ASSESSMENT OF POTENTIAL ANTIBIOTIC CONTAMINANTS IN WATER AND PRELIMINARY OCCURRENCE ANALYSIS

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ABSTRACT

Antibiotics are among the emerging microcontaminants in water because of concerns of their potential adverse effects on the ecosystem and possibly on human health. Antibiotics are likely to be released into the aquatic environment via wastewater effluent and agricultural runoff as a result of incomplete metabolism, ineffective treatment removal or improper disposal because large quantities of antibiotics are used annually in human therapy and in agriculture. Despite large quantities of use, published data on the amounts and use patterns of antibiotics are scarce. To assess the magnitude of the potential risks associated with antibiotics, a comprehensive literature review was conducted on the usage, occurrence, and behavior of antibiotics.

To identify antibiotics that are likely to be present in water sources, concentrations of antibiotics in municipal wastewater and animal waste in the United States (U.S.) were estimated and were classified according to chemical properties. The estimation of human health antibiotics was based upon the number of prescriptions administered. The estimated concentrations of antibiotics in untreated wastewater range from 3.9 ng/L to approximately 27,000 ng/L. The estimation of animal health antibiotics was based upon the subtherapeutic usage in feed for growth promoting. Considerable variation in antibiotic usage exists among different animal species. Reported data on the occurrence of antibiotics in the aquatic environment confirm the persistence of certain antibiotics. Although information is limited, studies on the transformation and sorption of antibiotics indicate that these processes significantly affect the fate of most classes of antibiotics. By combining information on environmental fate with the predicted concentrations, we identify that antibiotics of sulfonamides and fluoroquinolones are the most likely water contaminants, followed by macrolides. Among sulfonamide and fluoroquinolone antibiotics, sulfamethoxazole and ciprofloxacin are most likely to be present in municipal wastewater effluent and sulfamethazine is most likely to be present in agricultural runoff. Azithromycin and tylosin are the most likely macrolides present in municipal wastewater effluent and in agricultural runoff respectively.

An occurrence study, which is currently underway, focuses on three of the potential antibiotic contaminants, ciprofloxacin, sulfamethoxazole and sulfamethazine, identified by literature review. Solid phase extraction methods were developed. Recoveries ranged from approximately 45 to 106 percent. Analysis of the three antibiotics was conducted by liquid chromatography mass spectrometry (LC-MS). Additionally, high performance liquid chromatography (HPLC) with fluorescence detection was employed for ciprofloxacin analysis. Preliminary results indicated the presence of ciprofloxacin in secondary wastewater effluent at approximately 80 to 150 ng/L. Sulfamethoxazole was detected in one of the wastewater samples. Sulfamethazine was not detected. Concentrations of antibiotics were found to be much lower or below the detection limits in the effluent of advanced treatment processes including granular activated carbon and ozonation, indicating significant removal by those processes.

INTRODUCTION

Large quantities of antibiotics are administered to humans and animals to treat diseases and infection every year. Antibiotics are also commonly used at subtherapeutic levels to livestock to prevent diseases and promote growth. Despite the long history of antibiotic usage, information regarding antibiotic production and use patterns in the U.S. is severely limited due to the lack of coordinated and comprehensive monitoring and documenting efforts. The large amounts of antibiotic usage may result in their presence in environmental waters because up to 90 percent of the administered antibiotics can be excreted without undergoing metabolism.

Results of recent studies indicate the presence of low concentrations of antibiotics in municipal wastewater effluent and surface water (Hartmann et al., 1998; Hirsch et al., 1999; Hartig et al., 1999; Meyer et al., 2000; Alder et al., 2000; Nipales et al., 2000; Frick et al., 2001). Despite the detection of antibiotics, little is known about their distribution in the environment, their mobility and persistence in natural and engineered water systems. The presence of antibiotics in environmental waters is a concern because antibiotic contaminants could perturb microbial ecology, increase the proliferation of antibiotic-resistant pathogens, and could pose threats to human health (Daughton & Ternes, 1999). The contamination of antibiotics in waters also presents challenges for the water industry on the issues of water reuse and water resource planning.

To assess the magnitude of the potential risks associated with antibiotics as water contaminants, a better understanding of the occurrence and environmental fate of these compounds is strongly needed. Based upon this need, we conducted a comprehensive review on scientific literature and available data regarding the usage, occurrence, and behavior of antibiotics. Since a large number of antibiotic compounds are currently in use, conducting studies on the occurrence and fate of all compounds can be time-consuming and costly. Our objective is to review the commonly used antibiotics systematically to identify compounds that are likely to be water contaminants. Results of this review can facilitate prioritizing future research efforts.

Based upon the information on antibiotic usage, concentrations of antibiotics in municipal wastewater, animal manure, and liquid animal waste were estimated and were classified according to chemical properties. Although information is limited, studies on the transformation and sorption of antibiotics indicate that these processes significantly affect the fate of most classes of antibiotics. By combining information on environmental fate with the predicted concentrations, antibiotics that are likely to be present in water sources can be identified. After the potential antibiotic contaminants in waters have been identified, occurrence analysis for these compounds has been initiated. Our field measurements focus on three of the potential antibiotics, ciprofloxacin, sulfamethoxazole and sulfamethazine, identified by literature review. Robust and sensitive analytical techniques have been developed to extract, concentrate, and detect these compounds in complex water matrices. Some of the preliminary results of the field measurements are reported.

METHODOLOGY

Literature Review

The methodology for literature review involved several tasks in the following order: (1) identification of popular antibiotics commonly used in human therapy and in animal husbandry, (2) estimation of concentrations of antibiotics in untreated municipal wastewater and in liquid waste from confined animal feeding operations, (3) review of occurrence data of antibiotics in the aquatic environments, (4) review of information on sorption and transformation of antibiotics, (5) comparisons between human and veterinary antibiotics, and (6) assessment of potential antibiotic contaminants in water.

Antibiotics were categorized according to their chemical and structural properties. Members of the same class of antibiotics have similar structures, act by similar mechanisms, and are likely to behave similarly in the environment. Such an approach will yield results that can be used to predict structurally related compounds currently in use or being developed. More than ten antibiotic classes (aminoglycoside, ionophore, β-lactam, macrolide, polypeptide, quinolones, sulfonamide, tetracycline, streptogramin and other) are currently in use. Among the antibiotic classes, six are important in both human medicine and animal husbandry (aminoglycoside, β-lactam, macrolide, quinolone, sulfonamide and tetracycline). Our literature review focused on these six antibiotic classes because of the higher risks for human health if they are present in water as contaminants. The cross use of these antibiotic classes both in human and animals may lead to a more rapid development of bacterial resistance toward these drugs.

For antibiotics used in animal husbandry, we focused on beef, swine, and poultry production. Antibiotic usage in other food animal species and aquaculture was not included in the literature review because of the relatively small production quantities in the U.S. and thus less amounts of antibiotic usage. Our literature review also did not include antibiotic usage on pet animals.

Analytical Methods for Occurrence Survey

Effluent samples from local municipal wastewater treatment plants were collected in fluorinated high-density polyethylene bottles and kept in a cooler with ice during transportation. After being brought back to the lab, the samples were immediately filtered by 0.5 µm glass fiber filter and stored at 4°C until extraction, which occurred within two to three days. After acidification to approx. pH 3-4 with phosphoric acid, 1 L of effluent sample was extracted using a solid-phase extraction apparatus (Supelco). A combination of an anion-exchange cartridge (LC-SAX) (Supelco) and an Oasis HLB cartridge (Waters) was used in the solid-phase extraction. Prior to extraction, the cartridges were conditioned with methanol and deionized water (pH 4). The sample was pulled through the cartridges under a vacuum at a flow rate of less than 5 ml/min. Wastewater organics were eluted with 10 ml acidified methanol. The collected eluents were blown down to dryness under a gentle stream of nitrogen gas and redissolved in 1 ml acidified methanol/water mixture (1:4). For each wastewater sample, duplicate analyses were conducted for both unspiked and spiked samples. For spiked recovery analyses, 200 ng/L to 20 µg/L of antibiotics were added.

A reverse-phase HPLC system with a fluorescence detector (1100, Aligent Technology) was used to analyze ciprofloxacin. Detection of ciprofloxacin was conducted at an excitation wavelength of 278 nm and an emission wavelength of 450 nm. The mobile phases include a solution containing 20 mM H_3PO_4 and 20 mM NaH_2PO_4 (eluent A, pH ~ 2.4), acetonitrile (eluent B), and methanol (eluent C). The mobile phase begins with 0.5 minute isocratic 98 percent A (2 percent B), followed by a gradient decrease to 90 percent A in 0.5 minute, then a gradient decrease to 75 percent A in 9 minutes, followed by 5 minutes isocratic 75 percent A. The mobile phase is then switched to 15 percent A, and the column was flushed under these conditions for 5 minutes. The mobile phase then shifts to isocratic 100 percent C for 10 minutes to flush the column thoroughly and finally switches back to 98 percent A and 2 percent B for the final 6 minutes of the run.

A HPLC/UV/MS system (Hewlett-Packard, Series 100 MSD G1946A) was used to analyze ciprofloxacin, sulfamethoxazole, and sulfamethazine. The employed mobile phases include a solution containing 0.2 percent acetic acid with 10 percent acetonitrile (eluent A) and acetonitrile (eluent B). The flow rate was set at 0.2 ml/min with an injection volume of 20 µL. The column temperature was maintained at 30°C. Detection at 265 nm was used on the UV detector. MS analysis was conducted using electrospray ionization at positive ion mode. Both total-ion and single-ion monitoring analyses (SIM) were conducted. SIM was conducted for the molecular ions and some of the fragment ions of the antibiotics.

RESULTS AND DISCUSSION

Estimation of Introduction Concentrations

Antibiotics Used in Human Therapy

Antibiotics that are likely to be present in discharged municipal wastewater are primarily antibiotics used in human therapy. To identify the most important antibiotics present in U.S. municipal wastewaters, quantitative estimates on the concentrations of antibiotics in municipal wastewater were made. The estimation was focused on the most popular antibiotics in the U.S.; i.e., the antibiotics (18 of them) that were among the top 200 prescription drugs listed in a 1998 survey conducted by IMS Health (Health, 1999).

Our approach for estimating the concentrations of antibiotics involved dividing the mass of antibiotics excreted by patients by the volume of wastewater discharged to municipal wastewater treatment plants. Because numerous assumptions are needed to convert the number of prescriptions administered to the concentration of antibiotics in municipal wastewater, considerable uncertainties are associated with these estimates. Despite the uncertainties, the estimates should be useful in identifying antibiotics that are candidates for further study.

The mass of antibiotics excreted was estimated based upon the number of prescriptions administered, the number of doses per prescription, and the mass of antibiotic ingredient in each dose. To improve the accuracy of the estimation, we consulted medical reference books (Katzung, 1998; PDR, 1999) and interviewed a practicing pharmacist (Field, 1999). Antibiotics are normally given on a one-time basis. It was assumed that each prescription included a sufficient number of doses (typically ten days) to treat the ailment. When excretion data are readily available, we estimated the fraction of the dose excreted in its original form. However, excretion data were not readily available for all antibiotics, or when the data were available, it was unclear if conjugates (e.g., glucuronide or sulfate) were considered to be transformation products. Since the conjugates appear to be converted back into their original unconjugated forms prior to or during wastewater treatment, conjugated forms of antibiotics should be included with the original forms. As a result, estimated concentrations of antibiotics were made both with and without consideration of metabolism.

After estimating the mass of each antibiotic prescribed, we estimated the concentration present in untreated wastewater. For this calculation, it is assumed that a total amount of 8×10^{10} L of wastewater was produced daily (a population of 250 million and each person produces 320 L of wastewater per day) and that the excreted antibiotics are evenly distributed among all wastewater in the U.S. Estimated concentrations of antibiotics in untreated municipal wastewater range from 3.9 ng/L to approximately 27,000 ng/L.

Among the 18 most popular antibiotics, there are six β-lactams (amoxicillin, cephalexin, penicillin, cefprozil, cefuroxime, and loracarbef), three macrolides (azithromycin, clarithromycin, and erythromycin), two fluoroquinolones (ciprofloxacin and levofloxacin), two aminoglycosides (neomycin and tobramycin), one sulfonamide (sulfamethoxazole), one tetracycline (tetracycline), and three others. The β-lactam antibiotics account for the most antibiotic usage in human therapy followed by macrolide, sulfonamide, and fluoroquinolone antibiotics. Tetracycline, aminoglycoside, and other antibiotics account for smaller quantities of usage compared to the above four antibiotic classes. Among the β-lactam, macrolide, sulfonamide, and fluoroquinolone classes, our estimation indicates that the most important antibiotics are amoxicillin, azithromycin, sulfamethoxazole, and ciprofloxacin respectively. The estimated concentrations for these antibiotics in untreated municipal wastewater are listed in Table 1.

Antibiotics Used in Livestock

Information on the use of antibiotics in livestock is severely limited. The lack of data in this field is worse than in human therapy as a result of animal antibiotic use not being documented. Currently, publicly available information on the antibiotic usage or use patterns in livestock is not available. Recently, the Union of Concerned Scientists (UCS) (Mellon et al., 2001) issued a report on the estimates of antibiotic use in beef cattle, swine, and poultry production in the United States. Their estimates focused on nontherapeutic uses of antibiotics in livestock for growth promotion and disease prevention. Many assumptions were employed in these calculations due to information limitations. Despite the uncertainties associated with these assumptions, the UCS estimates were carefully based on a wide range of information (direct and indirect) related to the usage of antibiotics in livestock. The Animal Health Institute (AHI) (2001) also issued a report on antibiotic usage in livestock. This report was based upon surveys among their industry members. The information released by the AHI is difficult to utilize because information on specific antibiotic compounds was not provided. Therefore, we relied upon the UCS report to facilitate the identification of important antibiotic compounds used in agriculture. The information in the UCS report allows us to identify which antibiotics are used in the greatest quantities in livestock operations in the U.S.

Based upon the UCS estimates, considerable differences in antibiotic usage exist among different food animal species (beef vs. swine vs. poultry). Therefore, the types of antibiotic compounds that are likely to be found in surface water will strongly depend upon the types of livestock operations within the watershed. In considering all three animal productions, generally high quantities of tetracycline, macrolide, sulfonamide, aminoglycoside, and β-lactam antibiotics are commonly used. Chlortetracycline and oxytetracycline are the most commonly used tetracycline antibiotics. Among macrolides, tylosin is used in greatest quantity, followed by erythromycin and oleandomycin. Sulfamethazine is the most frequently used sulfonamide antibiotic. Among aminoglycosides, lincomycin is used in greatest quantity followed by apramycin. Penicillin is the most commonly used β-lactam antibiotic. Fluoroquinolone antibiotics are not used in livestock as growth promoters and are not included in the UCS estimates. Fluoroquinolones (enrofloxacin and sarafloxacin) have been used in poultry to prevent and treat diseases. However their use is expected to decrease as the USDA moves toward banning the use of these antibiotics in agriculture due to increases in bacterial resistance to fluoroquinolone drugs.

Other classes of antibiotics also play an important role in agricultural usage. These antibiotics include polypeptides (e.g., barcitracin), ionophores (e.g., monensin and lasalocid), arsenicals (e.g., roxarsone), and others (e.g., virginiamycin and bambermycin). These classes of antibiotics are used almost exclusively for livestock and thus may pose less threat to human health in contributing to antibiotic resistance, although exceptions exist. Land application of animal waste provides routes for agricultural antibiotics to enter the aquatic environments, which may eventually reach drinking water supplies. To assess the input of antibiotics from agricultural sources, concentrations of antibiotics in manure are desirable. The concentrations of antibiotics in the manure of both beef cattle and swine have been estimated and selected results are shown in Table 2 and Table 3. The concentrations were

Table 1. Predicted concentrations of antibiotics in untreated municipal wastewater.

Antibiotic	Class	Predicted Wastewater Conc. (ng/L) – excluding metabolism	Predicted Wastewater Conc. (ng/L) – including metabolism
amoxicillin	β-lactam	27,000	16,000
azithromycin	macrolide	9,200	NA
sulfamethoxazole	sulfonamide	3,800	3,200
ciprofloxacin	fluoroquinolone	3,100	1,400

NA = not available.

Table 2. Estimates of concentrations of antibiotics in manure of beef cattle in a confined feeding operation. cattle are in the 700-1200 pound (ave. weight of 1000 pounds) feedlot stage.

Antibiotics	Class	Antibiotic Concentration (μg of antibiotic/kg of manure)
chlortetracycline sulfamethazine	tetracycline sulfonamide	3973
tylosin	macrolide	1946
bacitracin	polypeptide	1703
oxytetracycline	tetracycline	1064
chlortetracycline	tetracycline	993
erythromycin thiocyanate	macrolide	730

Table 3. Estimates of concentrations of antibiotics in manure of swine in a confined feeding operation. Swine are in the 100-260 pound (ave. weight of 150 pounds) finishing stage.

Antibiotics	Class	Antibiotic Concentration (mg of antibiotic/kg of manure)
chlortetracycline sulfathiazole penicillin	tetracycline sulfonamide β-lactam	133
tylosin sulfamethazine	macrolide sulfonamide	89
chlortetracycline	tetracycline	37
bacitracin	polypeptide	27
oxytetracycline	tetracycline	21
lincomycin	aminoglycoside	11
tylosin	macrolide	11
oleandomycin	macrolide	6

calculated by dividing the amount of antibiotic excreted by the amount of manure produced by the animal. The amount of manure produced by the animal is estimated based upon data found in the literature (Miner et al., 2000). The amount of antibiotic excreted by the animal is estimated based upon the antibiotic dosage reported by the UCS and in the Feed Additive Compendium (2000), assuming up to 80 percent of the administered antibiotic is excreted unmetabolized.

The antibiotic concentrations in beef manure range from 0.73 mg/kg for erythromycin to 4.87 mg/kg for both of the two ionophores, lasalocid and monensin. The antibiotic concentrations in swine manure range from 1 mg/kg for bambermycin to 133 mg/kg for the mixture of chlortetracycline, sulfathiazole, and penicillin. Note that not all of the antibiotics listed in the tables will be administered to the animals at one feeding operation. The selection of antibiotics and use pattern could vary considerably among different operations; such information is particularly limited and difficult to obtain. In general, use of two to four antibiotics is a common practice according to results of surveys (Mellon et al., 2001). Our estimates represent the expected concentrations in manure when a particular antibiotic(s) is in use and are useful in assessing the magnitude of the source for particular antibiotic compounds from manure.

Furthermore, the concentrations of antibiotics in the liquid waste generated by a confined animal feeding operation (CAFO) were estimated. Our calculation focused on swine operations because CAFO is common for swine production and the facilities usually consist cement-based grounds, yielding limited on-site soil infiltration. The estimation involved dividing the mass of antibiotics excreted by animals by the volume of liquid waste generated. The calculation was conducted assuming a typical CAFO of 2500 head of swine (approx. 100-260 pound in the finishing-stage).

The liquid waste from a CAFO is comprised of (i) the waste quantities being generated by animals, and (ii) the water added to the waste from sources such as flushwater to remove manure from alleys and barns, water for cleaning, rainfall runoff from roofs and open lots, and direct rainfall on pretreatment facilities (Overcash et al., 1983). The volume of (i) is usually insignificant compared to that of (ii). Water uses varies considerably from one operation to another, depending on such factors as type of buildings, methods of flushing, and type of management. Based upon the suggestions by Overcash et al. (1983), the estimated flushwater flow is approx. 9000 gallons per minute (gpm). In addition, it is assumed that one thirty minute flushing period takes place per day.

The results of the calculation range from 12 µg/L for bambermycin to 1.4 g/L for the chlortetracycline-sulfathiazole-penicillin mixture. Selected results are shown in Table 4. Similar to the previous discussion, only two to three of the antibiotics will be used simultaneously on swine in this stage and the estimates represent the concentrations expected in the swine CAFO wastewater when the particular antibiotic(s) is in use.

Occurrence Data

Occurrence information of antibiotics is limited and most previous studies were conducted in Europe where antibiotic use could be different from that of the U.S. However, the previous studies provide guidance for identifying the classes of antibiotics that are more persistent in the environment.

The previous studies indicate that β-lactam antibiotics (many are widely used in Europe) were not detected in most environmental waters (Hirsch et al., 1999). Other classes such as fluoroquinolones (Hartmann et al., 1998), macrolides (Hirsch et al., 1999) and sulfonamides (Hirsch et al., 1999; Hartig et al., 1999) have been detected in wastewater effluent and surface water. Tetracyclines have been detected in the liquid hog lagoon waste (Meyer et al., 2000) and in general have

Table 4. Estimates of concentrations of antibiotics in the liquid waste of a swine confined feeding operation.

Antibiotics	**Class**	**Ave. Antimicrobial (mg/day-animal)[1]**	**Concentration in Flushwater (ug/L)[2]**
Chlortetracycline Sulfathiazole Penicillin	tetracycline sulfonamide β-lactam	741	1449
Tylosin Sulfamethazine	macrolide sulfonamide	496	971
Chlortetracycline	tetracycline	207	406
Oxytetracycline	tetracycline	118	232
Tylosin	macrolide	59	116
Lincomycin	aminoglycoside	59	116

(1) Based on data from the report issued by the Union of Concerned Scientist (UCS) (Mellon et al., 2001).
(2) Assumptions for the swine confined feeding operation: 2500 head in the 100-260 pounds (average weight of 180 pounds, finishing stage) and one thirty minute flushing period daily.
(3) It is assumed that up to 80 percent of antibiotics are excreted unmetabolized.

not been found in municipal wastewater effluent, surface water, or ground water (Hirsch et al., 1999; Meyer et al., 2000). A few other antibiotics including chloramphenical and trimethoprim have been detected in limited number of municipal wastewater effluent and surface water (Hirsch et al., 1999). More recent studies on the detection of fluoroquinolones and macrolides were reported at the spring 2000 national meeting of the American Chemical Society in San Francisco (Alder et al., 2000; Nipales et al., 2000). The U.S. Geological Survey (USGS) has recently conducted an occurrence survey on pharmaceutical compounds including antibiotics in surface waters collected from sites across the U.S. (USGS) and detection of antibiotics has been reported at the 2001 Georgia Water Resources Conference (Frick et al., 2001). The detected antibiotics included sulfonamide, macrolide, aminoglycoside, fluoroquinolone, and others.

Environment Fate of Antibiotics

Once antibiotics are excreted from the dosed animals, they are subject to various processes such as sorption, abiotic transformation and biotic transformation, in natural and engineered aquatic environments. These processes directly influence the fate and transport of these compounds in the environment as well as their biological activities. At present, published literature regarding the fate and transport of antibiotics in the aquatic environment is very limited, although more studies are being reported recently due to the increasing concerns over these compounds. We reviewed previous studies particularly focusing on the six antibiotic classes (aminoglycoside, β-lactam, macrolide, quinolone, sulfonamide, and tetracycline) that are important in both human medicine and animal husbandry. Results are discussed in the following paragraphs.

Sorption

Previous studies indicate strong adsorption of tetracyclines (tetracycline, chlortetracycline, and oxytetracycline) to clay materials (Sithole and Guy, 1987), soil and sediments (Rabolle & Spliid, 2000; Pouliquen & Lebris, 1996) throughout a wide range of environmental conditions. Although not as strong as that of tetracyclines, significant sorption of some macrolide (e.g., tylosin and avermectin B_{1a}) (Gruber et al., 1990; Rabolle & Spliid, 2000) and fluoroquinolone (Nowara et al., 1997) antibiotics to soil clay minerals has been reported. Sulfonamides exhibit weak adsorption to soil (Thiele, 2000; Fontaine et al., 1991) and activated sludge (Ingerslev & Halling-Sorensen, 2000). Information is scarce for the sorption of aminoglycoside and β-lactam antibiotics. Aminoglycosides are typically comprised by two or more sugars or amino sugars attached to an aminocyclitol ring, resulting in high polarity of the compounds. The amino groups of aminoglycosides can be positively charged by protonation under acidic conditions. The positive charge may facilitate adsorption to soil clay minerals that typically posses negative charge. β-Lactam antibiotics are highly polar compounds. Sorption of β-lactams to soil is expected to be weak due to their high polarity and carboxylic acid functional groups.

Abiotic Transformation

Hydrolysis is an important degradation pathway for organic pollutants in the aquatic environment. Among the six antibiotic classes, β-lactams, macrolides, and sulfonamides are susceptible to hydrolysis. However, hydrolysis of macrolides and sulfonamides at neutral pH range is very slow and can be considered negligible (Volmer & Hui, 1998). β-Lactams generally undergo hydrolysis fairly quickly under mild acidic and base conditions (Hou & Poole, 1969). Photodegradation is another abiotic transformation that can affect organic pollutant persistence in the surface layers of water bodies that receives appreciable amount of sunlight. Quinolones and tetracyclines are susceptible to photodegradation (Torniainen et al., 1996; Davies et al., 1979). Currently little information is available for abiotic reductive or oxidative transformation of antibiotics in the aquatic environments.

Biotic Transformation

Gavalchin and Katz (1994) examined the stability of a number of agricultural antibiotics in soil. The observed persistence of antibiotics followed the trend of chlortetracycline > bacitracin > erythromycin > bambermycin > tylosin > penicillin and streptomycin. The loss of antibiotic was attributed to biodegradation although other reactions are also possible. Oxytetracycline, quinolone derivatives, and sulfonamide antibiotics were found to be persistent in model marine aquaculture sediment (Samuelsen et al., 1994). In liquid manure, considerable degradation of tetracycline was reported (Kuhne et al., 2000). In manure-containing systems, rapid loss of tylosin from the aqueous phase was observed under both aerobic and methanogenic conditions (Loke et al., 2000). The loss of tylosin was caused by a combination of sorption, abiotic transformation, and biodegradation; however, no further details were examined. Low biodegradability of β-lactam (penicillin G, cefotiam, and meropenem), fluoroquinolone (ciprofloxacin), and sulfonamide (sulfamethoxazole) antibiotics was reported by Al-Almad et al. (1999). Partial biodegradation was observed with the β-lactam antibiotics and no biodegradation was observed with ciprofloxacin and sulfamethoxazole. Marengo et al. (1997) reported very slow aerobic biodegradation of sarafloxacin. Ingerslev and Halling-Sorensen (2000) examined biodegradation of several sulfonamides in activated sludge. Significant biodegradation occurred only after a considerable lag time and the employment of unusually high sulfonamide concentrations to stimulate particular degraders. It was suggested that biodegradation of sulfonamide in sewage treatment systems may be negligible. Based upon the previous studies, aminoglycosides, β-lactams, and some macrolides are likely to biodegrade to a greater extent than quinolones, sulfonamides, and tetracyclines.

Potential Antibiotic Contaminants in Water

The routes for entering the aquatic environment are different for human and agricultural antibiotics. For antibiotics used in human therapy, the excreted antibiotics undergo the treatment processes in the wastewater treatment facilities before entering surface water. After entering surface water, they are subject to various fate and transport processes in the environment. The routes of agricultural antibiotics entering the aquatic environment may be similar to those of many pesticides. Soil infiltration and biodegradation are likely to play an important role.

In general, antibiotics that degrade slowly and adsorb less strongly to soil and sediments are more likely to be emitted into surface and ground water flows and be transported to greater distance. Compounds with such properties also are more likely to pass through wastewater treatment. However, it is possible that persistent antibiotics with strong affinity toward particulate matters can be transported in the aquatic environment with particles and colloids.

As far as compound stability in the environment is concerned, sulfonamide antibiotics appear to be least susceptible to any transformation. Degradation of quinolone and tetracycline antibiotics is expected to be slow when exposure to sunlight is limited. Some macrolide antibiotics may be degraded to some extent. Aminoglycoside and β-lactam antibiotics are readily degraded in the environment and are unlikely to be persistent. As far as compound sorption to soil and sediments is concerned, tetracyclines adsorb most strongly followed by quinolones and macrolides. Sulfonamides exhibit weak to moderate adsorption to soils. Sorption of aminoglycosides and β-lactams to soils are likely to be weak. As a result, sulfonamide and fluoroquinolone antibiotics are the most likely water contaminants followed by the macrolides. Due to its strong affinity to soils and sediments, tetracyclines are likely to be found in water and soils near the antibiotic sources. Furthermore, the occurrence data on antibiotics generally correlate well with the above conclusion.

Comparison between the antibiotics commonly used in human therapy and animal husbandry indicate that, in general, different compounds within each antibiotic class are used separately for humans and for food animals. Among sulfonamide and fluoroquinolone antibiotics, sulfamethoxazole and ciprofloxacin are most likely to be present in municipal wastewater effluent and sulfamethazine is most likely to be present in agricultural runoff. Azithromycin and tylosin are the most likely macrolides present in municipal wastewater effluent and in agricultural runoff respectively.

Preliminary Results of Occurrence Analysis

After identifying the potential antibiotic contaminants in water, field measurements focusing on three compounds (ciprofloxacin, sulfamethoxazole, and sulfamethazine) were conducted. Robust and sensitive analytical techniques were developed for detection of these antibiotics in complicated matrices such as wastewater and surface water. The developed analytical methods yield good recoveries, ranging from approximately 45 to 106 percent for ciprofloxacin and 45 to 95 percent for the two sulfonamides.

Preliminary results indicated the presence of ciprofloxacin in secondary wastewater effluent (after activated sludge treatment) at approximately 80 to 150 ng/L. Utilization of advanced treatment such as granular activated carbon and ozonation reduced the concentrations of ciprofloxacin to around 10 ng/L and less. The presence of ciprofloxacin was also confirmed by LC/MS. The presence of sulfamethoxazole was detected in one of the secondary effluent samples. Sulfamethoxazole was not detected in effluent after granular activated carbon and ozonation treatment processes. In general, sulfamethazine was not found in any of the municipal wastewater effluent samples. Since the studied municipal wastewater treatment plants receive little wastewater from livestock operations, this observation is consistent with our expectation since sulfamethoxazole is used primarily in human medicine while sulfamethazine is used primarily in livestock, thus less likely to be present in municipal wastewater. The field measurements are currently in progress. Future occurrence study will include additional wastewater effluent from conventional and advanced treatment processes, surface water, and wastewater from animal feeding operations.

CONCLUSIONS

Six classes of antibiotics including aminoglycoside, β-lactam, macrolide, fluoroquinolone, sulfonamide, and tetracycline are commonly used in human therapy and in animal husbandry. The presence of these antibiotics in water as contaminants may pose risks to human health. A systematic literature review was conducted on antibiotic usage and environmental fate. The concentrations of antibiotics in municipal wastewater, animal manure, and liquid animal waste were also estimated.

The literature review indicates that sulfonamides and fluoroquinolones followed by macrolides are more likely to persist and transport in the aquatic environments. Tetracyclines may persist for a significant period of time but are less mobile. Aminoglycosides and β-lactams are least likely to persist in the environment. Among sulfonamide and fluoroquinolone antibiotics, sulfamethoxazole and ciprofloxacin are most likely to be present in municipal wastewater effluent and sulfamethazine is most likely to be present in agricultural runoff. Azithromycin and tylosin are the most likely macrolides present in municipal wastewater effluent and in agricultural runoff respectively.

Preliminary occurrence study using robust and sensitive analytical techniques was conducted for ciprofloxacin, sulfamethoxazole, and sulfamethazine. Field measurement of these three antibiotics is currently underway. Presently, the presence of ciprofloxacin in conventional municipal wastewater effluent has been detected at approx. 80 to 150 ng/L. The presence of sulfamethoxazole was detected in one sample. In general, sulfamethazine was not found in the municipal wastewater effluent. Concentrations of antibiotics are much lower in the effluent of advanced treatment processes such as granular activated carbon and ozonation, indicating significant removal by these processes.

As analyses of antibiotic contaminants in complex water matrices such as wastewater and surface water can be time-consuming and costly. The antibiotics identified by this literature review may serve as "indicators" in analytical survey for antibiotic contamination. Furthermore, the antibiotic use patterns change rapidly and new compounds are being developed and introduced continually. To assess the potential risks associated with antibiotics in water sources, consideration of chemical and structural properties of antibiotics is critical and allows predictions for structurally related compounds that are currently in use or being developed. Results of this literature review can also facilitate prioritizing future studies on the fate and transport of antibiotics in natural and engineered water systems.

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