Lives (t ½) of Antibiotics in the Presence and Absence of Photosensitizers[a]

	$t\,\frac{1}{2}_{(\text{sensitizers})}/t\,\frac{1}{2}_{(\text{distilled water})}$			
Antibiotics	With nitrate 5 mg L^{-1}	With nitrate 10 mg L^{-1}	With nitrate 15 mg L^{-1}	With humic acids 5 mg L^{-1}
Diclofenac	—	0.62	—	2.23
Sulfamethoxazole	—	0.24	—	0.33
Propanolol	—	1.02	—	0.75
Ofloxacin	—	0.12	—	0.20
Carbamazepine	—	0.43	0.22	4.22
Clofibric acid	0.80	—	—	0.48

—,Not determined.

[a]Source: Andreozzi *et al.*, 2003, *Chemosphere*, **50**(10), 1319–1330, Copyright 2003, with permission from Elsevier.

V. POTENCY OF RESIDUAL ANTIBIOTICS IN THE ENVIRONMENT

As mentioned earlier, there are several pathways by which antibiotics can enter the terrestrial environment. Their fate and persistence in that environment, however, depends upon many factors such as binding to soil, biodegradation, chemical complexation or chelation, hydrolysis, and photolysis. The implications of interests are whether or not the residual antibiotics in soils are potent against native bacteria.

Once antibiotics are released into the terrestrial environment, two processes are important in determining their antimicrobial activity:

(i). Chemical complexation or chelation and adsorption: Chemical complexation or chelation of antibiotics with various organic or inorganic compounds or ions may render the antibiotics inactive in soil or manure. It is well known that tetracyclines chelate with divalent and trivalent metal ions, such as Mg^{2+}, Ca^{2+}, Fe^{3+}, Zn^{2+}, and Al^{3+} (Halling-Sørensen *et al.*, 2002; Lunestad and Goksøyr, 1990). This suggests that the presence of specific metals in the soil may not affect only the potency of antibiotics but also their degradation products (Halling-Sørensen *et al.*, 2002). In many soils and manures, there is an abundance of these metal ions, which suggests that tetracyclines and their biodegradation products will lose their potency rather quickly. Avery *et al.* (2004) showed that iron blocks the accumulation and activity of tetracyclines in bacteria. Marengo *et al.* (1997) concluded that sarafloxacin strongly bound to the soil and was not bioavailable to microorganisms present in soil. These workers also reported that soil-bound sarafloxacin had no effect on known sensitive bacteria at 300 $\mu g\ g^{-1}$ soil, which is several thousand-fold higher than its MIC (0.1 $\mu g\ ml^{-1}$) in agar media. Chander *et al.* (2003) showed that soil-adsorbed tetracycline and tylosin were biologically effective in reducing the number of colony-forming units of both resistant and sensitive strains of *Salmonella*. However, the effectiveness decreased with a decrease in the concentration of soil-adsorbed antibiotics. At equivalent concentrations of field manure applications, the results showed that soil-adsorbed antibiotics will be minimally effective in reducing microbial population.

(ii). Degradation products: Another factor that is important in controlling the potency of an antibiotic on native bacteria is its degradation products. A study by Halling-Sørensen *et al.* (2002) showed that several degradation products of tetracycline, chlortetracycline, and oxytetracycline had similar potency against both the sludge and the soil bacteria as the parent compounds. However, the mode of action for some of the degradation products was different from that of the parent antibiotic. In another set of experiments, Halling-Sørensen *et al.* (2003b) showed that the potencies of oxytetracycline, tylosin, sulfadiazine, streptomycin, ciprofloxacin, and olaquindox declined with time under both aerobic and anaerobic conditions in activated sludge and selected soil bacteria, thus suggesting a lack of potency of degradation products. The potency of ciprofloxacin, however, remained high, suggesting that the degradation products probably had similar antimicrobial activity. In these studies, antibiotics such as oxytetracycline remained potent for as long as 100 days.

VI. ANTIBIOTIC TRANSPORT TO GROUND AND SURFACE WATERS

As stated earlier, significant amounts of antibiotics are present in manure. Thus, land application of manure presents several possibilities for antibiotic transport to ground and/or surface waters. The concentrations of antibiotics in the manure often exceed the resistance breakpoint concentrations of various antibiotics (Table XX). These may also be transported from the manure-applied fields to groundwater through percolation and to surface waters through runoff. Hirsch *et al.* (1999) noted that there is some possibility that highly mobile antibiotics may first leach into groundwater and then move to surface waters through lateral transport.

Hamscher *et al.* (2002) studied the fate of tetracycline and tylosin in manure-applied fields in Germany and concluded that these compounds

Table XX
Classes of Antibiotics and Their Resistance Breakpoints

Class	Antibiotic	Resistance breakpoint (g ml^{-1})
Cephalosporins	Cefoxitin	32
	Ceftiofur	8
	Ceftriaxone	64
	Cephalothin	32
Penicillins	Amoxicillin	32/16
	Ampicillin	32
Sulfonamides	Sulfamethoxazole	512
	Trimethoprim-sulfamethoxazole	4/76
Quinolones and fluoroquinolones	Ciprofloxacin	4
	Nalidixic acid	32
Phenicols	Chloramphenicol	32
Aminoglycosides	Gentamicin	16
	Tetracycline	16

Data from Shroeder *et al.*, 2002.

did not move in soil and there was no presence of these compounds in groundwater at 80 cm depth. Zhu *et al.* (2001) also did not detect any tetracycline in groundwater around areas of animal husbandry within the United States. This lack of deep percolation appears to be due to high sorption tendencies of tetracyclines and tylosin, especially in soils with higher clay content (Kumar *et al.*, 2002). Similar to the previously mentioned studies, De Liguoro *et al.* (2003) also did not detect any presence of oxytetracycline in watercourses from areas where high amounts of manure containing oxytetracycline had been applied.

In another study, Yang and Carlson (2003) found the presence of sulphonamides and tetracycline class antibiotics in the Poudre River in Colorado. They concluded that sulphonamides did not originate from agricultural sources in the river watershed, however; tetracyclines originated from both urban and agricultural settings but the concentrations of tetracyclines in the river waters were low (0.08 to 0.30 μg L^{-1}). In two reaches of an Italian river, Calamari *et al.* (2003) measured peak oxytetracycline loads at 4 mg s^{-1}. In a Swiss river, Golet *et al.* (2002) measured fluoroquinolone at 19 ng L^{-1} concentrations. Campagnolo *et al.* (2002) also found tetracycline antibiotic in water samples obtained from wells and streams which were close to poultry farms.

The occurrence of antibiotics is not restricted to surface waters only. Sacher *et al.* (2001) found sulfamethoxazole concentration as high as 410 ng L^{-1} in 10% of the tested groundwater wells in Germany. Lindsey *et al.* (2001) sampled 144 surface and groundwater samples throughout the United States and found sulphonamides in 7 groundwaters and tetracyclines in 6 surface waters (Table XXI). These authors concluded that sulphonamides were more mobile compared to tetracyclines. This may be because sulfonamides have little chelating ability, and have low sorption tendency to soils whereas tetracyclines are strong chelators and have high sorption coefficients (Halling-Sørensen *et al.*, 2002). However, small amounts of these antibiotics may be transported to groundwater through preferential flow via desiccation cracks and worm channels (Kay *et al.*, 2004). Another reason for greater occurrence of sulfonamides may be due to relatively lower removal efficiency (<60% for sulfamethoxazole) during sewage treatment (Carballa *et al.*, 2004). Kolpin *et al.* (2004) analyzed various antibiotics in streams during differing flow conditions in Iowa and found that antibiotics relevant to animal agriculture existed only in concentrations below 0.1 μg L^{-1}. These concentrations were similar to those reported earlier (Kolpin *et al.*, 2002). Greater numbers of antibiotics were found under low-flow than at normal- or high-flow conditions, thus suggesting some effects of dilution at normal and high-flow conditions. Frequency of occurrence of antibiotics was in the order of sulfamethoxazole (20%) > trimethoprim (17%) > erythromycin (10%) > tetracycline (3%). The other antibiotics like tylosin and virginiamycin were

Table XXI

Antibiotics ($\mu g\ L^{-1}$) Found in Groundwater and Surface Water Samples Collected throughout the United States

Site	Chlortetracycline	Oxytetracycline	Tetracycline	Sulfadimethoxine	Sulfamethazine	Sulfamethoxazole	Sulfathiazole
Groundwater Samples							
Groundwater, WA						0.22	
Surface Water Samples							
Snake Creek, GA	0.15		0.11				
Cuyahoga River, Steele, OH						1.02	
North Dry Creek, Kearney, NE				0.06	0.22		
Suwannee River, GA		0.34					
Four surface water samples, KS		0.07–1.34		0.24–15			0.08

Data from Lindsey *et al.*, 2001.

not detected at all. Boxall *et al.* (2004) have compiled detailed monitoring data about the occurrence of antibiotics in the terrestrial environment.

All these scenarios point out the risk of some antibiotics entering the drinking water supply, especially those antibiotics that are highly mobile and do not easily degrade during the water treatment process (Tolls, 2001). Less mobile antibiotics, on the other hand, present a potential of being toxic to some plants and soil organisms or they may just provide an environment for development of antibiotic resistance in native soil bacteria.

VII. ECOTOXICOLOGICAL IMPACTS OF ANTIBIOTICS ON THE TERRESTRIAL ENVIRONMENT

Limited information exists on ecotoxicological effects of antibiotics. Although antibiotics are designed to control bacteria in humans and animals, these pharmaceuticals can potentially be hazardous to other organisms in the terrestrial environment (Pursell *et al.*, 1995; Warman, 1980). Since antibiotic-laden manure is mainly land-applied as a source of nutrients for plants, there is some concern regarding the impact of antibiotics on plant growth, soil fauna, soil enzyme activities, and nutrient cycling. These impacts could be direct, such as antibiotics' toxicity to soil fauna and flora, or indirect effects, such as nutrient availability due to changed microfauna and microflora. Terrestrial ecotoxicity data (Table XXII) for a range of antibiotics used in agriculture show that some of these antibiotics may be toxic to soil organisms and plants at very low concentrations.

Tietjen (1975) reported that oats (*Avena sativa* L.) grown in a soil amended with manure from oxytetracycline-fed pigs contained 20% more N than oats grown in a soil amended with comparable rates of manure from the control animals. Patten *et al.* (1980) demonstrated no change in growth, yield, or elemental composition of 30-day-old corn (*Zea mays* L.) seedlings grown in a greenhouse experiment using manure from cattle fed with and without antibiotics. In a 2002 study, Jjemba reported that soybean plants were sensitive to low concentrations (>1 mg g^{-1} soil) of metronidazole, a drug used to control protozoa in animals and humans.

In a study on pinto beans (*Phaseolus vulgaris* var. Univ. of Idaho 114) grown in aerated nutrient media with chlortetracycline and oxytetracycline at 160 mg L^{-1}, top and root dry matter were reduced by 71 to 87% and 66 to 94%, respectively (Patten *et al.*, 1980). The results also showed that even relatively low antibiotic concentrations markedly affected pinto bean growth and development. Patten *et al.* (1980) found that neither chlortetracycline nor oxytetracycline affected the growth, development, or nutrient composition

Table XXII
Terrestrial Ecotoxicity for a Range of Antibiotics Used in Agriculture

Antibiotic	Test organism	Toxic effect	Concentration (mg kg^{-1})
Ceftiofur	Microbes	MIC or NOEC	0.25
Chlortetracycline	Soil respiration rate	NOEC	>0.6
Enrofloxacin	Pseudomonas putida	EC_{50}	0.0037
Lasalocid	Microbes	MIC or NOEC	0.20
	Plants	NOEC	2.0
Lincomycin	*Earthworms*	NOEC	1000
	Microbes	MIC or NOEC	0.78
	P. vulgaris (seedlings)	Reduction in leaf chlorophyl	100 g mL^{-1}
Monensin	*Earthworms*	NOEC	10
	Plants	MIC or NOEC	0.15
	Bobwhite quail	5-d LD_{50}	1090
	Mallard duck	5-d LD_{50}	>5000
Oxytetracycline	Mallard duck	8-d LD_{50}	>5620
	Northern bobwhite	8-d LD_{50}	>5620
	E. crypticus	EC_{50}	2701
	A. calignosa	EC_{50}	>5000
Sarafloxacin	Earhworms	NOEC	1000
	Microbes	MIC or NOEC	0.03
	Plants	NOEC	1.3
Sulfadiazine	Lupinus albus	Reduction in roots	100
Sulfadimethoxine	Amaranthus retroflexus	Development	<300 mg L^{-1}
	Pisum sativum	Development	<300 mg L^{-1}
	Zea mays	Development	<300 mg L
Tiamulin	Wheat	Plant vigor/ germination	No effect
	Lettuce	Plant vigor/ germination	No effect
	Microbes	MIC or NOEC	500
Tylosin	F fimetaria	EC_{50} reproduction	2520
	E. crypticus	EC_{50} reproduction	3109
	A caliginosa	EC_{50} reproduction	4530
	Earthwoms	28-d LD_{50}	918
	Aspergillus flavus	Inhibition	250
	Azobacter chroococcum	Inhibition	5
Virginiamycin	Microbes	MIC or NOEC	10

MIC, Minimum inhibitory concentration, EC_{50} – concentration causing 50% effect; NOEC, no observed effect concentration.
Data from Boxall *et al.*, 2004.

of corn grown in a sandy loam soil. However, yields of edible radish (*Raphanus sativus* L.) and nutrient uptake by wheat (*Triticum aestivum* L.) and corn grown on a clay loam soil were greater than the control for either antibiotic. This may be because antibiotics in the soil reduced pressure of pathogenic bacteria. In the same study, pinto bean yield, top and root dry matter, and nutrient uptake (Ca, Mg, K, and N) decreased in the presence of antibiotics in the sandy loam soil. There were also 52 and 67% fewer nodules on roots in the presence of chlortetracycline and oxytetracycline, respectively. However, for the same range of concentrations, there was no adverse effect of antibiotics on bean plants in a clay loam soil. These results show that the effects of antibiotics depend on soil characteristics and plant sensitivities (Batchelder, 1982).

Limited data is available on the effects of antibiotics on animal waste decomposition and nutrient availability. Manure decomposition depends on various microbial processes, which, in turn, depend upon the types and number of microorganisms actively participating. Morrison *et al.* (1969) suggested that excreted antibiotics might affect the decomposition of feedlot waste in two ways: (i) the antibiotics may decrease the conversion efficiency of the micro-flora and micro-fauna participating in the decomposition process, and/or (ii) the antibiotics may select resistant microorganisms that usually do not participate in manure decomposition, thus producing metabolites that may contribute to feedlot odor.

Patten *et al.* (1980) showed that feces from either oxytetracycline-fed heifers or chlortetracycline-fed heifers added to soil results in greater evolution of carbon dioxide as compared to similar quantities of control feces added to the same soil. However, the authors found no difference in N mineralization. The lack of significant differences in N mineralization may be due to alteration in the distribution of N between various organic fractions of the feces as a result of antibiotic feeding.

Differences in manure composition due to better utilization of some feed components in the presence of antibiotics have been reported by Hogue *et al.* (1956). These authors suggested that feces from animals fed with antibiotics contained a higher proportion of easily degradable C compounds than that of the control feces. Several other researchers have also reported the effect of antibiotic feeding on changes in manure quality (Elmund *et al.*, 1971; Klopfenstein *et al.*, 1964). Raun (1990) concluded that ionophore antibiotics such as monensin favor the growth of Gram-negative bacteria in the animal gut, which, in turn, changes the fermentation dynamics, improves dietary protein use efficiency, and results in less methane production. In a review article, Tedeschi *et al.* (2003) concluded that monensin in ruminant diet increased protein use efficiency by 3.5% and reduced the methane production by 25%. The effects of monensin in decreasing feed intake by animals and reduced excretion of nitrogenous compounds such as ammonia are environmentally beneficial. However, monensin cannot be fed to all animals—especially pigs—because of its toxicity.

Based on compiled data, Weldon (1997) showed that tylosin addition in swine feed results in reduced N excretion by as much as 10%. Roth and Kirchgessner (1993) also showed that avilamycin and tylosin addition reduced N excretion from growing pigs by 7 to 8%. These studies clearly show that banning antibiotic use in animal production may lead to a 7 to 10% increase in N loading of the terrestrial environment. Although it is well-established that antibiotic use in animal diet modifies manure quality, information on subsequent nutrient cycling when these manures are land-applied is unknown.

The other effect of manure antibiotics could be on nontarget microorganisms when manure is land-applied (Pursell *et al.*, 1995; Warman, 1980). For example, streptomycin decreased bacterial numbers in Georgia soils only for 14 days (Ingham *et al.*, 1991) as compared to a decrease of 50 to 75% over several months in semi-arid grassland soils (Ingham and Coleman, 1984). This implies that bacterial populations in the Georgia soils were either less susceptible or more resilient than the population in the semi-arid grassland soil. In the 1984 study of these authors, nitrate-N concentration was also significantly reduced after streptomycin application, indicating that nitrifying bacteria were especially susceptible to streptomycin.

Gram-negative bacteria such as *Nitrosomonas spp* are responsible for nitrification in soil. Therefore, the broad-spectrum antibiotics like tetracyclines, aminoglycosides, and sulphonamides are expected to inhibit the nitrification process (Halling-Sørensen, 2001). This researcher showed that oxytetracycline, chlortetracycline, tiamulin, and streptomycin inhibit nitrification in soils. However, the narrow-spectrum antibiotics such as sefadiazine, oxolinic acid, olaquindox, and tylosin stimulated the nitrification process. The differences between broad- and narrow-spectrum antibiotics may be partially due to (i) selective pressure on bacteria that do not participate in the nitrification process and (ii) stimulation of bacterial species responsible for nitrification. Veterinary antibiotics may also inhibit sulfate reduction as well as manure and soil organic matter decomposition (Sommer and Bibby, 2002). Westergaard *et al.* (2001) showed that a high tylosin concentration (2000 mg kg^{-1} dry soil) in soil caused a significant selective pressure on bacterial population, thus shifting bacterial communities from Gram-positive to a Gram-negative. The authors concluded that the effect of tylosin on protozoa population was not of direct toxicity, but rather through changes in the bacterial populations.

Lanzky and Halling-Sørensen (1997) showed that *Chlorella sps* were very sensitive to the antibiotic metronidazole. Ten (EC_{10}) and 50% (EC_{50}) reduction in reproduction of *Chlorella sps*, respectively, corresponded to 2.03 and 12.5 mg L^{-1} of metronidazole in a manure slurry. Literature from the aquatic environment shows that antibiotics might also be toxic to organisms other than the targeted bacteria. For example furazolidone, largely used in medicated fish feed, was found to cause acute toxicity (EC_{50}) in mosquito larvae (*Culex pipens*) at 40 mg kg^{-1} (Marci *et al.*, 1988). Acute toxicity tests on various terrestrial organisms show typical EC_{50} values in the range of 0.1 to >100 mg L^{-1} for various antibiotics such as bacitracin, carbadox, cloramphenicol, and kanamycin (Halling-Sørensen *et al.*, 1998).

Baguer *et al.* (2000) tested the effect of two widely used antibiotics, tylosin and oxytetracycline, on three species of soil fauna: earthworms, springtails, and enchytraeids. No effect of antibiotics was observed on soil fauna at environmentally relevant concentrations; the lowest effective concentration was 3000 mg kg^{-1}. Jjemba (2002) reported no effect of chloroquine and quinacrine on number of bacteria and protozoa in the soil, but in the rhizosphere, antibiotics concentration at 500 mg of antibiotic kg^{-1} soil was shown to reduce the protozoan population by 10-fold. This concentration is relatively higher than the antibiotic concentrations anticipated when manure-containing antibiotics are land-applied at the recommended rates. Jensen *et al.* (2003) reported that the toxic threshold levels (EC_{10}) of three antibacterial agents (tiamulin, olanquindox, and metronidazole) were 61 to 110 mg kg^{-1} dry soil for springtails (*Folsomia fimetaria*) and 83 to 722 mg kg^{-1} dry soil for enchytraeids (*Enchytraens crypticus*). However, ivermectin, an anthelmintic, was more toxic than tiamulin, olaquindox, and metronidazole, with EC_{10} values of 0.26 and 14 mg kg^{-1} dry soil for springtails and enchytraeids, respectively.

VIII. EMERGENCE OF ANTIMICROBIAL RESISTANCE IN THE TERRESTRIAL ENVIRONMENT

Widespread use of antibiotics and their subsequent release into the environment has led to the selection of antibiotic-resistant bacteria in the environment. Although antibiotic-resistant bacteria were shown to be present as early as 1954, soon after the introduction of antibiotics as human medication (Nordenberg, 1998), since then, we have further witnessed the shortening of time between the introduction of an antibiotic and development of resistance among microbial species. For example, methicillin was introduced in 1960 for the treatment of *Staphylococcus aureus* infections and within a few years methicillin-resistant *S. aureus* (MRSA) strains were reported (Swartz, 2002). Similarly, fluoroquinolones were introduced in the 1980s for treatment of MRSA but a majority of *Staphylococcus* strains became resistant to fluoroquinolones within one year (Neu, 1992). If spontaneous mutations were the only cause of antibiotic resistance, it would have been limited to only a few bacteria among the hundreds of billions in one antibiotic-treated host, and it would not be the epidemic problem it is today (Nandi *et al.*, 2004). Staphylococci and corynebacteria, common skin commensals of humans, have become resistant to treatment with cloxacillin and ofloxacin antibiotics (Terpstra *et al.*, 1999). It is suggested that similar phenomena of antibiotic resistance occur when antibiotics are routinely used at subtherapeutic levels for prophylaxis and metaphylaxis purposes in food animal production (Levy, 1992; Nandi *et al.*, 2004).

Van den Bogaard *et al.* (2002) reported a higher degree of vancomycin resistance (60%) in various enterococci isolates from broiler fecal samples than in laying hens (8%), even where this antibiotic was not used. Boothe and Arnold (2003) reported high levels of resistance among Gram-positive and Gram-negative isolates from various meat products; at least 4% of the isolates were found to be resistant to all six antibiotics tested (penicillin, erythromycin, sulfamethoxine, tetracycline, ceftiofur, and gentamicin) while more than 24% of isolates were resistant to four antibiotics (penicillin, erythromycin, sulfamethoxine, tetracycline). In a 2003 study, Malik *et al.* (2003) showed that *Ornithobacterium rhinotracheale* isolates from turkeys in Minnesota have steadily become resistant to gentamycin, ampicillin, tetracycline, and trimethoprim sulfa between 1996 and 2002. Sengeløv *et al.* (2003) showed that pig manure slurry containing tetracycline caused elevated levels of tetracycline resistance in soil bacteria after manure application. However, over time, the resistance level declined to a level corresponding to the unamended control soil.

In Table XXIII, we have summarized some of the studies that compared the effect of antibiotic feeding on antimicrobial resistance. All these studies showed that manure from animals fed with antibiotics contains bacterial isolates that are highly resistant to one or more antibiotics as compared to manure from animals which were not fed antibiotics. In an antibiotic feeding trial of nursery pigs, Kumar *et al.* (2004; unpublished data) showed that antibiotic resistance in manure bacteria develops quite rapidly; within three weeks of antibiotic feeding, more than 70% of fecal bacteria were resistant to penicillin and tetracycline. In all these studies, it is believed that antibiotic feeding of animals provides an environment that selects resistant strains and also encourages the transfer of genetic information from unrelated bacterial species.

Higher levels of antibiotic resistance in food-borne pathogens is a major concern because these infections can become difficult to treat with traditional antibiotics, thus threatening human and animal life. There have been several incidences of infection by multidrug resistant *Salmonella* Typhimurium DT104 in the past few years. Hudson *et al.* (2000) reported isolation of *S.* Typhimurium DT104 from nondomestic birds with multiple antibiotic resistances. Van den Boggaard *et al.* (2001) found 32% of *E. coli* strains

Table XXIII
Presence of Antibiotics-Resistant Bacteria in Manure or Feces from Antibiotic-Fed Animals and Animals Not Fed Antibiotics

Manure type/feces	Resistant isolates (%)		Country	Resistance type	Reference
	Antibiotics in feed	Without antibiotics			
Chicken dung	92	17	Denmark	Vancomycin-resistant enterococci	Bager *et al.*, 1997
Pig feces[a]	71	9	USA	Penicillin	Kumar *et al.*, (unpublished data)
	84	22		Tetracycline	
Turkey feces	60	8	Netherlands	Vancomycin-resistant enterococci	Stobberingh *et al.*, 1999
Number of CFUs per gram					
Pig manure	$2.87 \times 10^{7} \pm 4.45 \times 10^{6}$	$7.5 \times 10^{6} \pm 2.12 \times 10^{5}$	Denmark	Tetracycline resistance	Sengeløv *et al.*, 2003
Poultry litter	10^{1} to 10^{5}	10^{1} to 10^{2}	USA	Fluoroquinolone-resistant coliforms	Hofacre *et al.*, 2000

[a]Three weeks after feeding diets with and without antibiotics (antibiotics mixture fed contained aureomycin, penicillin, and sulphametazine).

resistant to more than five antibiotics in turkeys. Out of 125 isolates of *E. coli* O157:H7 and *E. coli* O157:NH from animals, food, and humans, 24% showed resistance to at least one antibiotic and 19% were found to be multidrug resistant. Bryan *et al.* (2004) showed a wide range of tetracycline-resistant genes in *E. coli* strains isolated from diverse human and animal sources. Contrary to the earlier belief that Gram-negative enterobacteria are the major source of antibiotic-resistant genetic elements, Nandi *et al.* (2004) showed that Gram-positive bacteria are also a major source of class 1 antibiotic-resistant integrons in animal litters.

In a 2002 study, Ash *et al.* found that more than 40% of bacteria in 16 rivers studied in the United States were resistant to one or more antibiotics. These resistant bacteria had at least one plasmid coded for resistance and 70% of the isolated plasmids exhibited resistance to ampicillin. Park *et al.* (2003) found that 54% of the coliform isolates obtained from a Korean river were resistant to at least one antibiotic. Similarly, Arvantidou *et al.* (1997) reported from Greece that 20% of *Salmonella* samples isolated from surface waters were resistant, and also, these resistant *Salmonella* bacteria were able to transfer resistance to *E. coli*.

IX. ANTIBIOTIC-RESISTANT BACTERIA AND HUMAN HEALTH CONCERNS

Animal waste is potentially a large source of both antibiotics and antibiotic-resistant bacteria. Their release into the environment on land application of manure thus presents problems for antibiotic therapy in humans and animals (Corpet, 1996; Klare *et al.*, 1995). Tetracycline is a commonly used antibiotic in animal agriculture. Selection of tetracycline resistance occurs in the swine gut (Aminov *et al.*, 2001), in swine waste lagoons (Chee-Sanford *et al.*, 2001), and upon release of resistance bacteria into the environment when these wastes are land-applied (Chee-Sanford *et al.*, 2001). These resistant genes can potentially mobilize and persist. There is also increasing evidence on the transfer of resistance genes from animal to human pathogens (Khachatourians, 1998). One possible pathway is through transmission from feed, meat, and animal wastes to foodborne illness-causing agents like *Salmonella, Campylobacter*, and other enteric pathogens (Gast *et al.*, 1988; Klare *et al.*, 1995; Koenraad *et al.*, 1995). Goyal and Hoadley (1979) showed that *Salmonella* sps and their r-plasmids in poultry processing wastes are capable of interspecial r-plasmid transmission, including transfer of antibiotic-resistance to *E. coli.* Another possibility of transmission of antibiotic resistance to animals or humans is through polluted waters or wastes, which are readily accessible to children and domestic animals (Chee-Sanford *et al.*, 2001; Goyal and Hoadley, 1979).

In a pioneering study, Levy *et al.* (1976) showed transfer of tetracycline-resistant genes from chicken *E. coli* to humans. Hummel *et al.* (1986) studied a pig farming community where antibiotic nourseothricin was added as a growth promoter in pig feed. After two years of antibiotic feeding, nourseothricin-resistant coliforms were found in 33% of fecal isolates of pigs suffering from diarrhea, in 18% of fecal isolates from workers and their families on the pig farms, and in fecal isolates from 16% of outpatients in adjacent communities. This study provided a clear indication that antibiotic resistance was transported to nearby communities, as antibiotic nourseothricin was never used in humans in this region. In the Netherlands, part-time farm workers showed less prevalence of antibiotic resistance in fecal *E. coli* compared to pig farmers (Nÿsten *et al.*, 1996a,b). These authors also showed that *E. coli* from fecal samples of pig farmers were 53 to 84% resistant to amoxicilin, tetracycline, trimethoprim, and sulfonamides, whereas samples from their pigs were 92 to 100% resistant. Hunter *et al.* (1994) found a widespread dissemination of apramycin-resistant plasmids in *E. coli* between the pigs and the stockman. These authors even found apramycin-resistant *Klebsiella pneumoniae* from the stockman's wife, despite the fact that she had no direct contact with the pigs. Earlier, Hunter *et al.* (1992) reported on the possible transfer of apramycin-resistant plasmids from *E. coli* to *Salmonella* Tryphimurium in calves.

Nikolich *et al.* (1994) showed horizontal transfer of *tetQ* genes among *Bacteriodes* sps. Early this decade, Schroeder *et al.* (2002) showed ceftiofur resistance in human *E. coli* isolates. Since ceftiofur is used exclusively in food animals and is not approved for human clinical medicine in the United States, this indicates a possible transfer of resistant genes from animal to human isolates. Van den Bogaard *et al.* (2001) showed identical pulsed-field gel electrophoresis patterns in ciprofloxacin-resistant *E. coli* from a turkey farmer and his turkeys and a broiler farmer and his broilers, thus suggesting the presence of identical clones in humans and poultry. The dissemination of antibiotic-resistant bacteria from turkeys to turkey farmers and slaughterers and from chickens to chicken handlers has also been reported in other studies (Levy *et al.*, 1976; Stobberingh *et al.*, 1999; Van den Bogaard *et al.*, 2001).

In the early 2000s, the use of the fluoroquinolone family of antibiotics came under increased public scrutiny because their use in animal agriculture was linked to gastroenteritis (food poisoning) caused by *C. jejuni* resistant to fluoroquinolones (FDA, 2001; Lipsitch *et al.*, 2002; Smith *et al.*, 1999). As a result of this scrutiny, Abbott Laboratories withdrew its sarafloxacin-based agricultural products for poultry use from the United States market (FDA, 2001).

It is not only the development of antibiotic-resistant bacteria that is cause for concern. The widespread use of antibiotics for nontherapeutic purposes has also jeopardized the effectiveness of new antibiotics because some of the new antibiotics for human use are similar to antibiotics being used in animal agriculture. For example, bacteria resistant to Synercid, an antibiotic used only in clinical trials for human use, were isolated from turkeys in the United States. The presence of synercid-resistance isolates in turkeys is mainly because synercid is a structural analogue of virginiamycin, a common antibiotic fed to turkeys in the United States. Similarly, bacteria resistant to synercid were also detected in humans in Germany even though it has not been approved for human use there (Anonymous, 1999). In Denmark, Bager *et al.* (1997) reported a good correlation between vancomycin-resistant *Enterococcus faecium* (VRE) and avoparcin, a glycopeptide antibiotic structurally similar to vancomycin that is used in swine feeding. This led to banning of avoparcin as a feed additive by the European Union in 1997 (Bager *et al.*, 1997). After avoparcin was banned in Denmark in 1995, the occurrence of VRE in poultry flocks has reduced from 82 to 12% in 1998. Similarly, after avoparcin was outlawed in Germany in 1996, VRE in poultry meat reduced from 100% in 1994 to 25% in 1997 (Wegener *et al.*, 1999).

Another concern of antibiotic use in animal agriculture is the development of microbes that are multidrug resistant. For example, infections caused by *Salmonella* Typhimurium, a bacterium resistant to five commonly used antibiotics, has risen from 1% in 1979 to 34% in 1996 in the United States (Anonymous, 1999). The net effect of increased resistance to commonly used antibiotics is increased costs of care from alternative and expensive antibiotics. According to the U.S. Congressional Office of Technology Assessment (1995), antibiotic resistance of just six different strains of bacteria has increased hospital costs by $1.3 billion in 1992 dollars.

Many researchers have been trying to prevent the expression of genes that make bacteria resistant to antibiotics and there has been some success in this regard. Guerrier-Takada *et al.* (1997) crafted synthetic genes and introduced them into bacteria through plasmids. The genetically modified bacteria did not express resistant genes, thus making them susceptible to antibiotics. These kinds of techniques may be exploited in the future to convert antibiotic-resistant bacteria to antibiotic-sensitive bacteria at various sites of infections in humans and animals.

X. CONCLUSIONS

A wide variety of antibiotics are routinely fed to food animals in subtherapeutic doses for growth promotion and disease prevention in confined facilities. A significant proportion of these antibiotics are excreted in their original form in animal urine and feces. These antibiotics can remain potent for a long period of time in manure during storage. When manure is land-applied, these antibiotics can also persist in soil for long periods, depending upon the type of antibiotic and other edaphic factors. The direct toxic effect of antibiotics on plants or soils microflora and -fauna appears to be unlikely because of low antibiotic concentrations in manure from the start as well as dilution effect when land-applied (Boxall *et al.*, 2003). However, the indirect effects of these antibiotic additions on the food web of terrestrial organisms can be significant.

One possible way to reduce antibiotic concentration in manure is by increasing its maturation period before land application. For example, data in Fig. 3 shows that >1200 mg kg^{-1} oxytetracycline present in fresh feces and bedding material reduced to <20 mg kg^{-1} and <1 mg kg^{-1} by day 30 (De Liguoro *et al.*, 2003). However, this will have minimal effect on the spread of antibiotic resistance in the environment.

Soil bacteria are the food source for many other soil organisms such as protozoa, nematodes, and microanthropods. Decrease in some microbial populations due to antibiotic residues could cause loss of food source for other soil organisms, thus reducing their populations. Cumulatively, this could affect nutrient cycling processes like decomposition and mineralization. On the other hand, exclusion of some antibiotics from animal diets may increase N excretion in manure, thus increasing the N loading of the terrestrial environment.

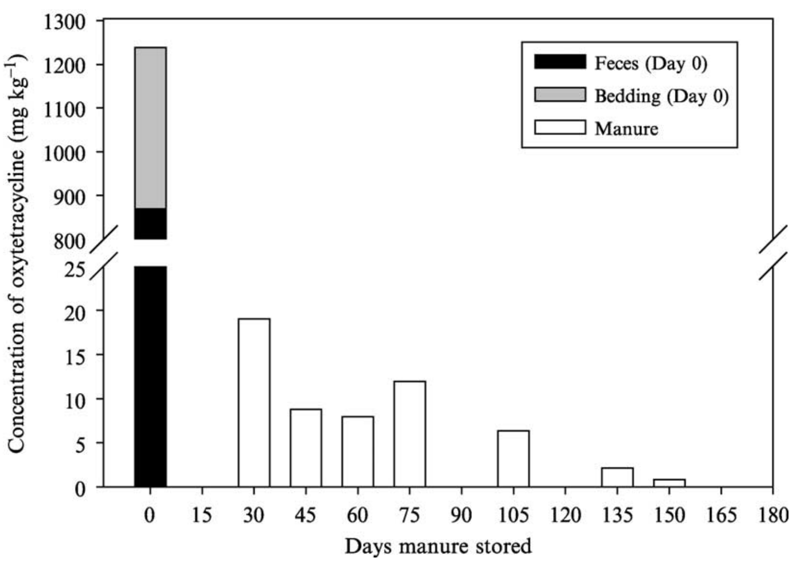

Figure 3 Concentration of antibiotic in relation to maturation period of manure (from De Liguoro *et al.*, 2003).

An important environmental issue on land application of antibiotic-laden manure is the presence of antibiotics in sources of potable water (Doughton and Ternes, 1999; Halling-Sørenson *et al.*, 1998; Hirsch *et al.*, 1999). Studies show that antibiotics such as tetracyclines, virginiamycin, and tylosin, which are tightly adsorbed on the soil clay fraction, have limited abilities to reach groundwater. However, these antibiotics will reach surface waters with soil particulates. Except for erythromycin and some sulfa drugs, most antibiotics in surface waters are present in only minute quantities and often below detectable limits.

The concern of antibiotic use in animal agriculture is not only in their presence as a micropollutant in sources of drinking water but also as a source for development of antibiotic-resistant bacteria on repeated application of antibiotic-laden manure. As has been suggested by O'Brien (2002), use of antibiotics anywhere can increase antibiotic resistance somewhere else, following the simple ecological principle proposed by Summers (2002) that "everything is connected to everything else." The transfer of resistant bacteria is not restricted to a particular country or a continent, because animal food products are traded worldwide. This suggests that prevention of further spread of resistance from the bacterial communities via animal products require global regulations (Witte, 2000).

With the exception of manures, the amounts of antibiotics in surface or ground waters are 100- to 1000-fold below what would cause selection of antibiotic resistance (Summers, 2002). Tetracyclines and penicillins, two most commonly used antibiotics in animal agriculture, have been seldom found in groundwater. Thus, it is reasonable to conclude that tetracycline and penicillin-resistant bacteria found in surface waters most likely traveled there via animal or insect vectors, in airborne dusts, or simply with runoff from some antibiotic-rich setting such as manure lagoons and lands where manure has been applied.

Every time some drug becomes ineffective against resistant bacteria, it adds to the cost of treatment. Discovery of new drugs is not only expensive but buys us only a short time. It is prudent that we use antibiotics to a bare minimum especially those antibiotics that are used by both animals and humans.

XI. FUTURE NEEDS

First and foremost, we need to collect precise data on antibiotic use in animal agriculture and the potential reservoirs for residual antibiotics in the terrestrial environment. There is also a need to generate more data on kinetics of biodegradation and potencies of degradation products of various antibiotics in different soils, manures, and waters. This will help us to better understand the ecotoxicological impacts of various antibiotic residues in the terrestrial environment. The data on transport of antibiotics and antibiotic-resistant bacteria from manure-applied fields is also needed.

We also need surveillance of antimicrobial resistance in all potential reservoirs. Specifically in the agriculture sector, we need information on levels of antimicrobial resistance on animal farms using antibiotics and not using antibiotics. Finally, we need to develop a mechanism for identification and rapid response to dangerous resistance trends. We also need to put efforts into developing novel alternatives to antibiotic use in agriculture.

ACKNOWLEDGMENTS

This research was supported with funds from the USDA-NRICGP; North Central Region SARE; Minnesota Pork Producers Association; USDA Cooperative State Research, Education, and Extension Service through the National Center for Manure and Animal Waste Management; U.S. Geological Survey through the National Institute of Water Resources; and the graduate school of the University of Minnesota.

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