OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Final Report

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First Progress Report
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LITERATURE REVIEW

INTRODUCTION

To identify pharmaceutically active compounds (PhACs) of significance to water suppliers, we reviewed scientific literature and available data for drug use and the occurrence of PhACs in the aquatic environment. The literature review was designed to identify a group of compounds to be measured as part of the occurrence survey. The selection criteria for compounds to be studied during the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and the availability of suitable analytical methods. Since use patterns for PhACs change rapidly, and it is time consuming and expensive to measure all of the PhACs potentially present in the aquatic environment, we attempted to identify compounds that can be used as surrogates in future studies. The use of surrogate compounds should expedite future assessments of PhACs in water supplies and their removal in water treatment systems.

During the first period of the project, we made considerable progress in the literature review. To estimate the concentrations of PhACs in municipal wastewater, we used pharmaceutical industry survey data on the most popular prescription drugs in the United States. Information on other PhACs likely to be present in municipal wastewater (e.g., over-the-counter drugs, drugs used exclusively in hospitals) was obtained by reviewing occurrence data for PhACs in municipal wastewater. In addition to predicting concentrations of PhACs in municipal wastewater, we also began a review of fate and transport properties of PhACs, occurrence data and analytical methods.

PREDICTION OF PhAC CONCENTRATIONS IN DIFFERENT SOURCE WATERS

The first step in assessing PhACs in the aquatic environment requires information on the concentrations of compounds present in water discharged by municipal and agricultural sources. During this phase of the project, quantitative estimates were made for the most popular U.S. pharmaceuticals in municipal wastewater. Estimates were not made for less popular drugs, non-prescription drugs, and drugs used mainly in hospitals (e.g., X-ray contrast media, cancer

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chemotherapy drugs) because sales data were unavailable. Discussions with pharmaceutical industry consultants indicated that information on less popular prescription drugs could be purchased at considerable cost from proprietary databases. Since our intention was to identify the most important PhACs present in U.S. waters, we chose to rely upon occurrence data to identify important PhACs not included in the survey. Data on PhACs discharged by agricultural sources are currently being reviewed by Dr. Huang, and will be presented in a future progress report.

Our approach for estimating the concentrations of prescription drugs involved dividing the mass of drug excreted by patients by the volume of wastewater discharged to municipal wastewater treatment plants. Calculations were performed for the top 200 prescription drugs listed in a 1998 survey conducted by IMS Health (1999). The top 200 prescription drugs include 136 PhACs, because some of the drugs contain the same active ingredient. Because numerous assumptions are needed to convert the number of prescriptions administered to the concentration of PhACs in municipal wastewater, considerable uncertainty is associated with these estimates. Despite the uncertainties, the estimates should be useful in identifying PhACs that are candidates for further study.

Estimation of the concentrations of PhACs in municipal wastewater required the conversion of the number of prescriptions administered into the mass of compound discharged. Because several formulations are available for each prescription drug, the mass of active ingredient in a dose varies between prescriptions. For example, the β-blocker timolol is prescribed at 40 mg/prescription in an oral formulation and 6 mg/prescription in an eye ointment. To estimate the mass of active PhAC associated with each dose, we consulted medical reference books (Katzing 1998, PDR 1999) and interviewed a practicing pharmacist who provided information on the most popular form of each prescription of interest (Field 1999). After estimating the mass of active ingredient in each dose of the most popular form of the drug, we estimated the number of doses per prescription. Estimates were made for the maximum and minimum masses per prescription assuming the most common drug formulations. For drugs that were given on a one-time basis (e.g., antibiotics), we assumed that each prescription included a sufficient number of doses to treat the ailment (typically 10 days). For drugs administered on a continuing basis (e.g., beta-blockers, birth control pills) we assumed that each prescription was

renewed monthly. The basis for this assumption was the current practices of many health maintenance organizations (HMOs) to refill prescriptions once per month (Field 1999).

After estimating the mass of each drug prescribed, we estimated the concentration present in untreated wastewater (Table 1). When excretion data were readily available, we estimated the fraction of the dose excreted in its original form. However, excretion data were not readily available for many drugs, or when the data were available, it was unclear if glucuronide or sulfate conjugates 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 drugs should be included with the PhACs. As a result of missing or ambiguous data, information on metabolism was only available for 30% of the PhACs in the table. Therefore, comparisons between estimated concentrations of PhACs are made without consideration of metabolism. No attempts were made to quantify pharmaceutically-active metabolites.

Estimated concentrations of prescription drugs in untreated wastewater (Table 1) range from less than 1 ng/L to approximately 133,000 ng/L. The estimated concentrations are distributed over a wide range, with the majority of compounds estimated to be present at concentrations between 100 and 1,000 ng/L (Figure 1). In general, the compounds expected to
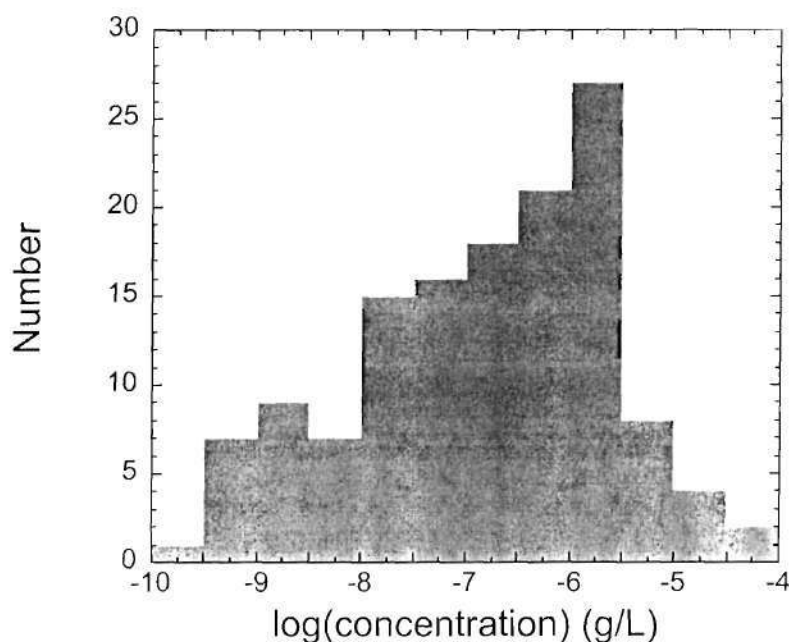

Figure 1: Histogram depicting the predicted distribution of wastewater concentrations for the compounds listed in Table 1.

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Table 1: Geometric mean for range of estimated concentrations of PhACs in municipal wastewater in the United States.

Name	Classification	Excluding Metabolism		Including Metabolism		Excretion (2)
		Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	
erythromycin	antibiotic	1,500	1,500	75	75	I
atenolol	β-blocker	1,500	520 to 4,100	1,500	520 to 4,100	D
sertraline	antidepressant	1,400	490 to 3,900	180	64 to 510	F
triamterene	diuretic	1,400	1,400			J
nefazodone	antidepressant	1,300	750 to 2,200	13	7.5 to 22	F
tetracycline	antibiotic	1,200	830 to 1,700			J
allopurinol	antigout	1,000	600 to 1,800			F
furosemide	diuretic	960	490 to 1,900			J
cefuroxime	antibiotic	900	450 to 1,800	900	450 to 1,800	D
nizatidine	H$_2$-receptor antagonist	860	610 to 1,200	520	370 to 730	D
fluoxetine	antidepressant	860	490 to 1,500			F
omeprazole	antiulcerative	850	480 to 1,500			F
amitriptyline	antidepressant	850	480 to 1,500			F
nifedipine	calcium channel blocker	760	530 to 1,100			J
codeine	opiod analgesic	730	230 to 2,300			C
trazodone	antidepressant	620	390 to 1,000			F
atorvastatin	cholesterol lowering	630	220 to 1,800	12	4.4 to 35	I
lisinopril	ACE Inhibitor	590	290 to 1,200	590	290 to 1,200	F
losartan	antihypertensive	560	320 to 970	23	13 to 39	F
loracarbef	antibiotic	480	340 to 680			J
fluconazole	antifungal	480	240 to 950			J
fexofenadine	antihistamine	470	470			J
paroxetine	antidepressant	440	180 to 1,100	9	3.6 to 22	F
valsartan	antihypertensive	440	220 to 880			J
levofloxacin	antibiotic	400	280 to 570	350	250 to 490	D
cisapride	gastroprokinetic	380	270 to 530	38	27 to 53	D
nitrofurantoin	antibiotic	350	250 to 500	130	90 to 180	E
loratadine	antihistamine	340	340	34	34	J
famotidine	H$_2$-receptor antagonist	320	160 to 630	85	43 to 170	J
venlafaxine	antidepressant	300	210 to 420	15	10 to 21	F

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Name	Classification	Excluding Metabolism		Including Metabolism		Excretion (2)
		Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	
isosorbide dinitrate	antianginal	280	280			F
quinapril	ACE Inhibitor	270	94 to 760			F
hydrocodone	opiod analgesic	270	98 to 720			J
propranolol	β-blocker	250	89 to 710			J
cyclobenzaprine	skeletal muscle relaxant	240	240			J
prednisone	glucocorticoid	240	69 to 830			F
enalapril	ACE Inhibitor	240	84 to 670	240	84 to 670	F
pravastatin	cholesterol lowering	240	120 to 470			I
simvastatin	cholesterol lowering	230	82 to 650			I
benazepril	ACE Inhibitor	220	110 to 460			F
fluvastatin	cholesterol lowering	180	130 to 250			I
Lovastatin	cholesterol lowering	180	88 to 350			I
nisoldipine	calcium channel blocker	150	110 to 210	15	11 to 21	B
glipizide	antidiabetic	130	60 to 290	13	6.0 to 29	A
fosinopril	ACE Inhibitor	110	54 to 220			F
zafirlukast	antiasthmatic	100	100			J
promethazine	antihistamine	91	63 to 130			J
tamoxifen	antiestrogen	79	57 to 110			F
sildenfil		79	57 to 110			J
warfarin	anticoagulant	80	36 to 180			J
medroxyprogesterone	hormone	65	51 to 82			F
cetirizine	antihistamine	58	41 to 82	29	20 to 41	D
sumatriptan	antimigraine	50	35 to 71	1.5	1.1 to 2.1	J
glyburide	antidiabetic	48	12 to 190			A
alendronate	suppressant - bone resorption	46	33 to 66			J
methylprednisolone	glucocorticoid	44	13 to 150			D
clotrimazole	antifungal	44	26 to 77			J
oxycodone	opiod analgesic	43	25 to 74			J
estrone	hormone	39	14 to 110			J
bisoprolol	β-blocker	35	13 to 100	1.8	0.6 to 5.0	E
doxazosin	antihypertensive	32	8.1 to 130			J
clonazepam	antianxiety	32	8.8 to 120			F
amphetamine	CNS stimulant	30	6.2 to 150			J
dextroamphetamine	CNS stimulant	30	6.2 to 150			J
terazosin	antihypertensive	27	8.5 to 85			B

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| Name | Classification | Excluding Metabolism | | Including Metabolism | | Excretion (2) |
		Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	
buspirone	antianxiety	24	5.7 to 100			F
hydrocortisone	glucocorticoid	22	22			D
estradiol	hormone	22	2.5 to 190			J
diazepam	antianxiety	21	3.2 to 140			F
equilin	hormone	20	7.0 to 56			J
risperidone	antipsychotic	19	16 to 24			A
amlodipine	calcium channel blocker	14	6.8 to 27			B
felodipine	calcium channel blocker	13	6.7 to 27			B
lorazepam	antianxiety	12	1.9 to 69			C
alprazolam	antianxiety	11	1.9 to 69			F
17-α-dihydroequilin	hormone	11	4.0 to 33			J
norethindrone	hormone	11	11			F
ramipril	ACE inhibitor	10	3.5 to 28			F
neomycin	antibiotic	7.6	7.6			J
levothyroxine	hormone	5.2	2.9 to 9.4			J
glimepiride	antidiabetic	5.2	2.6 to 10			A
digoxin	cardiotonic	5.1	2.6 to 10	3.1	1.5 to 6.2	D
tobramycin	antibiotic	3.9	2.5 to 6.1			J
triamcinolone	glucocorticoid	3.5	3.5			D
mometasone	glucocorticoid	2.9	1.4 to 5.8			D
betamethasone	glucocorticoid	2.2	1.3 to 3.8			D
beclomethasone	glucocorticoid	2.1	2.1			J
norgestimate	hormone	2.1	2.1			F
levonorgestrel	hormone	1.9	1.9			F
dexamethasone	glucocorticoid	1.3	0.82 to 2.0			J
ethinyl estradiol	hormone	1.2	1.2			J
fluticasone	antihistamine	1.2	0.19 to 7.6			J
timolol	β-blocker	1.2	0.58 to 2.3			J

| Name | Classification | Excluding Metabolism | | Including Metabolism | | Excretion (2) |
		Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	
clonidine	antihypertensive	0.90	0.37 to 2.2	0.40	0.16 to 1.0	D
nitroglycerin	antianginal	0.83	0.66 to 1.0			J
desogestrel	hormone	0.81	0.81			F
budesonide	glucocorticoid	0.70	0.70			J
tretinoin	keratolytic	0.67	0.12 to 3.7			J
latanoprost	antiglaucoma	0.60	0.60			J
salmeterol	bronchiodiolater	0.32	0.22 to 0.48			J
fluticasone	antiallergic	0.18	0.09 to 0.35			J
albuterol	bronchiodiolater	0.083	0.08			J

notes:

(1) This calculation was made assuming that the population of the U.S. is 250 million, that each person produces 320 L of wastewater per day, and that the excreted pharmaceuticals are evenly distributed among all wastewater in the U.S.

(2) (A) Extensive metabolism to inactive metabolites
(B) Extensive metabolism, possibly to conjugates
(C) Excreted mostly as conjugates
(D) Excreted mostly in original form (>50%)
(E) Excreted partially in original form (25-50%)
(F) Extensive metabolism to active metabolites
(G) Excreted as mixture of conjugates/original form
(H) Excreted partially as conjugates (25-50%)
(I) Little excreted in urine
(J) Data on metabolism not obtained

References for doses and metabolism:
Katzung, B.G. 1998. *Basic and Clinical Pharmacology.* Stamford, CT: Appleton and Lange.

Physicians' Desk Reference. 1999. Montvale, NJ: Medical Economics Company, Inc.

be present at the highest concentrations consisted of pain relivers (e.g., acetominophen, ibuprofen) and antibiotics (e.g., cephalexin, amoxicillin). Because some of the pain relievers on the list also are available as over-the-counter products, their concentrations in wastewater could be considerably higher. Compounds estimated to be present at the lowest concentrations tended to be potent drugs such as hormones (e.g., medroxyprogresterone, equilin). Therefore, compounds estimated to be present at low concentrations should not be eliminated from further consideration without considering issues related to potency.

It is instructive to compare our estimates with estimates based on drug use data from Germany, where PhACs have been detected in wastewater (Table 2). For example, clofibric acid precursors, such as benzafibrate, are extremely popular in Germany. However, they are rarely used in the United States because they have been replaced by HMG CoA reductase inhibitors. To illustrate difference between drug use in the U.S. and Germany, we have estimated concentrations of a group of PhACs in German wastewater by using the same approach as described in the previous section. Results of these calculations indicate that drug use patterns vary considerably between the two countries. Expected concentrations are significantly higher in the U.S. for fourteen of the PhACs while three of the compounds are expected at higher concentrations in Germany. The use of ten of the compounds varies by less than a factor of two between the U.S. and Germany. Seven of the compounds in the list do not appear in industry survey data for the United States and cannot be compared with German estimates.

As indicated in the previous section, antibiotics are used extensively in human therapy. Prescription data provide useful information on the commonly used antibiotic compounds and their approximate quantities in use in the United States. Based on the top 200 prescription drugs, β-lactams (e.g., amoxicillin and cephalexin), macrolides (e.g., azithromycin), and quinolones (e.g., ciprofloxacin) are most important antibiotics used in the treatment of human diseases. To a lesser extent, aminoglycosides, sulfonamides (sulfamethozaxole), tetracycline and other antibiotics are also used. The estimated concentrations of antibiotics in source waters range from more than 9,200 ng/L (azithromycin) and 27,000 ng/L (amoxicillin) to less than 25 ng/L.

Antibiotics are used in animal husbandry both therapeutically to treat diseases and subtherapeutically as feed additives to promote growth. The quantity of a specific antibiotic's use in agriculture is difficult to estimate because the instances of drug use are often not documented and some drugs can be purchased over-the-counter without prescription, usually

Table 2: Comparison of PhACs predicted in wastewater in the U.S. and Germany.

Compound	Predicted Concentration in Wastewater (ng/L) Germany (1)	United States (2)	Percent Difference
acetaminophen	8,200	62,000	153%
acetylsalicyclic acid	28,000	a	
amitriptyline	940	840	11%
amoxicillin	7,300	27,000	115%
atenolol	100	1,500	175%
azithromycin	1,800	9,200	135%
benzafibrate	4,900	b	
betaxolol	53	c	
bisoprolol	130	35	115%
carbamazepine	9,200	c	
cephalexin	67	14,000	198%
ciprofloxacin	590	3,000	134%
clarithromycin	210	2,800	172%
diclofenac	3,600	c	
diltiazem	1,600	2,600	48%
erythromycin	2,200	1,500	38%
fluoxetine	55	850	176%
gemfibrozil	1,400	3,400	83%
hydrochlorothiazide	610	1,900	103%
ibuprofen	13,000	37,000	96%
indometacine	660	c	
ketoprofen	70	c	
metoprolol	7,900	3,100	87%
metformin	25,000	24,000	4%
naproxen	150	2,500	177%
paroxetine	30	440	174%
penicillin	5,200	4,000	26%
phenytoin	1,400	2,700	63%
propranolol	690	250	94%
ranitidine	3,800	3,000	24%
sodium valproate	4,300	6,100	35%
sulfamethoxazole	3,400	3,800	11%
tramadol	1,500	2,200	38%
trimethoprim	680	2,200	106%

(1) This calculation was made assuming that the population of Germany is 81.4 million, that each person produces 250 L of wastewater per day, and that the excreted pharmaceuticals are evenly distributed among all wastewater in Germany.
(2) This calculation was made assuming that the population of the U.S. is 250 million, that each person produces 320 L of wastewater per day, and that the excreted pharmaceuticals are evenly distributed among all wastewater in the U.S.
a) over-the-counter medicine, not in top 200
b) Not in top 200, no longer commonly used in the U.S.
c) Not in top 200
Reference for German Calculations:
Schwabe, U. and D. Paffrath. 1999. Arzneiveordnungs-Report 1998. Berlin: Springer.

from distributors of animal feed and other animal production supplies. It was estimated that of the 8 million kilograms of antibiotics used in major species of food animals in 1985; 90% was used for subtherapeutic dose application (NAS 1989). In 1991, 34 million kilograms of antibiotics were used in humans and animals, and approximately 25% of that was used for food-animal production (NAS 1999).

Among the antibiotics used in agriculture, barcitracin, tetracyclines (chlortetracycline and oxytetracycline), macrolides (tylosin, erythromycin, streptomycin, virginiamycin), and penicillin are the most frequently used antibiotics for all major animal species. To a less extent, sulfonamides (e.g., sulfamethazine) and other antibiotics are used in therapeutic application. Fluoroquinolones (e.g., enrofloxacin and sarafloxacin) are used in broilers to control various infections (NAS 1999).

It is worth noting that antibiotics used commonly in food animals are usually different from the ones used in humans. This approach is used to reduce the risks of development of resistant bacteria in animals, which may in turn be passed on to humans, thus diminishing the effectiveness of antibiotics in treatment of human diseases. For instance, tetracyclines are much more important in agricultural application than in human therapy. Barcitracin, tylosin and virginiamycin were developed specifically for agricultural use. In general, different compounds of macrolides, β-lactams, sulfonamides, and fluoroquinolones are used separately for humans and for agricultural animals.

OCCURENCE DATA

Another important tool for identifying candidate compounds is occurrence data from other scientific studies. Published data on PhACs in municipal wastewater effluents and surface waters are limited to studies conducted in Germany and Switzerland. However, research is underway in Canada and the United States. The published data have been reviewed to identify compounds that are not included in the previously described estimates. In addition, the occurrence data provide guidance on the removal of compounds during sewage treatment.

Most data of PhACs in municipal wastewater have been collected by German and Swiss researchers (Table 3). The most comprehensive study of PhACs in municipal wastewater was

Table 3: Summary of occurrence data for PhACs. N.D. indicates compound not detected. >50%=compound detected in >50% of samples; <50%=compound detected in <50% of samples.

Compound	Wastewater Effluent			Surface Water			Drinking Water		
	N.D.	< 50%	>50%	N.D.	< 50%	>50%	N.D.	< 50%	>50%
acetaminophen		1		1					
acetylsalicyclic acid		1	5,6,7	7		1	7		
betaxolol			1,4		1	4	4		
bezafibrate			1,5,7	5		1,7		7	
bisoprolol			1,4		1	4	4		
carazolol		1,4			1,4		4		
carbamazepine			1			1			
clenbuterol		1,4		4	1		4		
clofibrate	1			1					6
clofibric acid			1,5,7		5	1,2,7		2,3,7	
cyclophosphamide		1		1					
diazepam		1	6	1		6			
diclofenac			1,5,7			1,7		7	
dimethylaminophenazone		1			1				
etofibrate	1			1					
fenofibrate		1		1					
fenofibric acid			1,5,7			1,7	7		
fenoprofen	1,7			1,7			7		
fenoterol		1,4			1,4		4		
gemfibrozil			1,5,7			1,7	7		
gentisic acid		1			1				
ibuprofen			1,5,7			1,5,7		7	
ifosfamide		1		1					
indometacine			1,5,7			1,7	7		
ketoprofen			1,5,7	7	1		7		
meclofenamic acid	1			1					
metoprolol			1,4			1,4	4		
nadolol			1,4	1	4		4		
naproxen			1,5			1,5			
o-hydroxyhippuric acid	1			1					
phenazone			1			1			
propranolol			1,4			1,4	4		
salbutamol		1,4		4	1		4		
salicyclic acid		1				1			
terbutalin		1,4			1,4		4		
timolol		1,4			1	4	4		
tolfenamic acid	1			1					

(1)Ternes (1998)

(2) Stan, Heberer, and Linkerhagner (1994)

(3) Heberer and Stan (1996)

(4) Hirsch et al. (1996)

(5) Stumpf et al. (1999)

(6) Richardson and Bowron (1985)

(7) Stumpf et al. (1996)

published by Ternes (1998). In this study, a total of 32 PhACs were measured in wastewater effluent samples collected at treatment plants throughout Germany. Other studies have considered the fate of a more limited number of PhACs (Table 3). Some of these data were reported at the spring 2000 national meeting of the American Chemical Society in San Francisco and will be published in a forthcoming book. As these data are published, our literature review will be modified accordingly.

Some of the compounds analyzed in previous studies do not appear in the list of popular U.S. prescription drugs (i.e., Table 1). Compounds detected with a high frequency in previous studies (i.e., in more than 50% of the wastewater effluents sampled) included:

- benzafibrate, clofibrate and their metabolites (clofibric acid, fenofibric acid). [As mentioned previously, these compounds are no longer popular in the U.S. and we do not expect them to be present at significant concentrations in U.S. wastewater.]
- Analgesics (diclofenac, indometacine, ketoprofen, phenazone).
- The β-blocker, nadolol.
- The antiepiletic, carbamazepine.

In addition to the aforementioned data on PhACs in wastewater, antibiotics may be present in water supplies. Published data on the occurrence of antibiotics in municipal wastewater and surface water are limited. Most of the studies were conducted in Europe. Fluoroquinolones (Hartmann et al 1998), macrolides, sulfonamides (Hartig et al., 1999) have been detected in wastewater and surface water.

More recent results on the occurrence of fluoroquinolone and macrolide, and sulfonamide antibiotics were presented at the spring 2000 National Meeting of the American Chemical Society in San Francisco. Tetracyclines were detected in agricultural runoff (Meyer et al. 2000). The concentrations of β-lactams such as amoxicillin and penicillin were found to be near or below the detection limits in most cases (Hirsch et al. 1999). This is probably due to the fact that β-lactams are readily hydrolyzed under environmental conditions (Hou and Poole 1969) and thus their half-lives in the environment are expected to be short. Occurrence data are not available for aminoglycosides and other less commonly used antibiotic compounds.

In addition to the studies listed above, the U.S. Geological Survey (USGS) presently is conducting an occurrence survey of PhACs and related compounds. The primary focus of the USGS study is to measure concentrations of contaminants in surface waters. Although they have

not yet published any data, they have listed their target analytes and sample locations on their internet site (http://toxics.usgs.gov/regional/contaminants.html). The target analytes in the USGS study include some of the PhACs described previously. In addition, their study also addresses personal care products, pesticides, detergent metabolites and other compounds not considered in our research. Contacts have been made with USGS personnel involved in the survey and results of their analyses will be considered in the design of our study and interpretation of our data.

OCCURENCE SURVEY

COMPOUND SELECTION

The intention of the literature review is to identify compounds to be analyzed in future research. Ideally, the occurrence survey would begin after completion of the literature review and receipt of feedback from the project advisory committee (PAC). However, the relatively short time frame of the project and complications associated with method development necessitate development of analytical methods and collection of data prior to completion of the literature review. During the first project period, we used results of the literature review to identify some of the compounds of interest in waters receiving municipal wastewater. Previously published analytical methods for PhACs were used to measure concentrations of selected PhACs in municipal wastewater and surface water. Antiobiotics originating in wastewater and in agricultural runoff will be addressed in a later progress report. Following comments from the PAC, we may add or delete compounds from this list.

Since data on drug use and contaminant fate are somewhat uncertain, and little is known about human health effects associated with low doses of PhACs, selection of compounds to be studied requires us to assess the importance of the compounds on the basis of limited information. To identify compounds that will be relevant to future discussions of PhACs in water supplies, we attempted to choose common PhACs that are likely to be present at detectable levels in municipal wastewater effluent and in drinking water supplies subjected to wastewater discharges. In addition, we also attempted to select compounds exhibiting a range of transport properties (e.g., polarity, susceptibility to transformation).

As a first step in compound selection, we eliminated compounds that we did not expect to detect at relatively high concentrations. We decided to eliminate PhACs likely to be present at extremely low concentrations in wastewater because their concentrations are expected to be significantly lower after dilution and it would probably be more difficult to study the fate and transport of these compounds. Compounds were eliminated if their geometric mean concentration predicted in wastewater was below 1,000 ng/L. Compounds detected in previously published studies, but not in the survey of popular U.S. drugs, were eliminated if they were not

detected in more than half of the wastewater effluent samples analyzed. In addition, several fibrates and their metabolites were eliminated because information from pharmaceutical industry sources suggests that they are no longer used in large quantities in the United States. The initial screening reduced the number of compounds to be considered to 47 compounds.
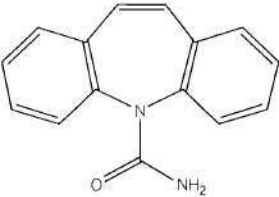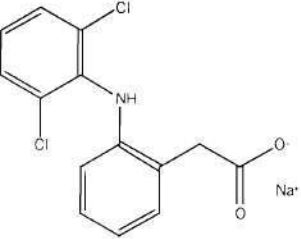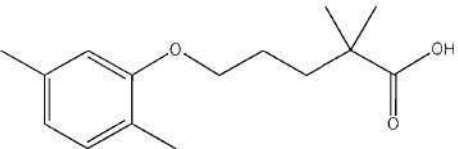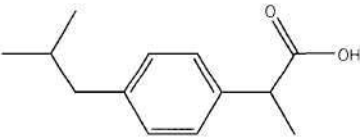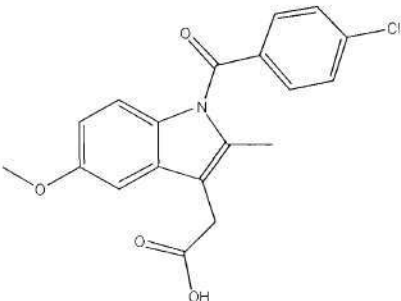
In the next step of our analysis, we reviewed the scientific literature to identify analytical methods for measuring the remaining PhACs in aqueous samples. Results of this review indicate that analytical methods employing solid-phase extraction (SPE) followed by GC/MS/MS under conditions encountered in wastewater effluents are available for fourteen of the compounds in Table 1. Some of the remaining compounds on the list may be amenable to analysis using the same techniques (i.e., compounds with similar structures can probably be analyzed using the same extraction and dervitization techniques). However, method development and testing would likely delay progress of the project, and new compounds were avoided in this stage of the research. Other compounds on the list are probably not amenable to SPE because they are relatively polar (e.g., gabapentin) or are difficult to derivitize (e.g., metformin).
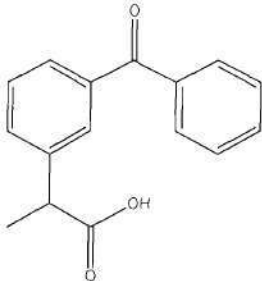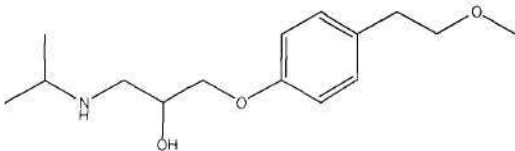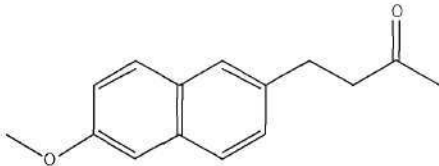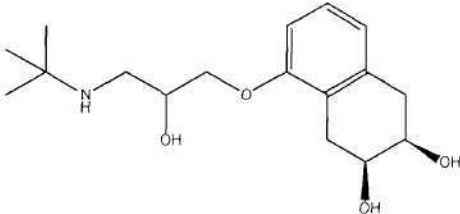
For compounds that could not be analyzed using GC/MS/MS, liquid chromatography/mass spectrometry (i.e., HPLC/MS or HPLC/MS/MS) may be viable alternative technique. HPLC/MS methods are particularly useful for analysis of antibiotics and thermolabile compounds. Because antibiotics are important contaminants in agricultural runoff, their analysis in agricultural and municipal sources will be addressed during our analysis of agricultural sources, in a later progress report.
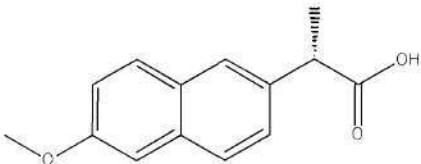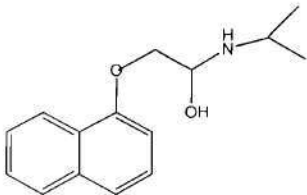
Immunochemical methods also were considered for the compounds remaining on the list. With the exception of antibiotics, which will be addressed in a later progress report, considerable effort would be required to develop immunochemical analytical methods. Because commercial ELISA kits are unavailable for most of the PhACs, the development of homemade immunoassays would be required. Therefore, we will focus our efforts on the development of immunochemical approaches for the antiobiotics for which ELISA kits and/or antibodies are available.

The compounds to be considered in the first phase of the occurrence survey are listed in Table 4. These compounds are analyzed using three different solid phase extraction and derivization techniques followed by GC/MS/MS (Appendix A). The compounds include six

Table 4: Compounds selected for initial phase of occurrence survey.

Compound	Structure	Type	Predicted U.S. Wastewater conc. (ng/L)[1]	Median observed concn. (ng/L)[2]
Carbamazepine		antipiletic	ND	2,100
Diclofenac		analgesic	ND	810
Gemfibrozil		β-blocker	3,400	400
Ibuprofen		analgesic	37,000	370
Indometacine		analgesic	ND	270

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Compound	Structure	Type	Predicted U.S. Wastewater conc. (ng/L)[1]	Median observed concn. (ng/L)[2]
Ketoprofen		analgesic	NA	200
Metoprolol		β-blocker	3,100	730
Nabumetone		analgesic	12,000	NA
Nadolol		β-blocker	ND	25

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Compound	Structure	Type	Predicted U.S. Wastewater conc. (ng/L)[1]	Median observed concn. (ng/L)[2]
Naproxen		analgesic	2,500	300
Propranolol		β-blocker	250	170

Notes:

[1] Estimated wastewater concentration (excluding metabolism) reported in Table 1.

[2] Median concentration reported in municipal wastewater effluent by Ternes (1998).

ND = no data reported for this compound reported in prescription survey.

NA = this compound was not analyzed by Ternes (1998).

acidic drugs, three compounds with alcohol functional groups and two neutral drugs. According to our estimates (Table 1), six of the compounds should be present in untreated municipal wastewater in the U.S. at concentrations greater than 1,000 ng/L and one should be present at 250 ng/L. Ten of the compounds were reported in wastewater samples collected in Germany. One compound (i.e., nabumetone) is expected to be present at concentrations above 1,000 ng/L but has not been measured in previous studies.

RESULTS

During the first phase of the project, samples were collected from a total of seven sampling points at four locations. As a result of difficulties associated with our first attempts to quantify these samples, all of the PhACs were not analyzed at each site. The sites are described below:

1. *The Dublin/San Ramon Advanced Wastewater Treatment Plant (Dublin, CA):* The Dublin/San Ramon Services District operates a 0.50 m^3 s^{-1} wastewater treatment plant equipped with primary treatment, secondary activated sludge treatment and chlorine disinfection. A pilot scale advanced wastewater treatment plant receives a portion of the secondary effluent. The advanced wastewater treatment plant consists of microfiltration, ultraviolet disinfection and reverse osmosis. During this round of sampling, samples were collected after secondary treatment, after microfiltration and after ultraviolet disinfection.

2. *Secondary Wastewater Effluent:* Wastewater effluent was sampled after secondary oxygen activated sludge treatment at a plant located in California.

3. *The Goodyear Wastewater Treatment Plant (Goodyear, AZ):* The Goodyear Wastewater Treatment Plant is a 0.13 m^3/s facility that is equipped with primary treatment, secondary activated sludge treatment, nitrification/denitrification, sand filtration and chlorine disinfection.

4. *The Prado Wetlands (Orange County, CA):* The Orange County Water District operates the Prado wetlands as a wildlife habitat and to improve water quality. During the dry season, the source of the wetlands mainly consists of effluent from wastewater treatment plants located on the Santa Ana River. One sample was collected near the entrance to the wetlands and a second sample was collected from a wetland cell after a hydraulic residence time of approximately five days.

Quality Assurance/Quality Control

To assess the validity of the analyses, a series of quality assurance/quality control measures were included in the analytical protocol. For each sample, spike recoveries were evaluated in duplicate by amending two separate aliquots of thew sample with 1,000 ng/L of

each analyte prior to extraction. To assess variability, all samples were analyzed in duplicate. Contamination of samples was monitored with deionized water blanks analyzed on each date when samples were extracted and analyzed. Contamination as detected for indometacine (i.e., 33 ng/L) on 8/8/00 and propranolol on 6/15/00 (i.e., 68 ng/L).

Results of spike recovery samples indicate that analyte recoveries improved as we gained more experience with the analyses (Tables 5,6). We attribute these results to slight improvements in our analytical procedures, particularly with respect to derivitization. For the acidic PhACs, recoveries improved from a mean of 32% (range 13-65%) on our first attempt to analyze the compounds to 71% (range 43-103%) during our most recent analyses. The recoveries varied with individual compounds, with a mean recovery in the most recent analyses of 48% for indometacine to 85% for gemfibrozil. Recoveries of the other drugs also improved from a mean value of 42% in the first round of sampling to 88% in the most recent analyses. For two spike recovery samples analyzed for neutral drugs, one or two of the analytes were not detected. In both cases, the accompanying duplicate sample exhibited good recovery.

Problems were encountered in the analysis of carbamazepine, which is known to be unstable in the injection port of the GC. Carbamazepine was detected in one sample of secondary wastewater effluent, but not in standards prepared in deionized water. As a result, concentrations were quantified by standard additions. Efforts are currently underway to improve the analysis of this compound.

Duplicate analyses of samples and standards exhibited good agreement between samples. More variability was observed in samples containing PhACs at concentrations near the limit of quantification. In general, more variability was observed in the first round of samples than the most recent analyses.

Preliminary Results

Results from the preliminary sampling indicate that PhACs are present in municipal wastewater effluent and that concentrations vary between locations (Tables 5,6 and Figures 2,3). For the acidic PhACs, concentrations were highest in effluent samples collected from the secondary wastewater treatment plant, with concentrations ranging from approximately 30 to

Table 5: Concentrations of acidic drugs detected during the first project period.

Compound	Location	Date	Concentration (ng/L)	Spike Recovery
Ibuprofen	Goodyear Wastewater Effluent	8/8/00	17, 18	84%, 58%
	Prado Wetlands Influent	7/31/00	<10, <10	61%, 65%
	Prado Wetlands Effluent	7/31/00	<10, <10	75%, 103%
	Secondary Wastewater Effluent	5/30/00	180, 220	54%, 34%
Naproxen	Goodyear Wastewater Effluent	8/8/00	21, 23	90%, 78%
	Prado Wetlands Influent	7/31/00	21, 11	70%, 67%
	Prado Wetlands Effluent	7/31/00	8, 9	70%, 91%
	Secondary Wastewater Effluent	5/30/00	55, 53	56%, 32%
Gemfibrozil	Goodyear Wastewater Effluent	8/8/00	40, 44	99%, 76%
	Prado Wetlands Influent	7/31/00	25, 14	76%, 73%
	Prado Wetlands Effluent	7/31/00	14, 19	87%, 98%
	Secondary Wastewater Effluent	5/30/00	1840, 3660	65%, 25%
Ketoprofen	Goodyear Wastewater Effluent	8/8/00	9, 16	80%, 81%
	Prado Wetlands Influent	7/31/00	<5, <1	69%, 69%
	Prado Wetlands Effluent	7/31/00	<5, <1	68%, 87%
Diclofenac	Goodyear Wastewater Effluent	8/8/00	16, 22	74%, 63%
	Prado Wetlands Influent	7/31/00	<5, <2	59%, 55%
	Prado Wetlands Effluent	7/31/00	<5, <2	57%, 71%
	Secondary Wastewater Effluent	5/30/00	40, 21	53%, 30%
Indometacine	Goodyear Wastewater Effluent	8/8/00	<10, <30	46%, 43%
	Prado Wetlands Influent	7/31/00	<30, <10	48%, 45%
	Prado Wetlands Effluent	7/31/00	<30, <10	48%, 56%
	Secondary Wastewater Effluent	5/30/00	510, 510	22%, 13%
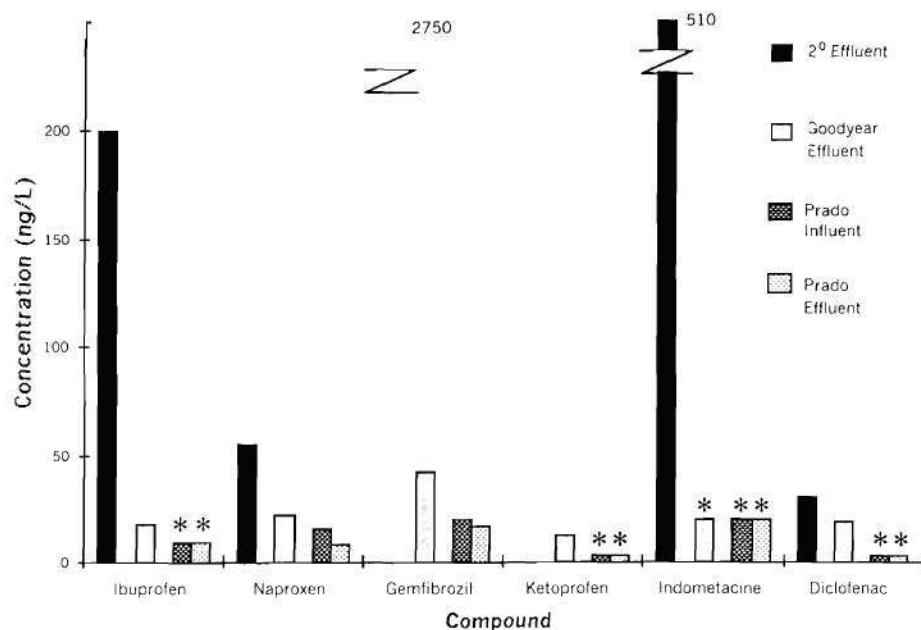
Figure 2: Concentrations of acidic drugs detected during first project period. * indicates concentration below limit of quantification.

Table 6: Concentrations of other drugs detected during the first project period.

Compound	Location	Date	Concn. (ng/L)	Spike Recovery
Metoprolol	Goodyear Wastewater Effluent	8/8/00	30, <8	136%
	Prado Wetlands Influent	7/31/00	<8, <8	89%, ND*
	Prado Wetlands Effluent	7/31/00	<8, <8	53%, 115%
	Dublin/San Ramon Secondary Effluent	6/15/00	130, 110	39%
	Dublin/San Ramon Microfiltration Effluent	6/15/00	90, 110	56%, 58%
	Dublin/San Ramon UV Effluent	6/15/00	40, 120	34%, 31%
	Secondary Wastewater Effluent	5/30/00	150, 120	27%, 31%
Propranolol	Goodyear Wastewater Effluent	8/8/00	14, <10	96%
	Prado Wetlands Influent	7/31/00	<10, <10	76%, ND*
	Prado Wetlands Effluent	7/31/00	<10, <10	37%, 105%
	Dublin/San Ramon Secondary Effluent	6/15/00	210, 150	36%
	Dublin/San Ramon Microfiltration Effluent	6/15/00	90, 100	48%, 48%
	Dublin/San Ramon UV Effluent	6/15/00	55, 97	47%, 40%
	Secondary Wastewater Effluent	5/30/00	140, 80	18%, 25%
Nadolol	Secondary Wastewater Effluent	5/30/00	180, 160	60%, 66%
Nabumetone	Dublin/San Ramon Secondary Effluent	6/15/00	1020, <1	ND*, 115%
	Dublin/San Ramon Microfiltration Effluent	6/15/00	<1, <1	110%, 102%
	Dublin/San Ramon UV Effluent	6/15/00	<1, 10	118%, 111%
	Secondary Wastewater Effluent	5/30/00	70, 150	88%, 105%
Carbamazepine	Secondary Wastewater Effluent	5/30/00	1130, 980	49%, 61%

* ND = compound not detected in spike recovery sample
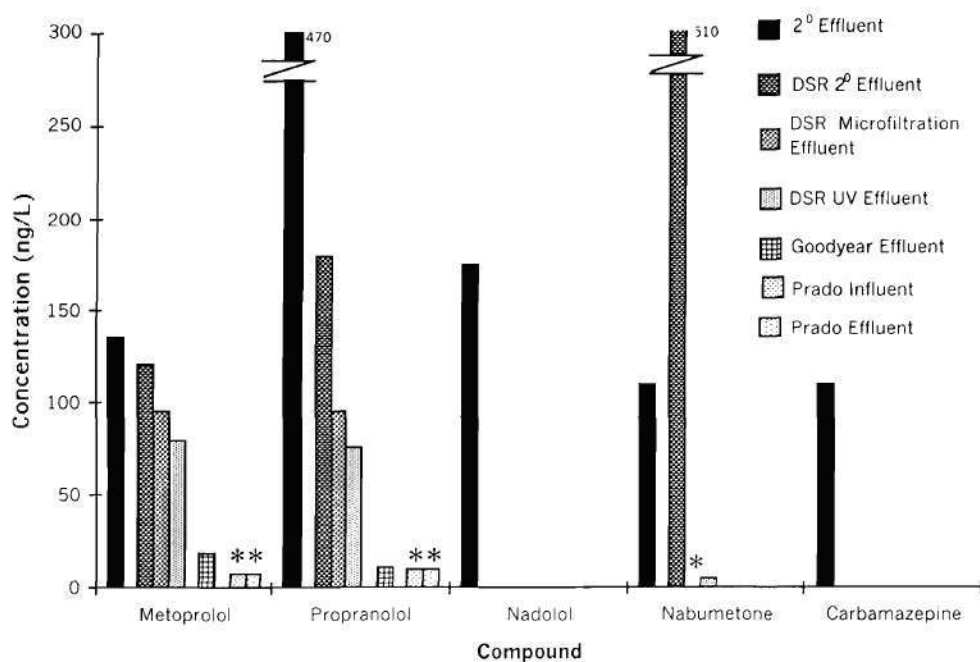
Figure 3: Concentrations of other drugs detected during the first project period.

2,000 ng/L. Concentrations of acidic PhACs were approximately an order of magnitude lower in the effluent samples collected from the Goodyear wastewater treatment plant. Only two of the six PhACs (i.e., naproxen and gemfibrozil) were detected in the Prado wetland samples. In both cases, the concentrations of PhACs decreased slightly between wetland influent and effluent samples.

The PhACs with neutral and alcohol functional groups were detected in the two secondary effluent samples at concentrations ranging from 120 to 1,050 ng/L. Concentrations of metoprolol and propranolol were approximately five to ten times lower in the effluent from the Goodyear wastewater treatment plant compared to effluent samples from the secondary treatment plants. Analysis of samples from the advanced wastewater treatment plant suggest a slight decrease in the concentrations of metoprolol, propranolol and nabumetone during microfiltration and ultraviolet disinfection. Metoprolol and proranolol were not detected in samples collected from the Prado wetlands.

The preliminary results provide some insight into the fate of PhACs in engineered systems. For example, detection of lower concentrations of PhACs in the effluent from the Goodyear wastewater treatment plant suggests that removal of the compounds may occur during nitrfication/denitrification. Furthermore, both engineered wetlands and advanced wastewater treatment plants appear to remove a small portion of the PhACs. These preliminary findings will be examined in more detail during future sampling of these and other sites.

FUTURE RESEARCH

During the next project period we plan to continue our efforts related to the literature review, the development of new analytical methods and collection of new data. As part of the literature review, additional information will be collected on the use of antibiotics in agriculture and their occurrence in the aquatic environment. After identification of two or three antibiotics of concern, analytical methods (i.e., HPLC/MS and immunochemistry) will be developed and tested.

Preliminary results on the occurrence of PhACs will be augmented by the collection and analysis of additional samples. To provide data on contaminant removal during conventional and advanced wastewater treatment, samples will be collected from several locations. Removal of PhACs during nitrification/denitrification will be evaluated by collection of samples before and after nitrification/denitrification systems at one or more facilities. Samples also will be collected from several advanced wastewater treatment plants (e.g., the Livermore Advanced Wastewater Treatment Plant in Livermore, CA) and a water reuse system (i.e., Rio Hondo Basins in Los Angeles, CA). In addition, background samples will be collected from the Sacramento River Delta.

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APPENDIX A: SUMMARY OF ANALYTICAL METHODS

The methods used to analyze these compounds were adapeted primarily from those developed by Ternes et al. (1998).

Collection of Samples

The samples were collected in 10 or 15 liter LDPE bottles that had been washed with Micro detergent, methanol and deionized water. The samples were filtered with a 0.5 μm glass fiber filter and stored at 5°C until extraction, which occured within three days.

Analysis of Acidic Compounds

To analyze the samples for the acidic PhACs (i.e. ibuprofen, ketoprofen, indometacine, naproxen, acetylsalicyclic acid, and gemfibrozil), one liter of the sample was acidified to a pH of less than 2 with sulfuric acid. A 6 mL glass column packed with 250 mg endcapped C18 (Supelco) solid phase resin and 100 mg Lichrolut EN (Merck). The column was rinsed with 10 mL of methanol and 20 mL of deionized water that had been adjusted to a pH of less than 2 with sulfuric acid. The sample was then pulled through this column under a vacuum at a flow rate of 15 mL per minute. After all of the sample had passed through the column, the column was dried by pulling air through it for approximately five minutes. The column was then eluted with 5-10 mL of methanol. This extract was evaporated to dryness in a vacuum oven overnight at a temperature of 50 °C. Standards were also prepared and evaporated to dryness. The standards and the extracts from the samples were then redissolved in 1 mL of isooctane.

To derivatize the analytes prior to GC analysis, 250 μL of a diazomethane/diethylether mixture was added to each standard and sample extract. Ten μL of acetic acid/acetone (1:10) mixture was then added to each to remove any excess diazomethane. The samples and standards were then evaporated to near dryness using nitrogen. They were then redissolved in isooctane and spiked with hexachlorobenzene, the internal standard, and analyzed using a GCQ (Finnigan) equipped with a 30 m DB-5 column. The injection parameters are: 2 μL splitless, 270 °C. GC

temperature parameters are: 50 °C isothermal for 0.75 min, 20 °C/min to 120 °C, 2 °C/ min to 200 °C, 9 °C/min to 290 °C, isothermal for 4 min.

Analysis of Beta Blockers

The beta blockers (metoprolol, propranolol, and nadolol) were extracted and analyzed in a similar way to the acidic compounds. A one liter sample was extracted by SPE using a six mL glass column packed with 500 mg C18. The SPE was washed with 10 mL of methanol and 20 mL of deionized water prior to use. The sample was pulled through the column at a rate of approximately 15 mL per minute. Air was then pulled through the column in order to dry it for approximately five minutes to remove excess water. The column was eluted with 5 mL of methanol into a test tube. This extract was then dried in a vacuum oven at 50 °C. The dried extracts were redissolved in 1 mL of methanol which was transfered to 2 mL volumetric flasks. The test tubes were then rinsed with an additional 0.5 mL of methanol, which was added to the 2 mL volumetric flask. The methanol was blown to complete dryness with nitrogen. The dried extracts were redissolved in 250 μL of acetonitrile. The β-blockers were derivitized by first adding 50 μL of MSTFA to each flask. This was allowed to react at room temperature for 45 minutes. After this, the flasks were placed in a 60 °C oven for five minutes. Next, 10 μL of MBTFA was added to each and the flasks were placed in the oven for an additional five minutes. The extracts were then blown to near dryness with nitrogen. They were then redissolved in isooctane, spiked with hexachlorobenzene, and analyzed using the GC/MS/MS. The injection parameters are: 2 μL splitless, 230 °C. GC temperature parameters are: 50 °C isothermal for 2 min, 16 °C/min to 180 °C, 5 °C/ min to 290 °C, isothermal for 3 min.

Analysis of Neutral Compounds

The neutral compounds, carbamazepine and nabumetone, were analyzed in the same way as the β-blockers except that the derivitization steps were omitted since these compounds do not need

to be derivitized prior to their analysis. The injection parameters are: 2 μL splitless, 200 °C. GC temperature parameters are: 50 °C isothermal for 2 min, 16 °C/min to 180 °C, 4 °C/ min to 290 °C, isothermal for 7 min.

QA/QC

A total of four extractions of each sample were made. Two extractions were made of the unspiked sample, two of the spike recovery samples were analyzed after the addition of 1000 ng/L of the analytes of interest. In addition, a blank, which consisted of 1 L of deionized water was included on each date in which extractions were performed. The standard curve used to quantify the analytes consisted of seven points in the range of 37.5 μg/L to 1200 μg/L

OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Second Progress Report

January 15, 2001

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SUMMARY

During the second project period, we completed the literature review and focused our attention on development and testing of analytical methods to be used during the occurrence survey. A site visit was conducted by the Project Advisory Committee (PAC) on November 10, 2000. This progress report summarizes information presented during the site visit and research conducted during November and December 2000.

Efforts related to the literature survey were focused on analysis of antibiotics. Results of the literature review indicated that antibiotics are used in large quantities in association with production of cattle, chicken and swine. Antibiotics used for human therapy are usually different from those used in animal husbandry. Evaluation of antibiotic use patterns, fate and transport properties and reports of environmental occurrence resulted in the identification of five candidate compounds to be evaluated as part of the method development.

Method development for drugs other than antibiotics continued during this project period. Spike recovery samples, blanks and samples were collected and analyzed from three additional locations being considered for inclusion in the occurrence survey. Results indicated that the methods yield reproducible, accurate data in most samples. However, decreased sensitivity of the GC/MS system affected data for some of the compounds. In addition, we have not yet completed our evaluation of approaches for improving methods suggested by the PAC. Preliminary results indicate the presence of PhACs in secondary wastewater effluent and samples collected from sites throughout an engineered treatment wetland. PhACs detected in a groundwater infiltration basin apparently were removed during transit to a downgradient groundwater well.

Method development for antibiotics began during this project period. As a first step in this process, the antibiotics ciprofloxacin, sulfamethoxazole and sulfamethazine were analyzed by HPLC with fluorescence or UV detection.

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TABLES

FIGURES

PROGRESS THIS PERIOD

TASK 1: LITERATURE REVIEW

The objective of this task is to identify compounds to be studied during the occurrence survey. The selection criteria for compounds in the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and availability of suitable analytical methods. Progress on the literature review was presented in the first progress report and during the site visit on November 10, 2000. The Project Advisory Committee (PAC) made suggestions for improving the literature review and stated that the review was complete for the current purpose of the project. Progress made during this project period is described below along with a brief summary of progress from the first project period.

Sub-Task 1A: Drugs Used in Human Therapy

In the first progress report, we reviewed data on prescription drugs likely to be present in wastewater effluent in the United States. Using data on the number of prescriptions for the top 200 drugs and information on prescription formulations and metabolism we estimated ranges of concentrations of 136 drugs expected to be present in wastewater effluent. In addition, we reviewed published occurrence data to identify other compounds of interest that did not appear among the common prescription drugs. A total of eleven drugs were identified for further consideration.

During the current project period no activity was conducted in association with this sub-task.

Sub-Task 1B: Drugs Used in Animal Husbandry

Antibiotics are used in livestock both therapeutically to treat diseases and sub-therapeutically as feed additives to promote growth. We have assumed that other drugs used in animal husbandry are insignificant compared to the antibiotics. Quantification of antibiotic use in livestock and aquaculture is challenging because drug use is often not documented and some drugs can be purchased from distributors of animal feed without reporting requirements. Furthermore, the formulae of antibiotics employed as feed additives are often not revealed by the feed manufacturers.

Since published data on the amount of antibiotics used in agriculture are not available, predictions were made based upon annual animal feed consumption and the recommended doses of antibiotics added in feed. Although antibiotics are also given to animals to treat disease, it is difficult to estimate the frequency and quantities of antibiotics use for these purposes. It is assumed that antibiotics used as feed additives to promote animal weight gain and feed efficiency account for the majority of antibiotic consumption in livestock because of their continuing usage. For instance, it was estimated that 8.2 million kilograms of antibiotics were used in major species of food animals in 1985; 90% of that was used for sub-therapeutic dose application (IOM, 1989).

Antibiotics are also used in aquaculture. In the United States, aquaculture production is relatively small compared to livestock production and is concentrated in the coasts and estuaries of a few states such as Washington and Mississippi. Antibiotic use in aquaculture could result in localized water pollution. Because of the relatively small quantities of use and localized contamination, antibiotics used in aquaculture are not included in our estimation.

Our predictions are based on an estimate of the mass of each antibiotic consumed in promoting livestock growth, which is converted into concentrations of antibiotics in the liquid waste generated by animal feeding operations (AFOs). The prediction methods are described in the following paragraphs.

Masses of Antibiotics Used for Promoting Weight Gain and Feed Efficiency

The mass of each antibiotic used to promote animal growth is calculated in two ways: (1) the annual consumption of each antibiotic per animal, and (2) the annual consumption of each antibiotic by all animal species. The first approach provides information needed to calculate antibiotic loading in raw liquid animal waste at a feeding operation. The second approach provides information on the total consumption for each antibiotic.

The annual consumption of an antibiotic per animal was calculated by multiplying the quantities of feed consumed per animal by the concentration of antibiotic in feed. The amount of feed consumed per animal per year can be calculated by multiplying the grain used per "grain consuming animal unit" (1.87×10^3 kg/year, Feed Yearbook, USDA, 2000) by the equivalency factors for animal species. The "animal unit" (AU) is a unit of measurement used to standardize sizes of animal feeding operations (AFOs). The number of AUs is determined by multiplying

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the number of animals of each species (other than poultry) by an equivalency factor. Species equivalency factors are 1.0 for slaughter/feeder cattle, 1.4 for mature dairy cattle and 0.4 for swine (>55lbs.).

The amount of feed consumed by poultry (broilers and layers) was calculated in a different way. (A broiler is raised for consumption while a layer is raised for egg production.) A broiler has a 6-7 week lifespan and consumes approximately 8 lbs (i.e., 3.6 kg) of feed during its lifespan. In addition, there is a typical 2-week downtime between two crops of broilers in poultry operations. It is assumed that there are 6.5 crops of broilers per year at a poultry operation. This leads to approximately 52 lbs (i.e., 23.6 kg) of feed consumption per year per broiler space. Information was also gathered on the amount of feed consumed by a layer. A layer has a typical 65-week lifespan and consumes approximately 0.23 lbs (i.e., 0.10 kg) of feed per day. This figure can be converted to a feed consumption rate of 38.1 kg/year-layer.

The amount of feed consumed per animal is then multiplied by the concentration of antibiotic in feed to obtain the mass of each antibiotic consumed per animal per year. Because information on the feed formulation and antibiotic additives are not publicly accessible and are difficult to obtain, the recommended dosages of antibiotics listed in the Feed Additive Compendium (2000) were used. The Feed Additive Compendium is updated on a yearly basis and information for year 2000 was used for calculation. The calculated results are listed in Table 1. When a range of dosages is recommended, calculations were performed with the minimum and maximum dosages, respectively.

Table 1

Antibiotic (in feed) consumption per year per animal (g/year-animal).

Antibiotic	Category	Broiler		Layer		Cattle		Swine	
		min.	max.	min.	max.	min.	max.	min.	max.
Ampraymycin	aminoglycoside	NA		NA		NA		33.7	67.4
Arsanilic Acid	other	2.1		2.1		NA		33.7	67.4
Bacitracin MD*	polypeptide	0.1	1.2	0.1	1.2	NA		33.7	67.4
Bacitracin Zinc	polypeptide	0.1	1.2	0.2	0.6	65.5	131.0	15.0	30.0
Bambermycins	aminoglycoside	<0.1	<0.1	NA		1.9	74.9	1.5	3.0
Carbadox	other	NA		NA		NA		7.5	18.7
Chlorotetracycline	tetracycline	0.2	1.2	0.2	1.2	131.0		7.5	18.7
Lincomycin	aminoglycoside	<0.1	0.1	<0.1	<0.1	NA		15.0	
Oxytetracycline	tetracycline	1.2	2.4	1.2	2.4	140.4		7.5	37.4
Penicillin	β-lactam	0.1	1.2	<0.1	1.2	NA		7.5	37.4
Roxarsone	other	0.5	1.1	0.5	1.1	NA		17.0	25.5
Tiamulin	other	NA		NA		NA		7.5	
Sulfamethazine	sulfonamide	NA		NA		NA		37.4	
Tylosin	macrolide	0.1	1.2	0.1	1.2	NA		37.4	
Virginiamycin	macrolide	0.1		<0.1		20.6	30.0	3.7	7.5

NA = Not Applicable; *Bacitracin Methylene Disalicylate

To calculate the masses of antibiotics consumed by all animals, information on the total annual feed consumption by all livestock in the United States were needed. The total feed consumption in year 2000 was calculated by multiplying the number of "grain consuming animal units" (30.1, 23.0 and 21.1 million units for poultry, swine and cattle respectively) by the amount of grain consumed per "grain consuming animal unit" (i.e., 1.87×10^3 kg) (Feed Yearbook, USDA, 2000). The mass of antibiotics consumed was then calculated by multiplying the total mass of feed consumed by the recommended dosages of antibiotics.

It is also necessary to consider the fact that only one or two of the recommended antibiotics are used in feed at any time and the selection of antibiotics is dependent upon the feed manufactures. Information regarding the selection of antibiotics is difficult to obtain, thus we assumed that each antibiotic was added in approximately 50% of the consumed feed. The results are listed in Table 2. Combining the amounts of antibiotics consumed by all animal species yields the total mass of consumption for each antibiotic. These calculations are only rough

Table 2

Antibiotic consumption rates (kg/year) for poultry, cattle and swine in the United States.

Table 3. Antibiotic Consumption Per Year (10^3kg/year).

Antibiotic	Category	Poultry		Cattle		Swine		Total	
		min.	max.	min.	max.	min.	max.	min.	max.
Bacitracin*	polypeptide	225	2817	711	1422	1399	2799	2336	7038
Oxytetracycline	tetracycline	1409	2817	1523		215	1076	3147	5417
Arsanilic Acid	other	2536	2536	NA		969	1938	3504	4473
Chlorotetracycline	tetracycline	282	1409	1422		215	538	1919	3369
Penicillin	β-lactam	56	1409	NA		215	1076	272	2485
Tylosin	macrolide	113	1409	NA		1076		1189	2485
Roxarsone	other	640	1279	NA		489	732	1128	2011
Ampraymycin	aminoglycoside	NA		NA		969	1938	969	1938
Sulfamethazine	sulfonamide	NA		NA		1076		1076	
Bambermycin	aminoglycoside	28	56	20	812	43	86	92	955
Virginiamycin	macrolide	6	141	223	325	108	215	337	681
Lincomycin	aminoglycoside	56	113	NA		431		487	543
Carbadox	other	NA		NA		215	538	215	538
Tiamulin	other	NA		NA		215		215	

NA = Not Applicable; *Bacitracin Zinc and Bacitracin Methylene Disalicylate
Note: It is assumed that each antibiotic accounts for 50% of use in feed.

approximations for the quantities of growth-promoting antibiotics; however they allow us to identify "high-use" veterinary antibiotics.

As shown in Table 1 and 2, there are fourteen antibiotics commonly used for promoting livestock growth. Considerable differences in antibiotics exist among different animal species. For example, only 4 antibiotics are used in cattle while 14 are used in swine. Among the 14 antibiotics, bacitracin, oxytetracycline, chlorotetracycline, bambermycin and virginiamycin were used among all animal species. According to our estimation, bacitracin, oxytetracycline, and arsanilic acid are the top three most common growth-promoting antibiotics.

Concentrations of Antibiotics in the CAFO Liquid Waste

There were approximately 450,000 animal feeding operations (AFOs) throughout the United States, ranging from small livestock production facilities with a few animals to the large

and geographically concentrated facilities generating a mass of animal waste equivalent to the waste produced by humans in a medium-sized city. Under Section 502 of the Clean Water Act, confined animal feeding operations (CAFOs) are point sources and must apply for a National Pollutant Discharge Elimination System (NPDES) permit. Of approximately 6,600 CAFOs, fewer than a quarter have NPDES permits (EPA, 1996). CAFO liquid waste usually undergoes some type of pretreatment prior to land application. In some cases, it is combined with other wastewater for further treatment before discharge.

The wastewater volume from CAFOs is comprised of (i) the waste quantities being generated by animals (also called pollutant load), 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).

Water use varies considerably from one operation to another, depending on such factors as type of buildings, methods of flushing, and type of management. In general, Overcash et al. (1983) suggest that the volume of flushwater used in swine and poultry facilities can be estimated by calculating approximately 2 gallons per minute (gpm) of water per 100 pounds (lbs) of animal weight for the flushing period. For cattle and dairy facilities, 40 to 50 gallons per cow per day are assumed in flushing requirements for freestall alleys. The frequency of daily flushing will determine the total volume of flushwater used.

Using the above information and assuming an average poultry weight of 8 lbs and an average swine weight of 60 lbs, the flushwater flows computed for CAFOs (2500 head) of poultry and swine are approximately 400 and 3000 gallons per minute respectively. It is also assumed that one flushing period of 30 minutes is employed per day; this leads to 4.54×10^4 and 3.41×10^5 L/day of flushwater for poultry and swine CAFOs respectively. The volumes of animal wastes can also be estimated based upon species populations (The Agricultural Waste Management Field Handbook, USDA, 1992). It was found that animal waste volume was insignificant compared to the flushwater volume, thus flushwater volume roughly represents the total volume of the CAFO wastewater. For a typical cattle CAFO (2500 head), 50 gallons of flushwater was assumed per cow per day, leading to a flushwater flow of 4.73×10^5 L/day.

The estimated concentrations of antibiotics in raw CAFO wastewater are obtained by dividing the amounts of antibiotics consumed per animal per day (Table 1) by the wastewater

volume generated per animal per day. The calculated results are listed in Table 3. The calculations also assume 80% of antibiotics are excreted without undergoing metabolism. The estimated concentrations ranged from 0.2 µg/L to greater than 1.6 mg/L. There are considerable differences in antibiotics among different animal species.

Antibiotic compounds in municipal wastewater are primarily those used in human therapy. Estimates of the concentrations of antibiotics in municipal wastewater were described in the first progress report. A summary of the results of antibiotics is included in Table 4. The estimated concentrations of antibiotics in untreated municipal wastewater range from 2.5 ng/L to approximately 38,000 ng/L. Among the 19 antibiotics, there were 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 four others.

Table 3

Estimated concentrations of antibiotics in CAFO wastewater (µg/L)

Table 4. Estimated Concentrations of Antibiotics in CAFOs Wastewater (µg/L).

Antibiotic	Broiler		Layer		Cattle		Swine	
	min.	max.	min.	max.	min.	max.	min.	max.
Ampraymycin	NA		NA		NA		542	1,084
Arsanilic Acid	256		256		NA		542	1,084
Bacitracin MD*	11	142	11	142	NA		542	1,084
Bacitracin Zinc	11	142	28	71	759	1,517	241	482
Bambermycins	3	6	0.2		22	867	24	48
Carbadox	NA		NA		NA		120	301
Chlorotetracycline	28	142	28	142	1,517		120	301
Lincomycin	6	11	0.5	0.5	NA		241	241
Oxytetracycline	142	285	142	285	1,626		120	602
Penicillin	7	142	7	142	NA		120	602
Roxarsone	65	129	65	129	NA		273	409
Tiamulin	NA		NA		NA		120	
Sulfamethazine	NA		NA		NA		602	
Tylosin	11	142	11	142	NA		602	
Virginiamycin	14		0.6		238	347	60	120

CAFO = Confined Animal Feeding Operation

NA = Not Applicable; *Bacitracin Methylene Disalicylate

Note: Assuming 80% of unmetabolized excretion and 0% of treatment removal

Table 4
Geometric mean for range of estimated concentrations of antibiotics in municipal wastewater
in the United States (from the first progress report).

Name	Category	Excluding Metabolism		Including Metabolism	
		Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)	Predicted Wastewater Conc. (1) (ng/L)	Predicted Range (ng/L)
1 amoxicillin	β-lactam	27,000	19,000 to 38,000	16,000	11,000 to 23,000
2 cephalexin	β-lactam	14,000	6,800 to 27,000	12,000	6,100 to 24,000
3 azithromycin	macrolide	9,200	1,400 to 61,000		
4 penicillin	β-lactam	4,000	3,100 to 5,200		
5 sulfamethoxazole	sulfonamide	3,800	1,700 to 8,400	3,200	1,400 to 7,200
6 ciprofloxacin	fluoroquinolone	3,100	2,200 to 4,300	1,400	970 to 1,900
7 mupirocin	other	2,800	2,000 to 4,000		
8 clarithromycin	macrolide	2,800	2,000 to 3,900	680	390 to 1,200
9 trimethoprim	other	2,200	670 to 7,100	1,500	450 to 4,700
10 clavulanic acid	β-lactamase inhibitor	2,100	2,100	680	680
11 cefprozil	β-lactam	1,700	1,200 to 2,400	1,000	710 to 1,400
12 erythromycin	macrolide	1,500	1,500	75	75
13 tetracycline	tetracycline	1,200	830 to 1,700		
14 cefuroxime	β-lactam	900	450 to 1,800	900	450 to 1,800
15 loracarbef	β-lactam	480	340 to 680		
16 levofloxacin	fluoroquinolone	400	280 to 570	350	250 to 490
17 nitrofurantoin	nitrofuran	350	250 to 500	130	90 to 180
18 neomycin	aminoglycoside	7.6	7.6		
19 tobramycin	aminoglycoside	3.9	2.5 to 6.1		

Comparing tables 1 and 2 with table 4, it is evident that antibiotics used in livestock are different from the ones used in human therapy. This approach has been adopted to reduce the risks of development of resistant bacteria in animals, which may in turn be passed on to humans, thus diminishing the effectiveness of antibiotics in treatment of human diseases.

To assist comparisons among all antibiotics, common antibiotics currently in use for human therapy or veterinary applications are listed in Table 5. The antibiotics are listed according to their applications and their structural classes. For instance, tetracyclines are much more important in agricultural applications than for human therapy. Barcitracin, tylosin and virginiamycin are used almost exclusively for agricultural purposes.

In general, different compounds of β-lactams, macrolides, fluoroquinolones, and sulfonamides are used separately for humans and for food animals (see Table 5). For instance, fluoroquinolones (e.g., ciprofloxacin) play an important role in treating human diseases; many of the new antibiotics being used to fight antibiotic-resistant bacteria are members of fluoroquinolones. Other fluoroquinolones such as enrofloxacin and sarafloxacin were developed to prevent and cure diseases in poultry. However, due to the concern of fluoroquinolone residues in meat products, USDA has recently issued a ban on the use of enrofloxacin and sarafloxacin in poultry industry (C&EN, 2000). For the early-developed sulfonamide antibiotics, only sulfamethazine is used as a feed additive. A variety of sulfonamide antibiotics are used in livestock for disease treatment, burt at much lower application rates.

The U.S. Geological Survey presently is conducting an occurrence survey of PhACs and related compounds. The primary focus of the USGS study is to measure concentrations of contaminants in surface waters. Although their results have not yet published, information regarding their target analytes and samples locations are available on their website (http://toxics.usgs.gov/regional/contaminants.html). The antibiotic target analytes in the USGS study are also indicated in Table 5 for comparisons.

Table 5
Comparisons of common antibiotics with respect to applications and structural classes.

Compound	Human Therap.[a]	Agric. Feed	Agric. Ailment	USGS Study[b]	Compound	Human Therap.[a]	Agric. Feed	Agric. Ailment	USGS Study[b]
β-lactam:					*aminoglycoside:*				
amoxicillin	X		X	X	neomycin	X		X	
cephalexin	X				tobramycin	X			
penicillin	X	X	X		apramycin		X	X	
cefprozil	X				bambermycin		X	X	
cefuroxime	X				lincomycin		X	X	X
loracarbef	X"				efrotomycin			X	
ampicillin	X"		X		gentamycin			X	
					streptomycin			X	
macrolide:					*sulfonamide:*				
azithromycin	X				sulfamethoxazole*	X			X
clarithromycin	X				sulfamethazine		X	X	X
erythromycin	X		X	X	sulfachloropyridazine			X	X
oleandomycin			X		sulfadimethoxine			X	X
roxithromycin	X"			X	sulfaethoxypyridazine			X	
spectinomycin			X	X	sulfamerazine			X	X
tilmicosin			X		sulfathiazole			X	X
tylosin		X	X	X	sulfamethiazole			X	X
virginiamycin		X	X						
fluoroquinolone:					*tetracycline:*				
ciprofloxacin	X			X	tetracycline	X"		X	X
levofloxacin	X				doxycycline	X"		X	X
norfloxacin	X"			X	chlortetracycline		X	X	X
enrofloxacin			X	X	oxytetracycline		X	X	X
sarafloxacin			X	X					
β-lactamase inhibitor:					*other:*				
clavulanic acid	X"				trimethoprim	X			X
					mupirocin	X			
					barcitracin MD*		X	X	
					barcitracin zinc		X	X	
					arsanilic acid		X	X	
					carbodox		X	X	X
					roxarsone		X	X	X
					ivermectin			X	X

Note: *barcitracin methylene disalicylate

Antiboitcs are indicated according to their applications in human therapy, livestock feed additive, or livestock disease treatment.

a: Rank by the Rxlist (1999), www.rxlist.com.
   X = rank among the top 200 prescription drugs
   X" = rank below the top 200 prescription drugs
b: USGS's current study on the occurrence survey of antibiotics.

Occurrence data for antibiotics are reviewed and summarized in Table 6. Most previous studies were conducted in Europe where antibiotic use could be different from that of the United States. However, the previous studies provide guidance for identifying the classes of antibiotics that are more persistent in the environment.

In general, β-lactam antibiotics were not detected in most environmental waters. The β-lactam compounds are readily hydrolyzed in the environment (Hou and Poole, 1969), and thus are less likely to be persistent. Other classes such as fluoroquinolones, macrolides, sulfonamides and tetracyclines have been detected. 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, and will be published in a forthcoming book. Occurrence data are not available for aminoglycoside antibiotics and most of the other types of antibiotics.

Table 6

Summary of occurrence data for antibiotics.

N.D. indicates compound not detected. >50%=compound detected in >50% of samples;

<50%=compound detected in <50% of samples.

Compound	Wastewater Effluent					Surface Water					Ground Water		
	N.D.	<50%	ng/L	>50%	ng/L	N.D.	<50%	ng/L	>50%	ng/L	N.D.	<50%	>50%
β-Lactams:													
Cloxacillin	2					2					2		
Dicloxacillin	2					2					2		
Methicillin	2					2					2		
Nafcillin	2					2					2		
Oxacillin	2					2					2		
Penicillin G	2					2					2		
Penicillin V	2					2					2		
Macrolides:													
Clarithromycin				2, 5	90-240		2	150			2		
Erythromycin-H$_2$O				2, 5	110-5100				2	630	2		
Roxithromycin				2, 5	20-800		2	200			2		
Quinolones:													
Ciprofloxacin				3	3000-87000						2		
Sulfonamides:													
Sulfadiazine				1	26-81		1	7					
Sulfamethazine	2, 5					2					2		
Sulfamethizole				1	6	1							
Sulfamethoxazole				1,2,5	300-2000				1, 2	30-140	2		
Tetracyclines:				4	1000-7x10^5		4	<1000					
Chlorotetracycline	2					2					2		
Doxycycline	2					2					2		
Oxytetracycline	2					2					2		
Tetracycline	2					2					2		
Others:													
Chloramphenicol	5	2	560				2	60			2		
Trimethoprim				2, 5	40-620		2	90			2		

References:
(1) Hartig et al., (1999)
(2) Hirsch et al., (1999)
(3) Hartmann et al., (1998). Concentrations were measured in hospital wastewater.
(4) Meyer et al., (2000) Concentrations were measured in liquid hog lagoon waste and in groundwater.
(5) McArdell et al., (1999)

A preliminary review on the fate of antibiotics is summarized in Table 7. Among the six classes of antibiotics, strong sorption to soils and sediments was observed with tetracyclines throughout a range of solution pH values (Pouliquen and Lebris 1996; Rabolle and Spliid, 2000) while weaker sorption was observed for other antibiotics. The stronger the adsorption of antibiotics to sediments, the less likely the compounds will be present as contaminants in aqueous solutions.

Hydrolysis is an important degradation pathway for organic pollutants in aquatic environments. β-lactams, macrolides and sulfonamides are susceptible to hydrolysis; however hydrolysis of sulfonamides under typical environmental conditions is extremely slow and can be considered irrelevant. The β-lactams generally undergo hydrolysis fairly quickly under environmental conditions (Hou and Poole, 1969). Macrolides are only susceptible to hydrolysis under low pH conditions. Photodegradation is another abiotic transformation that can affect organic pollutant persistence in the surface layers of water bodies that receives appreciable amount of sunlight. Only quinolones (Torniainen et al., 1996) and tetracyclines (Davies et al., 1979) are susceptible to photodegradation.

Studies of biodegradation of antibiotics in soils have been reported. Despite the fact that antibiotics may inhibit microbial activities, some degree of degradation by indigenous microbial population have been reported for antibiotics of all six classes (Gavalchin and Katz, 1994; Weerasinghe and Towner, 1997; Marengo et al., 1997; Al-Almad et al., 1999). Based upon the previous studies, β-lactams, aminoglycosides and some macrolides degrade to a greater extent than quinolones, sulfonamides and tetracyclines.

Table 7. Summary of environmental fate of antibiotics.

Antibiotics	Hydrolysis	Photodegradation	Biodegradation	Adsorption
Aminoglycosides			Y	Low
β-Lactams	Y	N	Y	Low
Macrolides	Y*	N	Moderate	Low
Quinolones	N	Y	Slow	Moderate
Sulfonamides	Very slow**	N	Slow	Low
Tetracyclines	N	Y	Slow	Strong

* Macrolides may hydrolyze under acidic pH ranges.
** Sulfonamide hydrolysis is extremely slow under typical environmental pHs and temperatures.

Since the antibiotic use patterns change rapidly, and it is time consuming and expensive to measure all the antibiotics potentially present in the aquatic environment, it is necessary to identify compounds that can be used as indicators of antibiotic contamination in the future occurrence survey. To select these antibiotic indicators, the following criteria were considered: compound structural class, quantities in use, occurrence data, and environmental fate. Members of the same class of antibiotics have similar structures, act by similar mechanisms, and thus share similar fate and transport processes. In addition, analytical methods are usually applicable for compounds within the same class with minor modifications. Thus, the compound selection covered a range of structural classes, rather than only one class.

In addition, selection of antibiotics was conducted for human antibiotics and veterinary antibiotics separately. For municipal wastewater effluent and surface water, antibiotics that are important in human therapy need to be considered. For agricultural runoff and surface water receiving significant agricultural input, veterinary antibiotics should be considered.

As illustrated in Figure 1, antibiotics used in the greatest quantities for each structural class were selected; antibiotics that were used less commonly were eliminated from the indicator candidate list. This is a reasonable approach since all structural classes play an important role in either human therapy or veterinary applications (i.e., no particular structural class dominates the antibiotic use). Next, we eliminated the antibiotics that were not detected in the aquatic environment in previous studies. Furthermore, antibiotics that degrade fairly quickly in the environment (e.g., aminoglycosides and polypeptides) and adsorb strongly to soil and sediments (i.e., tetracyclines) are unlikely to be present as water contaminants and thus were also eliminated. As a result, azithromycin, sulfamethoxazole and ciprofloxacin (representing macrolides, sulfonamides, and fluoroquinolones) were selected as indicator antibiotics that primarily originate from human therapy. Tylosin and sulfamethazine (representing macrolides and sulfonamides) are the two selected indicators representing antibiotic contaminants primarily originate from veterinary applications. The structures of these antibiotics are shown in Figure 2.

Among the five antibiotics, ciprofloxacin, sulfamethazine and sulfamethoxazole are considered to be more persistent than azitrhomycin and tylosin since macrolides are more susceptible to biodegradation than fluoroquinolones and sulfonamides. Therefore, our method development and analysis will first focus on ciprofloxacin, sulfamethazine and

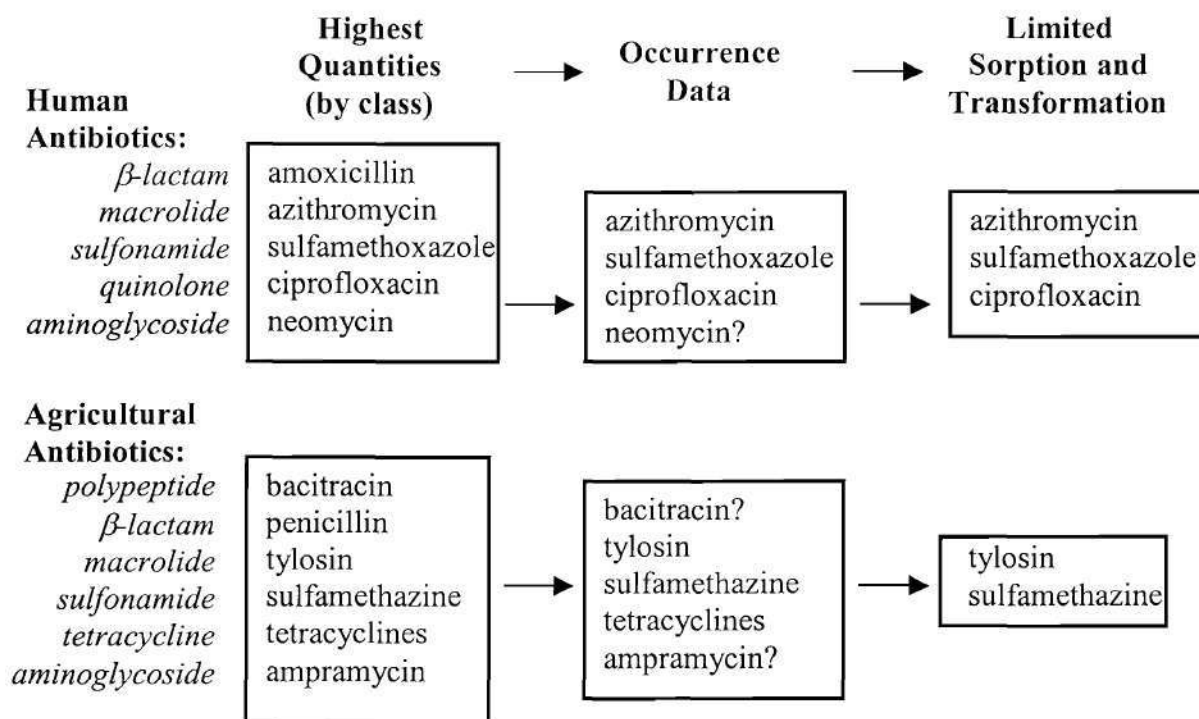sulfamethoxazole. Time permitting, methods will be developed for the two macrolides after the other compounds.
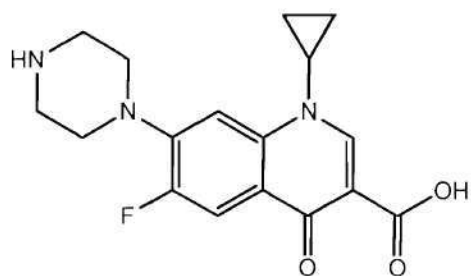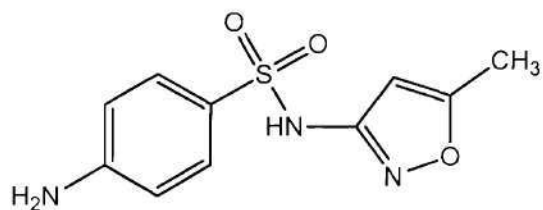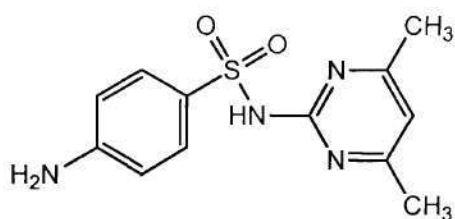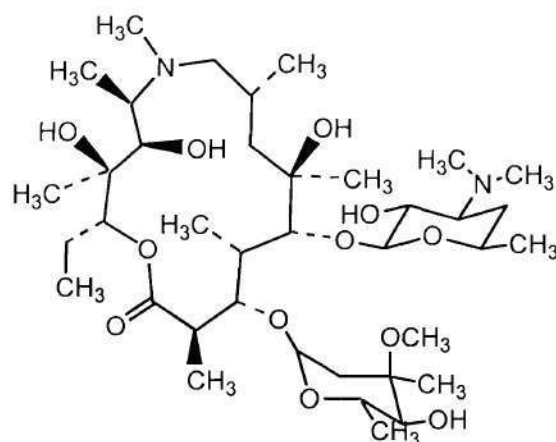

Figure 1. Selection of antibiotics for the occurrence survey.

Ciprofloxacin
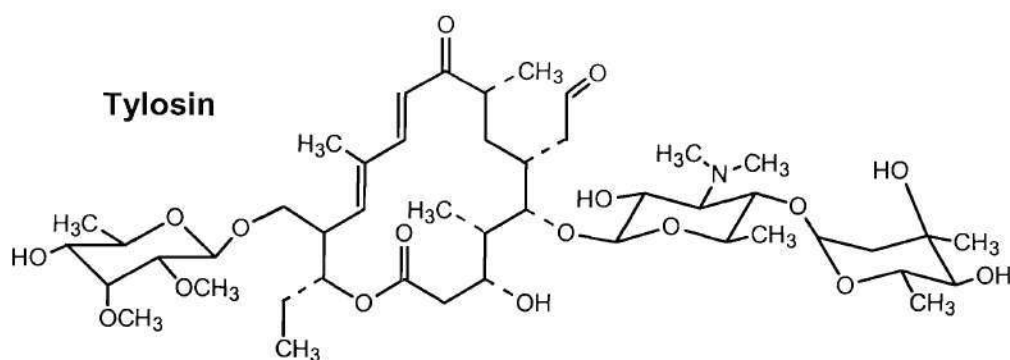
Sulfamethoxazole

Sulfamethazine

Azithromycin

Tylosin

Figure 2. Structures of the antibiotics selected for further study.

Analytical methods for quantifying antibiotics have been reported by previous researchers (e.g., Hartmann, et al., 1998; Hartig, et al., 1999). Many methods were developed for quantifying antibiotic residues in animal food products and for analysis and diagnosis in human. Some methods have been modified and applied in analysis of environmental water samples. A number of recent review articles on the analytical methods for antibiotics are also available (e.g., Kanfer, et al., 1998; Niessen, 1998; Belal, et al., 1999). Since these review articles already provide relatively thorough information, our literature review does not include a detailed review on the analytical methods.

A summary of available methods for analyzing antibiotics is shown in Table 7. All antibiotics can be analyzed by high performance liquid chromatography (HPLC) with UV detection. Fluoroquinolones can be detected by fluorescence detection (HPLC/FLD) with higher selectivity and sensitivity (e.g., Hartman et al., 1998). Liquid chromatography with mass spectrometry (LC/MS) and tandem mass spectrometry (LC/MS/MS) is thus far the most accurate method for quantifying low concentrations of antibiotics in complex matrices. Published LC/MS methods are available for all classes of antibiotics. Due to the low volatility and thermal instability of antibiotics, gas chromatography is generally not suitable. Among antibiotics, sulfonamides can be analyzed by GC/MS after derivatization. Immunoassays such as enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay are available for many aminoglycoside, β-lactam, macrolide and sulfonamide antibiotics including sulfamethazine and tylosin. Immunoassys have the advantages of simple procedures and higher sensitivity, and can serve as useful screening tools.

The literature review also indicates that extraction and detection methods for macrolides are considerably different from those used for fluoroquinolones and sulfonamides due to their much higher polarity. Analysis of all three classes of antibiotics will complicate the analytical procedures, resulting in longer run times. Therefore, we focused our method development for ciprofloxacin, sulfamethazine and sulfamethoxazole.

Table 7

Summary of analytical methods for antibiotic compounds.

Compound	HPLC-FLD	HPLC-UV	GC/MS	GC/MS/MS	LC/MS	LC/MS/MS	ELISA	Radioimmu.
Aminoglycosides		Y			Y			Y
β-Lactams		Y			Y	Y		Y
Macrolides[a]		Y			Y	Y	Y	Y
Quinolones[b]	Y	Y	Y*		Y	Y		
Sulfonamides		Y	Y	Y	Y	Y	Y	Y
Tetracyclines		Y			Y		Y	Y
Others:								
Chloramphenicol		Y			Y	Y		
Trimethoprim		Y			Y	Y		

*only for a few quinolone compounds

Review articles on analytical methods: a: Kanfer, et al., 1998; b:Belal, et al., 1999

TASK 2: ANALYTICAL METHOD DEVELOPMENT AND TESTING

The objective of this task is to identify and test analytical methods that will be used during the occurrence survey. As part of the task, analytical methods for extraction, cleanup and derivitization will be tested by adding drugs to deionized water and to samples collected from sites being considered for inclusion in the occurrence survey. Analysis of antibiotics is included as a separate task from the other pharmaceutically active compounds (PhACs) because the analytical methods for antibiotics (e.g., liquid chromatography and immunoassays) are fundamentally different from the gas chromatography methods used for the other compounds.

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the first progress report, we described our preliminary efforts to develop suitable analytical methods for eleven compounds identified as part of the literature survey. We used solid phase extraction followed by derivitization and gas chromatography/tandem mass spectrometry (GC/MS/MS) to quantify pharmaceuticals in deionized water and in samples collected from several candidate sampling sites. To quantify recoveries, all samples also were analyzed after addition of known quantities of analytes.

During the current project period, we continued our efforts to develop and test the analytical methods by collecting samples from three additional candidate sites. Data are included in Appendix A. Furthermore, we began to pursue several suggestions made by PAC members to expand the number of compounds analyzed and to modify the analytical methods to improve recoveries. Because the suggestions from the PAC were made late in the project period, we have not completed our evaluation of these modifications. Results of our analysis will be reported in the next progress report.

Analytical Methods: Protocols for extraction, derivitization and analysis of compounds were reported in Appendix A of the first progress report. During the current project period, we used these methods to analyze samples from several additional locations. Suggested changes to these methods are being evaluated and will be employed in future sampling.

As suggested by members of the PAC, we also began an evaluation of the possibility of increasing the number of compounds to be analyzed. We have purchased several potentially important drugs that were not included in our initial selection and will report on the ability of existing analytical methods to detect these compounds in the next progress report. Compounds to be tested include: allopurinol, atenolol, bupropion, caffeine, carisoprodol, cimetidine, diltiazem, gabapentin, hydrochlorothiazide, ipratropium, metformin, phenytoin, ranitidine, triamterene, valproic acid and verapamil.

Quality Assurance/Quality Control (QA/QC): Prior to our meeting with the PAC, we had developed a rigorous QA/QC plan designed to identify problems with analytical methods encountered during method development. This approach involved the time consuming analysis of duplicate samples and duplicate spike recovery samples from all sites. It was our intention to use this rigorous approach until we gained enough experience with the techniques to reduce the number of QA/QC samples. During the PAC meeting, we discussed modifications to the QA/QC plan that would streamline the analysis by reducing the number of samples to be analyzed. As a result, we are currently developing a new QA/QC plan, which will be described in a future progress report.

To evaluate the reproducibility of the analytical methods, duplicate aliquots of each sample were always extracted. Each extract was subject to blow down, derivitization, solvent

transfer and gas chromatographic analysis in the same sample batch. Sample positions were randomized throughout the analysis to eliminate bias. Results generally indicate good reproducibility between duplicate samples (Figure 3). The greatest variability was observed for propranolol and metoprolol, which are β-blockers that are dervitized with MSTFA. In cases where metoprolol and propranolol spiked samples exhibited poor reproducibility, one sample usually exhibited recoveries between 60% and 100% while the other was considerably lower. We have noticed that traces of water in the samples lead to lower derivitization efficiency. To ensure consistent data for these two compounds, we extend the length of the drying step in future analyses. Excluding metoprolol and propranolol, the duplicate samples exhibited a mean of 26% error.
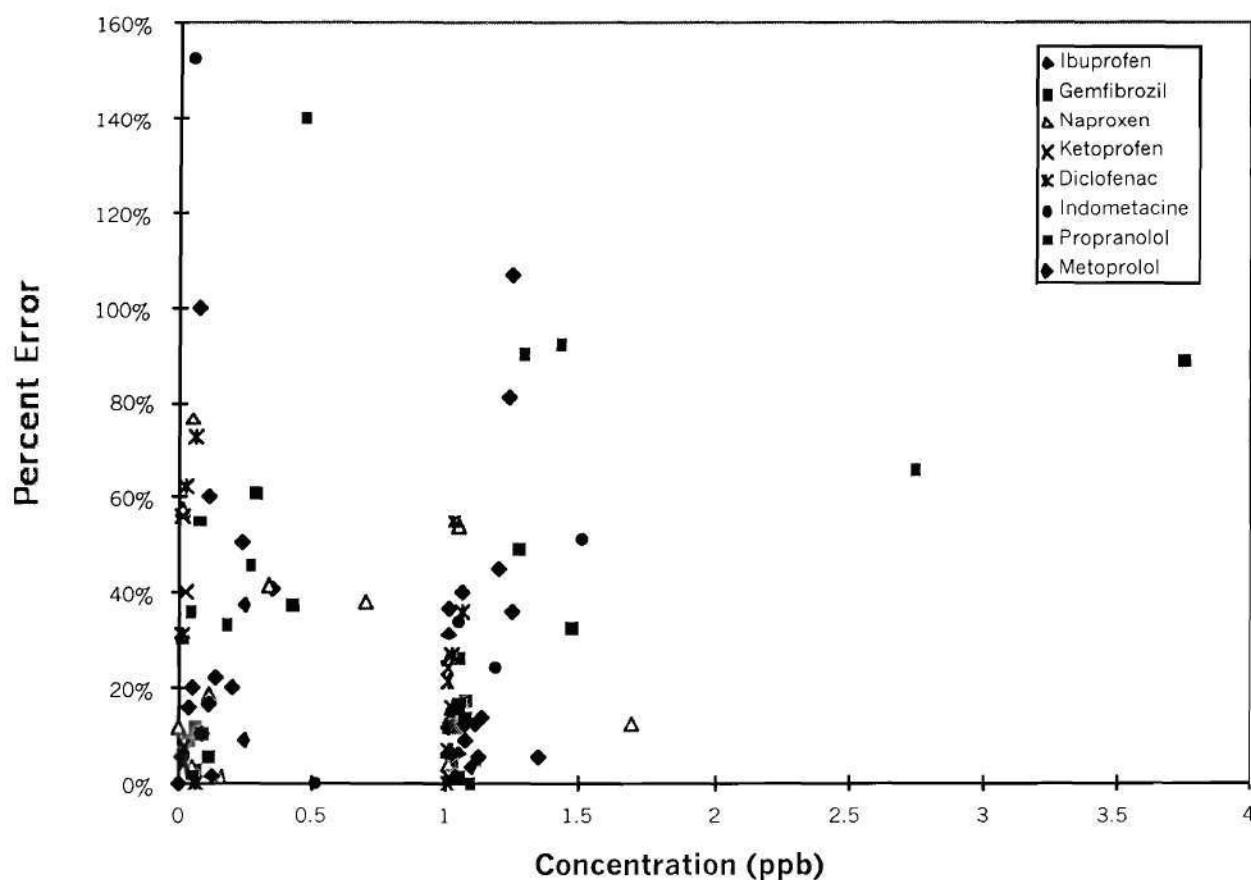
Figure 3. Percent error for spiked and unspiked duplicate samples as a function of measured concentration.

To assess the potential for sample contamination, one deionized water blank sample was analyzed with each sample batch. The blank was extracted and derivitized with the other samples. Results indicate that contamination is not a significant issue. In a total of 10 complete analyses of blank samples conducted during the first two project periods, propranolol and naproxen were detected once and indometacine was detected twice. In all cases, the concentrations detected were less 20 ng/L. We believe that these false positives are attributable to sample carryover or instrument noise. Although we have not completed our determination of detection limits, we believe that the false positives are below the limit of quantification.

To evaluate analyte recovery, duplicate aliquots of each sample were amended with approximately 1 µg/L of each analyte prior to analysis. Results of the spike recovery analyses indicate improvement in recoveries since the first project period for some of the compounds. Unfortunately, intermittent problems with our GC/MS system occurred during this project period. As discussed in the following paragraphs, these problems resulted in our inability to analyze several of the compounds in certain samples. We are currently working with the instrument's manufacturer (i.e., Thermoquest/Finnigan) to correct this problem.

Ibuprofen (Figure 4) exhibited recoveries ranging from approximately 40 to 140%. No trends were evident in the data.

Naproxen (Figure 5) exhibited recoveries ranging from approximately 30 to 90%. During the current project period, recoveries consistently ranged from 40 to 60%.
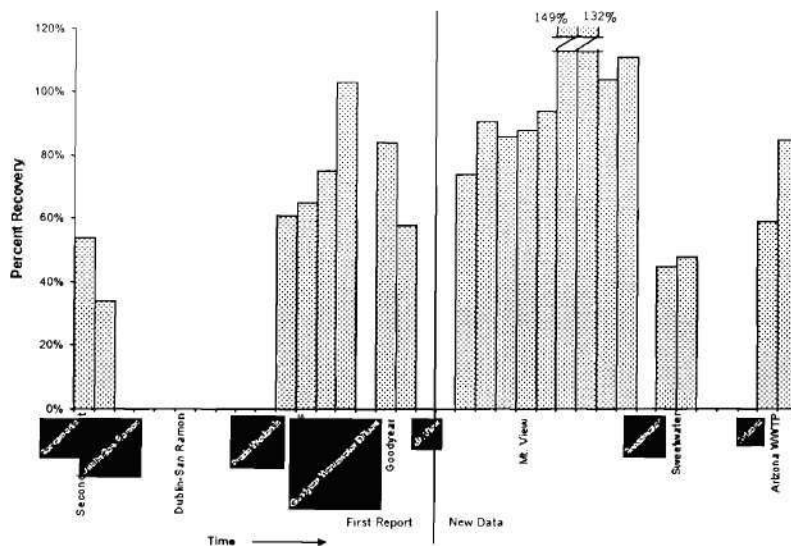
Figure 4. Ibuprofen recoveries as a function of time during the first two project periods. Recovery samples were not analyzed at the Dublin/San Ramon WWTP.
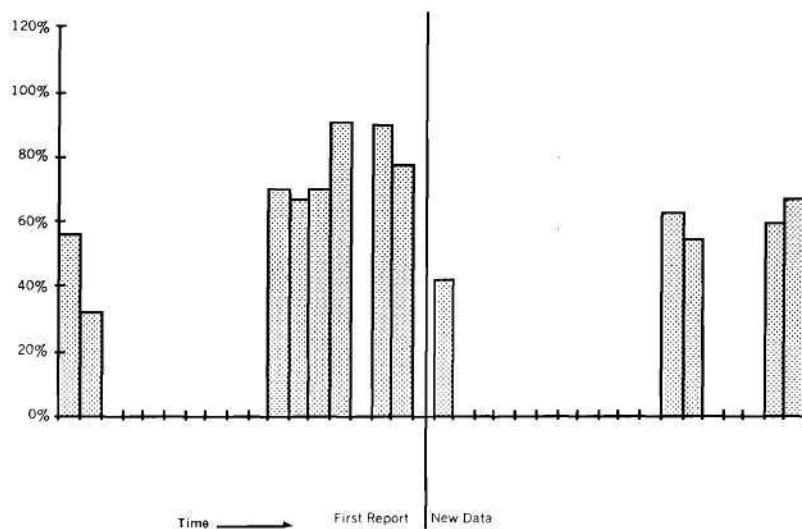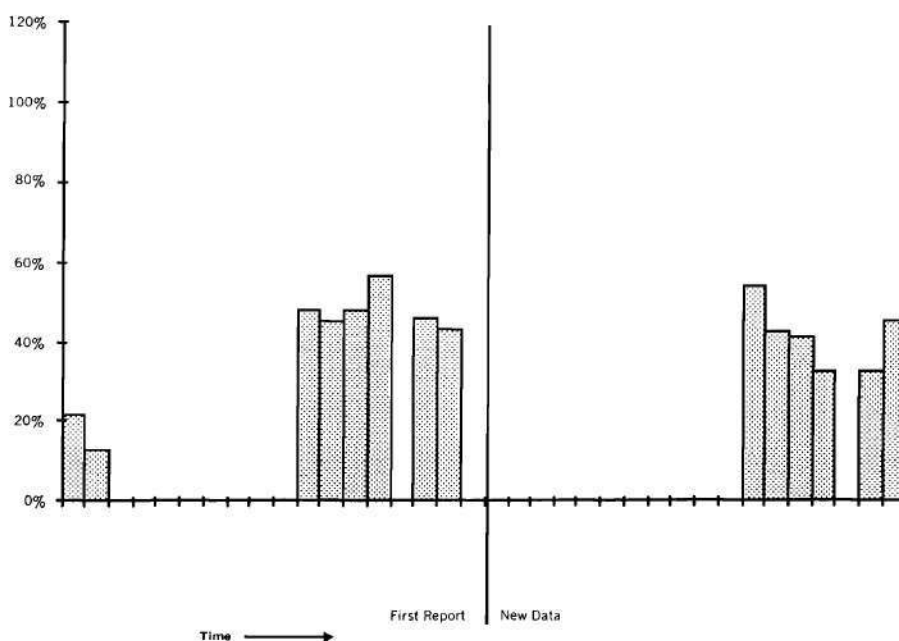

Figure 5: Naproxen recoveries as a function of time during the first two project periods. Recovery samples were not analyzed at the Dublin/San Ramon WWTP.

New data for recoveries of gemfibrozil, ketoprofen, nadolol and diclofenac were not obtained during this project period because we experienced problems with our GC/MS system. The nature of the problems with the system (i.e., decreased sensitivity for high mass fragments) had a greater effect on these compounds than the other analytes. We are currently working with the manufacturer to correct the problem.

With the exception of the first sampling event, indometacine exhibited recoveries have ranged from approximately 10 to 50% (Figure 6). Although these recoveries are lower than our target of 70 to 110%, the recoveries are consistent and we plan to use the method without further modification.

Figure 6: Indometacine recoveries as a function of time during the first two project periods. Recovery samples were not analyzed at the Dublin/San Ramon or Mt. View sites.

Propranolol (Figure 7) exhibited recoveries ranging from 20 to 143%. During the current project period, recoveries generally improved, compared to the first project period. In the most recent sampling event, recoveries ranged from 60 to 100%.

Metoprolol (Figure 8) exhibited recoveries ranging from approximately 20 to 136%. Recoveries have improved and have become more consistent with time. In the most recent samples, recoveries ranged from 70 to 100%.
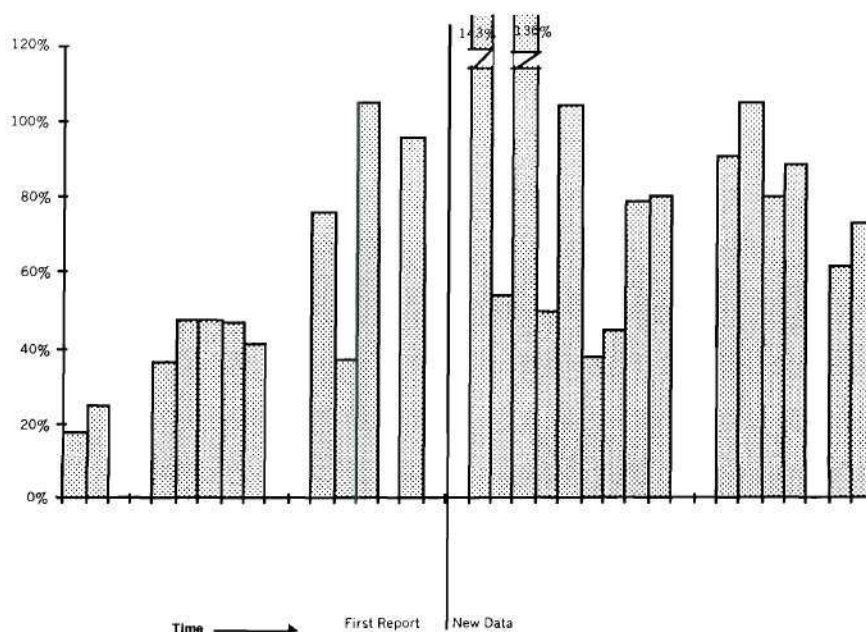

Figure 7: Propranolol recoveries as a function of time during the first two project periods.
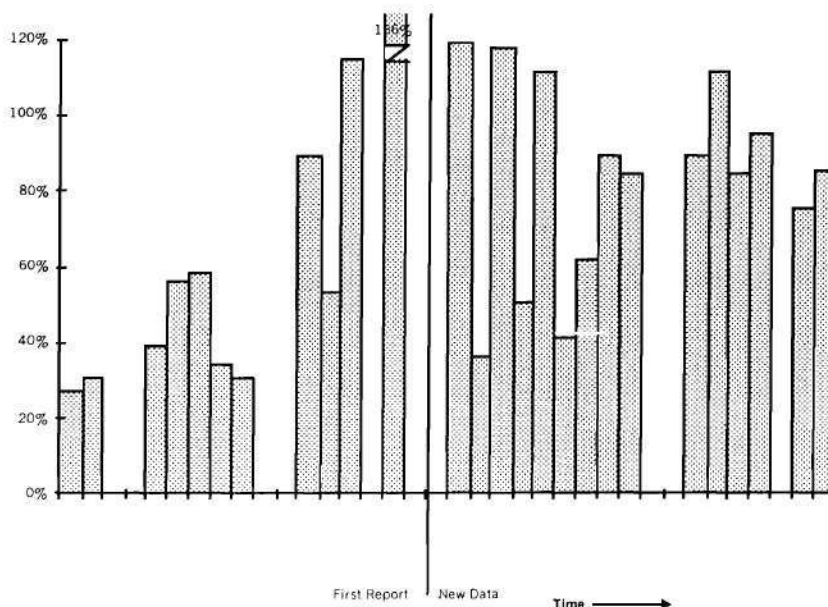
Figure 8: Metoprolol recoveries as a function of time during the first two project periods.

No new data are available for carbamazepine and nabumetone. As discussed during the site visit, carbamazepine is very difficult to analyze by GC/MS/MS and we are still attempting to optimize the analytical method for this compound. As a result, we also have not analyzed nabumetone because it was run along with carbamazepine. In the future, nabumetone will be analyzed with the other compounds.

Preliminary Results: During method development and testing, duplicate samples were analyzed from sites that may be included in the occurrence survey. These results are considered preliminary because method development is incomplete. However, these preliminary results will guide us in the design of the occurrence survey. During the current project period we collected samples from the Mt. View Sanitary District's WWTP and three sites in the associated treatment wetland, the Sweetwater groundwater replenishment site located in Tuscon, Arizona and a municipal WWTP located in Phoenix, Arizona. Results are included in Appendix B and are discussed under task 3a.

Sub-Task 2B: Antibiotics

During the second quarter of the project, we began method development for the selected antibiotics ciprofloxacin, sulfamethazine and sulfamethoxazole. Our initial efforts focused on the development of HPLC-fluorescence methods for ciprofloxacin and HPLC-UV methods for the two sulfonamides. The use of HPLC/FLD to analyze fluoroquinolone antibiotics has been reported by previous studies to have sensitivities as low as several ng/L in wastewater effluent matrices (Hartmann, et al., 1998). In addition, we will develop LC/MS methods to confirm the results of HPLC/FLD. It is likely that HPLC/UV may not be sensitive or selective enough to accurately quantify very low concentrations of sulfonamides in environmental matrices. Therefore, we will develop LC/MS methods for sulfamethazine and sulfamethoxazole as the primary quantification methods. The developed HPLC methods can serve as guidance for our method development with LC/MS or can be applied as clean-up steps when dealing with particularly complicated matrices.

The HPLC methods are being developed using wastewater concentrated extracts spiked with 10-500 µg/L of antibiotics to assess matrix effects. Method development focused on separation of interfering compounds, detection limits of analysis, and calibration curves. From

the preliminary results, our developed methods showed effective separation of antibiotics from other interfering compounds in the wastewater matrices as shown in Figures 9 and 10. However, it is likely that the interference will present more of a problem when lower concentrations of antibiotics are analyzed. The details of the methods are described in the Appendix C.

We also initiated method development for extracting ciprofloxacin, sulfamethazine and sulfamethoxazole from wastewater. Solid phase extraction methods are being developed for these antibiotics in both wastewater and deionized water matrices. Preliminary results will be discussed in the next progress report.
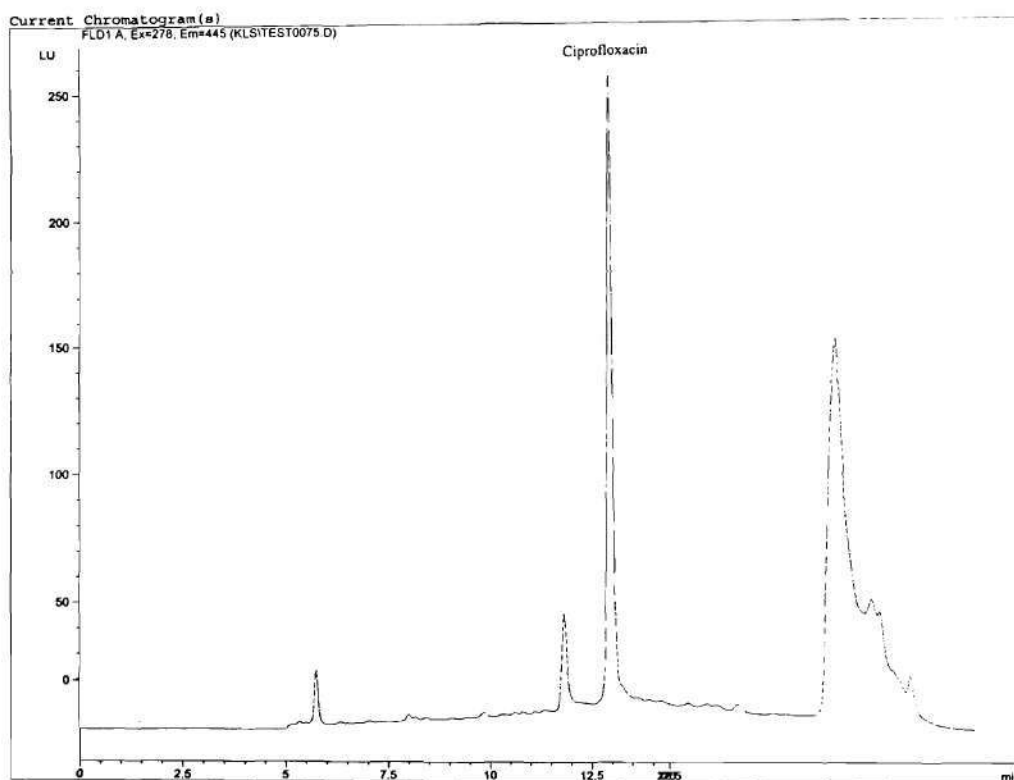
Figure 9. HPLC/Fluorescence chromatogram for 300 µg/L ciprofloxacin in a wastewater effluent extract.
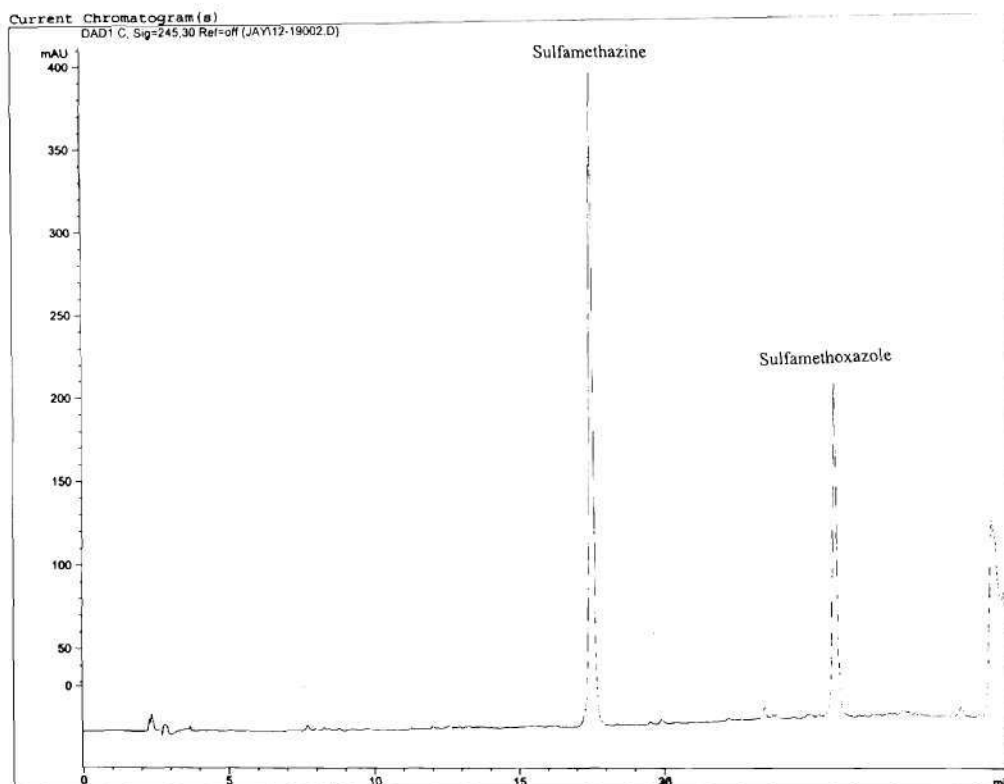
Figure 10. HPLC/UV chromatogram for 14 mg/L of sulfamethazine and 12.7 mg/L of sulfamethoxazole in a wastewater effluent extract.

TASK 3: OCCURRENCE SURVEY

Sub-Task 3A: Site Selection

As part of the site selection process, preliminary samples have been collected during the first and second project periods from sites that we are considering for inclusion in the occurrence survey. Although we have not yet finalized site selection, a brief description of the sites sampled during this project period and interpretation of the preliminary data are included below:

Municipal Wastewater Treatment Plants: Samples will be collected from municipal wastewater treatment plants to assess the sources of pharmaceuticals entering advanced treatment systems, engineered treatment wetlands and groundwater infiltration systems. During the first project

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period, we sampled the Dublin/San Ramon (CA) Municipal Wastewater Treatment Plant (WWTP and the Goodyear (AZ) Municipal WWTP. During this project period, we sampled the Mt. View (CA) Sanitary District Municipal WWTP and a WWTP located in Phoenix, Arizona. The Mt. View WWTP was sampled because we are interested in measuring the removal of pharmaceuticals as wastewater passes through the engineered treatment wetland associated with the facility. The Phoenix, Arizona WWTP was sampled because it is being considered as a wastewater source for a soil column study being conducted by the USDA Laboratory. We plan to collaborate with the USDA laboratory to assess mechanisms of removal of PhACs during groundwater infiltration.

Concentrations of pharmaceuticals measured in secondary effluent from the Mt. View WWTP (Figure 11) are considerably higher than those detected in other secondary WWTPs sampled to date. These unexpectedly high concentrations were greater than the highest point on our standard curve and we were unable to dilute the samples to obtain exact quantification because samples were discarded after the analysis. These differences could be attributable to differences in the efficacy of the trickling filter used at the Mt. View WWTP and the activated sludge systems employed elsewhere. levels comparable to those measured elsewhere. After
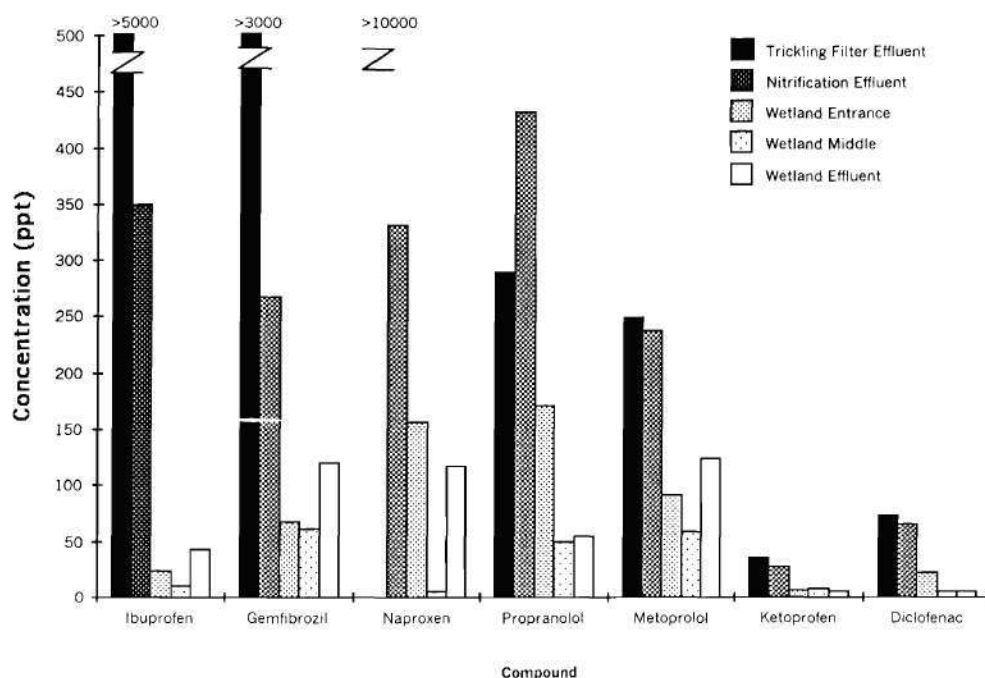

Figure 11. Concentrations of target analytes measured at the Mt. View WWTP and the associated treatment wetland.

nitrogen removal in a biotower, followed by sand filtration, concentrations of pharmaceuticals at the Mt. View WWTP decrease to levels comparable to those measured in other WWTPs. In our opinion, these differences merit further examination during occurrence survey.

Concentrations of pharmaceuticals measured in secondary effluent from the Phoenix, Arizona WWTP are higher than those detected at the Goodyear WWTP, which is a secondary treatment plant equipped with sand filtration and nitrification (Figure 12). These data are consistent with our observation from a previous study that nitrification decreases concentrations of hormones in wastewater effluent. Although wastewater from the Phoenix, Arizona WWTP may be more appropriate for use in USDA's soil column study, we believe that the removal of pharmaceuticals during nitrification merits further examination during the occurrence survey.
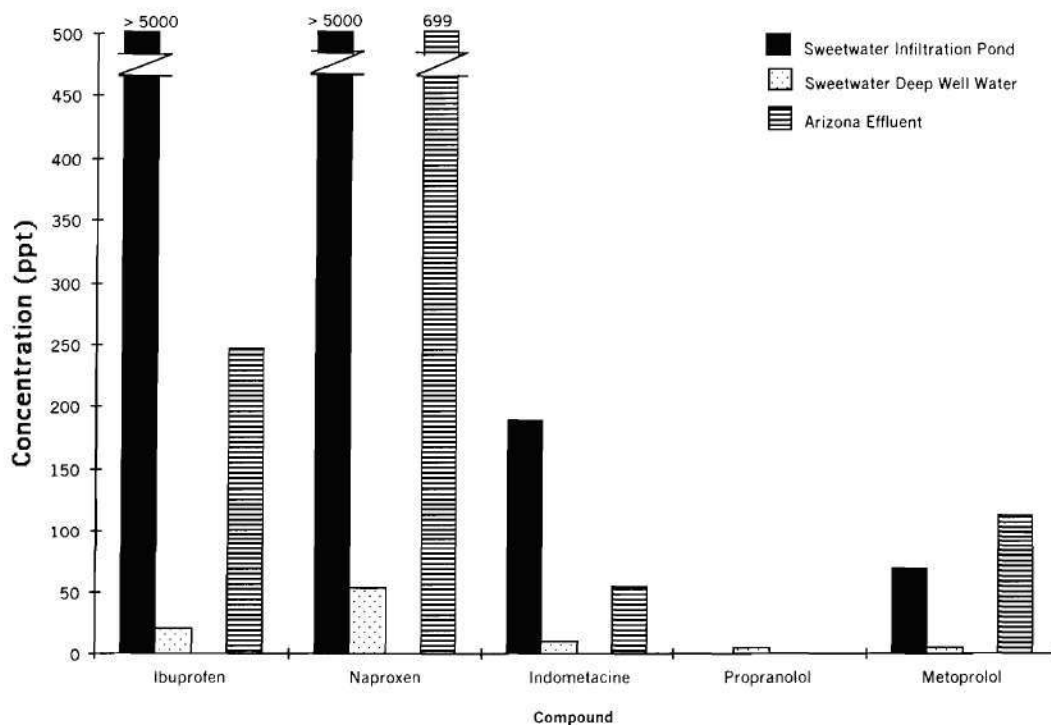

Figure 12. Concentrations of target analytes measured at the Phoenix, Arizona WWTP and the Sweetwater Groundwater Recharge Site. Indometacine, propranolol and metoprolol were observed in deep well water samples at concentrations below 10 ng/L.

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Engineered Treatment Wetlands: During the occurrence survey, samples will be collected from engineered treatment wetlands to assess the removal of pharmaceuticals during transport in surface waters. Engineered treatment wetlands will be studied because they receive relatively high concentrations of pharmaceuticals through undiluted wastewater effluent and they exhibit considerable biological activity. During the first project period, samples from the Prado (CA) treatment wetlands were found to contain pharmaceuticals at concentrations near or below the method detection limit.

During this project period, samples were collected from the Mt. View (CA) Sanitary District's Engineered Treatment Wetland (Figure 11). Almost all of the water entering the wetland originates at the Mt. View WWTP and flows through a series of ponds with a residence time of approximately 10 days. Samples were analyzed from three of the ponds: near the discharge point of the WWTP, midway through the wetland and near the discharge point of the wetland. Results indicate that concentrations of pharmaceuticals were considerably higher than those detected in the Prado treatment wetlands. Furthermore, none of the compounds are completely removed during passage through the wetlands. Concentrations of compounds known to be removed in WWTPs (e.g., propranolol) appeared to decrease during passage through the wetland. However, it is difficult to draw any conclusions from three grab samples collected on the same date. Therefore, synoptic sampling will be employed during the occurrence survey.

Groundwater Recharge Systems: Samples will be collected from groundwater infiltration systems to assess the removal of compounds during recharge. During this project period, we analyzed samples from a groundwater recharge site located in Tuscon, Arizona (Figure 12). Samples were collected from a basin that receives secondary wastewater effluent and a groundwater well downgradient of the basin screened at a depth of approximately 30 meters. According to researchers at the University of Arizona, a tracer study indicated that the travel time from the basin to the groundwater water ranges from two to four weeks. Boron isotope analysis indicates that the water in the deep well is 100% wastewater in origin. Our results suggest nearly complete removal of pharmaceuticals during passage through the infiltration zone. Of the four compounds detected in the wastewater pond, two (i.e., ibuprofen and naproxen) were detected in the deep groundwater at concentrations to less than 5% of those detected in the

ponds. The removal of pharmaceuticals during groundwater infiltration merits further investigation during the occurrence survey.

Sub-Task 3B: Sample Collection and Analysis

No activities related to this sub-task were conducted during this project period.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task were conducted during this project period.

PLANS FOR NEXT PERIOD

The following section describes research planned during the next project period. In addition, plans for the remainder of the project are described at the end of each section. A revised schedule for the project is presented in Appendix C.

Task 1: Literature Review

Sub-Task 1A: Drugs Used in Human Therapy

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Sub-Task 1B: Drugs Used in Animal Husbandry

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Task 2: Analytical Method Development and Testing

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

During the third project period we plan to continue to improve our analytical methods in preparation for the occurrence survey. Planned activities include assessment of additional compounds to be included in the analytical protocol, use of additional internal standards to evaluate recoveries and assessment of sampling protocols for quantification of PhAC concentrations in municipal wastewater effluent. Our research plans are summarized below:

Assessment of additional compounds: As recommended by the PAC, we are evaluating the possibility of including sixteen additional PhACs (i.e., allopurinol, atenolol, bupropion, caffeine, carisoprodol, cimetidine, diltiazem, gabapentin, hydrochlorothiazide, ipratropium, metformin, phenytoin, ranitidine, triamterene, valproic acid and verapamil) in our analytical protocols. The first step in this evaluation involves an assessment of our ability to measure these compounds by GC/MS/MS with existing derivitization protocols. After completion of these tests, we will

evaluate recovery efficiencies for these compounds in deionized water and in wastewater effluent. Compounds which can be readily analyzed with existing protocols will be included in further analyses.

Internal Standards: As recommended by the PAC, we are evaluating the use of radiolabeled internal standards to be added prior to sample extraction. The radiolabeled internal standards being considered (i.e., caffeine, propranolol, and mecoprop) will provide information on the efficiency of sample extraction, derivitization and sample transfer steps. As a first step in this analysis, we will evaluate the use of at least two of the three compounds in deionized water and samples collected from candidate sites.

Evaluation of Sampling Protocols: To minimize potential losses following sample collection, we have limited our sampling to the collection of grab samples. However, data collected at the Mt. View Treatment Wetland and data published from other studies suggest that concentrations of PhACs in municipal wastewater effluent may vary considerably over periods of several hours. Sampling during the occurrence survey will be conducted to quantify the mass of PhACs discharged by different sources as well as their removal by different treatment processes. These two goals may require different sampling approaches. To assess the mass of compounds discharged by a wastewater treatment plant, regulators prefer 24-hour composite samples. As part of the occurrence survey, we plan to collect 24-hour composites of wastewater effluent samples. To assess the removal of compounds during individual treatment processes, a different approach is required. During the next project period, we will examine the use of synoptic sampling and composites collected over shorter time intervals as a means of sampling water entering and leaving different unit processes. We will conduct side-by-side sampling at either the Dublin/San Ramon or Mt. View WWTP. Results from composite samples, collected over a 24-hour period will be compared with results of grab samples collected at intervals approximating those of unit processes in the treatment pant (i.e., hours). Results of these studies will be used to guide the sampling frequency to be employed during the occurrence survey.

In addition to evaluating different sampling procedures, we plan to evaluate recovery of compounds from PFE-lined polyethylene containers and glass containers. Until now, have used

PFE-lined containers and, for logistical reasons, would like to continue using them if possible. If losses are observed, glass containers will be used during the occurrence survey.

After completion of the activities described above, method development should be complete. A finalized version of the QA/QC plan and analytical protocol will be submitted to the PAC for review prior to implementation of the occurrence survey.

Sub-Task 2B: Antibiotics

During the next quarter, we plan to continue the method development for extraction and detection of ciprofloxacin, sulfamethazine and sulfamethoxazole. Analytical and extraction methods will be tested in conventional secondary and tertiary wastewater effluents to assess matrix effects. We will evaluate the use of HPLC/Fluorescence for quantification of ciprofloxacin. HPLC/UV will be used to assess recoveries of sulfamethazine and sulfamethoxazole spiked into wastewater effluents.

After the above method development is accomplished, LC/MS methods will be developed for confirmatory analysis of ciprofloxacin and for quantification of sulfamethazine and sulfamethoxazole. The Environmental Engineering Laboratory of Georgia Tech is equipped with a Hewlett Packard LC/MS. When tandem mass spectrometry analysis is necessary, we plan to seek collaboration with experts on LC/MS/MS. In addition, GC/MS/MS analysis is an alternative for sulfamethazine and sulfamethoxazole after derivatization. A Varian ion-trap GC/MS is available at the Environmental Engineering Laboratory of Georgia Tech.

Task 3: Occurrence Survey

Sub-Task 3A: Site Selection

During the third project period, we plan to collect samples at several additional candidate sampling sites including the Rio Hondo Groundwater Infiltration System, located in Los Angeles (CA), the Livermore Advanced Wastewater Treatment System, located in Livermore (CA) and the Sacramento River Delta (CA), which will serve as one of our background sites. In addition, we plan to collect an additional sample from a well located between the infiltration basin and the previously sampled well at the Tuscon groundwater infiltration site.

After completion of analyses from these sites, we should be ready to begin the occurrence survey. A final list of sites will be submitted to the PAC for review and additional sites will be added if the PAC and research team agree that it is merited.

Sub-Task 3B: Sample Collection and Analysis

No activities related to this sub-task are planned during this project period. During the second year of the project we plan to collect samples from each of the sites selected as part of sub-task 3A. Samples will be collected on at least two different dates to assess temporal variability. Results from these analyses will be used to identify follow-up sampling to be conducted during the third year of the project. The follow-up sampling will be designed to address specific questions about removal mechanisms suggested by the occurrence survey. For example, if PhACs appear to be removed effectively at treatment plants that employ nitrification, additional samples will be collected from a larger number of plants equipped with nitrification systems.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task are planned during this project period. After completion of the occurrence survey, data will be evaluated to identify trends meriting further study. Data will be compared with expectations based on physical/chemical properties of the compounds as well as results reported by other researchers.

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APPENDIX A: Summary of Data from Second Project Period

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Ibuprofen	Mt. View Trickling Filter Effluent	9/6/00	>5000, >5000	74%
	Mt. View Nitrification Effluent	9/6/00	278, 421	91%, 86%
	Mt. View Wetland Entrance	9/6/00	25, 23	88%, 94%
	Mt. View Wetland Middle	9/6/00	10, 10	149%, 132%
	Mt. View Wetland Effluent	9/6/00	47, 40	104%, 111%
	Blank	9/6/00	<5	
	Sweetwater, Arizona Pond Water	10/30/00	> 5000, >5000	
	Sweetwater, Arizona Deep Well Water	10/30/00	<10, 30	45%, 48%
	Blank	10/30/00	<5	
	Arizona	11/9/00	201, 293	59%, 85%
Naproxen	Mt. View Trickling Filter Effluent	9/6/00	>10,000 , >10,000	42%
	Mt. View Nitrification Effluent	9/6/00	263, 400	
	Mt. View Wetland Entrance	9/6/00	156, 158	
	Mt. View Wetland Middle	9/6/00	<5, <5	
	Mt. View Wetland Effluent	9/6/00	128, 106	
	Blank	9/6/00	<5	
	Sweetwater, Arizona Pond Water	10/30/00	>5000, >5000	
	Sweetwater, Arizona Deep Well Water	10/30/00	74, 33	63%, 54%
	Blank	10/30/00	17	
	Arizona	11/9/00	565, 833	59%, 67%
Gemfibrozil	Mt. View Trickling Filter Effluent	9/6/00	3540, 5000	132%
	Mt. View Nitrification Effluent	9/6/00	206, 329	319%, 193%
	Mt. View Wetland Entrance	9/6/00	72, 64	141%, 168%
	Mt. View Wetland Middle	9/6/00	60, 62	286%, 255%
	Mt. View Wetland Effluent	9/6/00	123, 116	214%, 225%
	Blank	9/6/00	<5	
Ketoprofen	Mt. View Trickling Filter Effluent	9/6/00	28, 42	
	Mt. View Nitrification Effluent	9/6/00	28, 26	
	Mt. View Wetland Entrance	9/6/00	7, <5	
	Mt. View Wetland Middle	9/6/00	11, <5	
	Mt. View Wetland Effluent	9/6/00	<5, <5	
	Blank	9/6/00	<5	
Diclofenac	Mt. View Trickling Filter Effluent	9/6/00	46, 99	120%
	Mt. View Nitrification Effluent	9/6/00	65, 65	141%, 98%
	Mt. View Wetland Entrance	9/6/00	23, 22	84%, 64%
	Mt. View Wetland Middle	9/6/00	<5, <5	128%, 118%
	Mt. View Wetland Effluent	9/6/00	<5, <5	96%, 110%
	Blank	9/6/00	<5	
Indometacine	Sweetwater, Arizona Pond Water	10/30/00	173, 206	41%, 32%
	Sweetwater, Arizona Deep Well Water	10/30/00	<10, <10	54%, 42%
	Blank	10/30/00	10	
	Arizona	11/9/00	13, 96	32%, 45%

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Propranolol	Mt. View Trickling Filter Effluent	9/6/00	378, 201	143%, 53%
	Mt. View Nitrification Effluent	9/6/00	511, 351	135%, 50%
	Mt. View Wetland Entrance	9/6/00	171	105%
	Mt. View Wetland Middle	9/6/00	59, 41	38%, 45%
	Mt. View Wetland Effluent	9/6/00	<5, 53	78%, 80%
	Blank	9/6/00	<5	
	Sweetwater, Arizona Pond Water	10/30/00		80%, 89%
	Sweetwater, Arizona Deep Well Water	10/30/00	<5, <5	91%, 105%
	Blank	10/30/00	<5	
	Arizona	11/9/00		62%, 73%
Metoprolol	Mt. View Trickling Filter Effluent	9/6/00	238, 261	119%, 35%
	Mt. View Nitrification Effluent	9/6/00	296, 197	118%, 50%
	Mt. View Wetland Entrance	9/6/00	147	110%
	Mt. View Wetland Middle	9/6/00	65, 54	41%, 61%
	Mt. View Wetland Effluent	9/6/00	125, 122	89%, 84%
	Blank	9/6/00	<5	
	Sweetwater, Arizona Pond Water	10/30/00	69	84%, 95%
	Sweetwater, Arizona Deep Well Water	10/30/00	<5 , <5	89%, 111%
	Blank	10/30/00	<5	
	Arizona	11/9/00	147, 79	75%, 85%

APPENDIX B: Method Development for Antibiotics

Unless otherwise specified, all chemicals were obtained from Fisher Scientific (Pittsburgh, PA) at the highest possible purity. Ciprofloxacin and sulfamethoxazole were purchased from ICN (Costa Mesa, CA). Sulfamethazine was purchased from Sigma (St. Louis, MO). Aqueous solutions were prepared in deionized water produced by a Nanopure system (Barnsted, Dubuque, IA). Stock solutions of ciprofloxacin, sulfamethazine and sulfamethoxazole were initially prepared in methanol at around 10 to 28 mg/L, and were subsequently diluted with deionized water to lower concentrations (1 ng/L to 1mg/L) as standard solutions when HPLC analysis was performed.

Wastewater concentrated extracts were obtained from extracting 500-750 ml conventional secondary wastewater effluent (after disinfection) with 500 mg ENVI-18 (octadecyl, endcapped) cartridges (Visiprep, Supelco, Bellefonte, PA). Wastewater organics were eluted with 6-10 mL of methanol, blown down to dryness under a gentle stream of nitrogen gas and in a water bath of 37 °C. The dried extract was then reconstituted in 100 μl methanol and 600 μl of deionized water.

Analysis was performed using a reversed-phase HPLC system (1100, Agilent Technology, Santa Clara, CA) with a quaternary pump, an autosampler, a diode-array UV/Vis detector and a multiple-wavelength fluorescence detector. A 250 mm Eclipse XDB-C18 column (4.6 mm, 5 μm particles, Agilent Technology) was used. Column temperature was maintained at 25 °C and a flow rate of 1.0 ml/min was employed. Injection volumes range from 50 to 100 μl.

For ciprofloxacin, the method from Hartmann, et al. (1998) was adapted with some modification. The mobile phases include a solution containing 20 mM trifluoroacetic acid (eluent A, pH ~2.4) and HPLC-grade acetonitrile (eluent B). The mobile phase begins with 0.5 minute isocratic 98% A, followed by a gradient decrease to 90% A in 0.5 minute, then a gradient decrease to 75% A in 10 minutes, followed by a 5 minutes isocratic 75% A. The mobile phase is then switched to 15% A, and the column was flushed under these conditions for 5 minutes. Detection of ciprofloxacin was conducted with the UV detector at 278 nm, and with the fluorescence detector at an excitation wavelength of 278 nm and an emission wavelength of 445 nm. UV spectra were collected for some analyses.

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From our preliminary results, ciprofloxacin appears to be stable in DI-water. Based on the preliminary results, the instrument detection limit (HPLC/FLD) is close to 5 µg/L. During our efforts to establish calibration curves for ciprofloxacin, a carryover problem was discovered. Therefore, DI-water and methanol was injected between samples. This significantly reduces carryover, however does not completely eliminate carryover associated with high-concentration standards (e.g., > 1 mg/L). Recently, we also observed deterioration of peak shape resulting in considerable variation in peak areas. Currently, we are modifying our method to resolve the above problems. A similar column of higher tolerance to strong acids (RX-C18, Agilent Technology) is selected. In addition, 20 mM H_3PO_4/NaH_2PO_4 buffer is used instead of trifluoroacetic acid, and modification of the mobile phase gradient program is made. The preliminary results indicated improvement with the new method and will be discussed in the following progress report.

For sulfamethazine and sulfamethoxazole, the method from Hartig, et al. (1999) was adapted with some modification. The mobile phases include a solution containing 6.5 mM trifluoroacetic acid (eluent A, pH ~ 2.5) and HPLC-grade acetonitrile (eluent B). The conditions for HPLC were as follows: 2 minutes isocratic 3% B, followed by a gradient increase to 33.8% B in 28 minutes. The column is then flushed with 64.6% B for 2 minutes followed by a 3 minute equilibration time for 3% B before the next injection. The retention time for sulfamethazine and sulfamethozaxole were approximately 17 min and 25 min respectively. Sulfamethazine and sulfamethoxazole was detected by the UV detector at both 245 nm and 270 nm.

Both sulfonamides appeared to be very stable in DI-water solutions. Linear relationships between peak areas and sulfonamide concentrations were obtained. No significant carryover was observed after injecting high-concentration standards.

APPENDIX C: Revised Schedule

Year

| | 1 | 2 | 3 |

1a. Draft literature review-drugs

1a. Update review

1b. Draft literature review-antibiotics

1b. Update review

2a. Method development-drugs excluding antibiotics

2b. Method development-antibiotics

3a. Site selection

3b. Sample collection and analysis

3c. Data analysis and synthesis
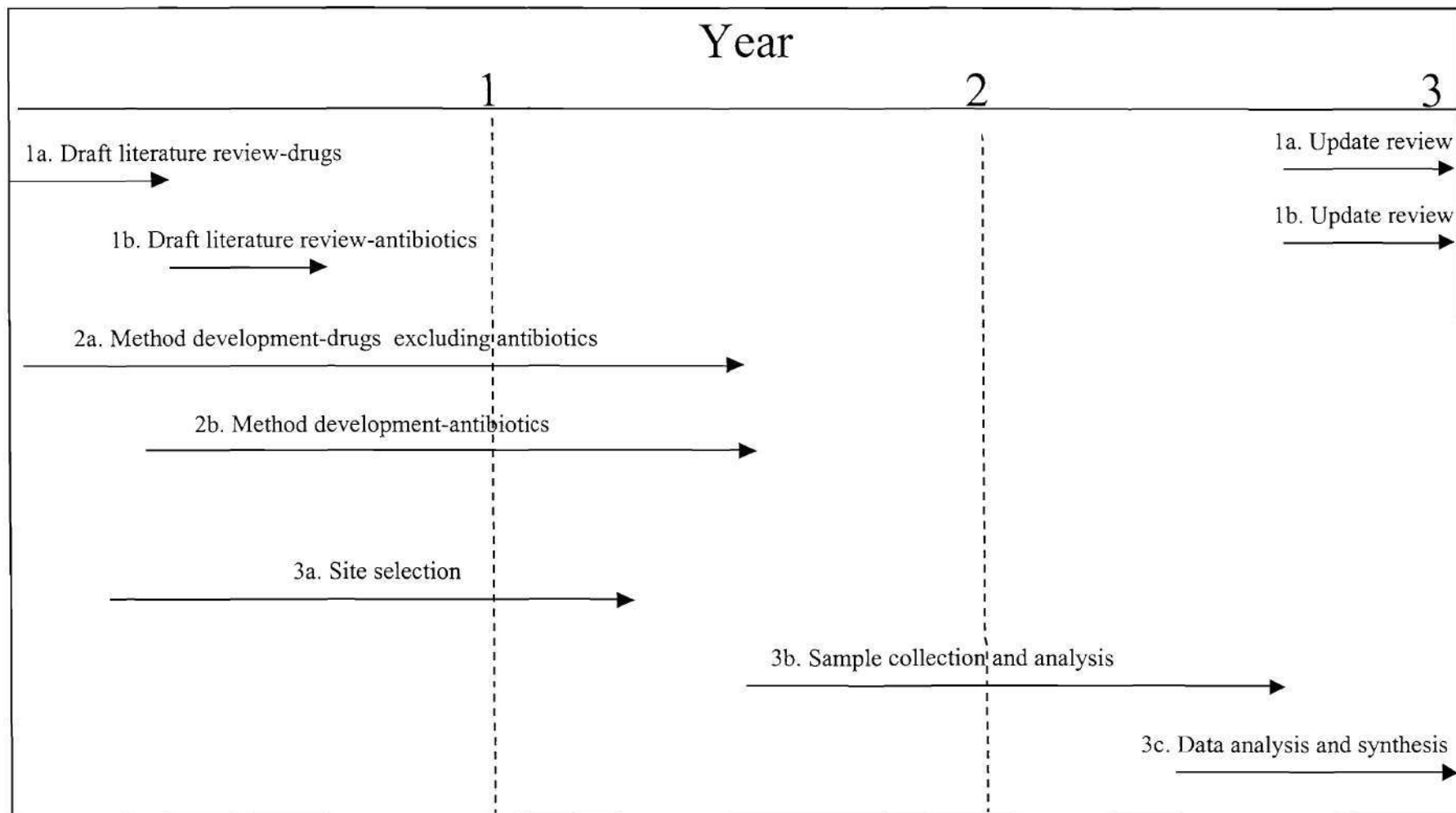
OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Third Progress Report

May 15, 2001

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SUMMARY

During the third project period, we concentrated on method development for compounds to be analyzed during the occurrence survey. To evaluate method performance and matrix effects, we collected samples from sites being considered for the occurrence survey and subjected them to solid phase extraction (SPE) and analysis before and after amendment with target analytes.

Method development activities for drugs other than antibiotics focused on approaches for improving recovery efficiency and reproducibility of our GC/tandem mass spectrometry (GC/MS/MS) analyses. Evaluation of the SPE method used for acidic drugs revealed that use of a mixed resin extraction system was responsible for the low and variable recoveries measured previously. Evaluation of the method used for beta-blockers suggested that occasional low recoveries were related to the presence of water during the derivitization step. After addressing both of these issues, recoveries and reproducibility improved considerably. In addition, we incorporated the use of radiolabeled mecoprop as an internal standard in the analysis of acidic drugs and made progress in evaluation of radiolabeled propranolol as a possible internal standard for the beta-blockers. We also evaluated potential losses of compounds by sorption to containers. Preliminary data obtained during method development suggest that the target analytes are present in secondary wastewater effluents and that most of the target pharmaceuticals are removed during reverse osmosis and groundwater infiltration.

Method development activities for antibiotics focused on the development and testing of SPE techniques and detection of the compounds using high performance liquid chromatography (HPLC) with fluorescence or mass spectrometry for detection. For the three target antibiotics (i.e., ciprofloxacin, sulfamethoxazole and sulfamethazine) improved recoveries were obtained using a SPE method with an anion exchange resin coupled to a hydophilic-lipophilic resin. Analysis of ciprofloxacin was performed by HPLC/fluorescence while the sulfonamides were analyzed by HPLC/UV and HPLC/MS. Low concentrations of ciprofloxacin and sulfamethoxazole were tentatively identified in secondary wastewater effluent but not in effluent treated with activated carbon and ozone. Further method development and inclusion of internal standards is required prior to quantification of the antibiotics in environmental samples.

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TASK 1: LITERATURE REVIEW

The objective of this task is to identify compounds to be studied during the occurrence survey. The selection criteria for compounds in the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and availability of suitable analytical methods. Progress on the literature review was presented in the first and second progress reports. The literature review has been completed and will be updated prior to preparation of the final report. No additional progress related to task one was made during the current project period.

Sub-Task 1A: Drugs Used in Human Therapy

In the first progress report, we reviewed data on prescription drugs likely to be present in wastewater effluent in the United States. Using data on the number of prescriptions for the top 200 drugs and information on prescription formulations and metabolism, we estimated ranges of concentrations of 136 drugs expected to be present in wastewater effluent. In addition, we reviewed published occurrence data to identify other compounds of interest that did not appear among the common prescription drugs. A total of eleven drugs were identified for further consideration.

During the current project period no activity was conducted in association with this sub-task.

Sub-Task 1B: Drugs Used in Animal Husbandry

In the second progress report, we reviewed data on antibiotics used in livestock both therapeutically to treat diseases and sub-therapeutically as feed additives to promote growth. We focused our review on antibiotics used in animal feeding operations, because these sources could result in concentrated discharges of antibiotics. We also reviewed fate and transport properties and available analytical methods. On the basis of these results and our review of antibiotics used in human therapy (sub-task 1a), we identified five antibiotics that merit further consideration.

During the current project period no activity was conducted in association with this sub-task.

TASK 2: ANALYTICAL METHOD DEVELOPMENT AND TESTING

The objective of this task is to identify and test analytical methods that will be used during the occurrence survey. As part of the task, analytical methods for extraction, cleanup and derivitization will be tested by adding drugs to deionized water and to samples collected from sites being considered for inclusion in the occurrence survey. Analysis of antibiotics is included as a separate task from the other pharmaceutically active compounds (PhACs) because the analytical methods for antibiotics (e.g., HPLC/MS) are fundamentally different from the gas chromatography methods used for the other compounds.

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the first progress report, we described our preliminary efforts to develop suitable analytical methods for eleven compounds identified as part of the literature survey. We used solid phase extraction followed by derivitization and GC/MS/MS to quantify pharmaceuticals in deionized water and in samples collected from several of the candidate sampling sites. To quantify recoveries and matrix effects, all samples also were analyzed after addition of known quantities of analytes.

During the second project period, we focused our attention on improving the accuracy and precision of the analytical methods by analyzing samples from three sites being considered for inclusion in the occurrence survey. As a result of analytical difficulties, we did not analyze three of the compounds (i.e., carbamazepine, nabumetone and nadolol) during the second project period. Analysis of duplicate samples indicated that, with the exception of two beta-blockers, results were reproducible (i.e., we observed an average of 26% error between duplicate samples). Analysis of blank samples indicated that sample carryover and cross contamination were not significant problems. Analysis of spike recovery samples indicated variable recoveries, with recoveries as low as 30% for some analytes. All of the drugs were detected in one or more of the unspiked samples.

During the third project period, we continued to improve the analytical methods. To address the incomplete and variable recoveries, we performed a series of experiments designed to identify the step(s) where analytes were lost. We also evaluated several internal standards that could be used to evaluate the efficacy of extraction and derivitization procedures. In addition, we evaluated several additional compounds that could be included in the occurrence survey

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using the extraction and derivitization methods developed for acidic drugs and beta-blockers. Finally, we evaluated the sorption of the analytes onto glass and PFE-lined sampling containers in preparation for the occurrence survey. Our progress in each of these areas is described below.

Analytical Methods: As suggested by members of the PAC, we evaluated the possibility of increasing the number of compounds to be analyzed. We attempted to analyze several potentially important drugs that were not included in our initial selection (Table 1). As a first step in our analysis, we prepared standards of each compound in isooctane at relatively high concentrations (i.e., 0.1-1 g/L). These standards were analyzed using the GC conditions developed for the acidic drugs and beta-blockers after derivitization with diazomethane or MSTFA/MBTFA, respectively. On the basis of total ion chromatograms, we identified the retention times and major ions for the compounds or their derivatives. For acetaminophen, more than one peak was identified because the derivitization was incomplete or the compound partially decomposed during analysis. Of the seventeen compounds, only seven were detected using one or both of the derivitization schemes (Table 1).

Following detection of the compounds at high concentrations, we optimized selected ion monitoring and MS/MS conditions for the seven compounds detected using the method developed for beta-blockers. After optimizing the data collection parameters, we analyzed standards of the derivitized compounds at a concentration of 1,000 µg/L (Table 1). For almost every compound, the response was considerably lower than that observed for comparable concentrations of the other compounds. One of the compounds (i.e., diltiazem) was not detected at 1,000 µg/L. Using peak areas and signal-to-noise ratios, we estimated detection limits for all of the compounds. Assuming pre-concentration factors comparable to those being used for the other compounds (i.e., 4000:1), and assuming quantitative recovery during SPE, we would be able to detect these compounds in water samples at concentrations ranging from 12 to 125 ng/L.

SPE recovery was analyzed for the six compounds detected in the 1,000 µg/L standards. An aqueous solution containing 1,000 ng/L of each compound was extracted using the C-18 SPE method developed for the beta-blockers. After elution and derivitization, none of the compounds were detected. Therefore, we conclude that the SPE methods would have to be modified considerably to include these compounds in the occurrence survey. Because method development is not the main objective of our research, we are not planning any further attempts to analyze these compounds.

Table 1: Results of attempts to include additional compounds in analytical methods developed for beta-blockers and acidic drugs.

Compound	Peaks observed at high concentration?		Peak observed at 1,000 µg/L?	Estimated MDL (µg/L)
	β-blocker	acidics		
acetaminophen	X[1]		X	100
allopurinol	X		X	50
atenolol				
bupropion	X	X	X	130
caffeine	X	X	X	500
carisoprodol				
cimetidine				
diltiazem	X	X		
gabapentin				
hydrochlorothiazide				
ipratropium				
metformin				
phenytoin	X		X	500
ranitidine				
triamterene				
valproic acid				
verapamil	X	X	X	50

Notes:

[1] Two peaks observed

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Quality Assurance/Quality Control (QA/QC): In the previous progress report, we reported our evaluation of recovery efficiencies for samples amended with target analytes. Because recoveries often were lower than desired, during the current project period we attempted to identify steps in the analysis where analytes were lost. We also modified the analytical methods to minimize steps where losses could occur. For example, we increased the duration of drying of the SPE prior to elution to avoid the presence of water in samples to be analyzed for beta-blockers because water could decrease the efficiency of the derivitization process.

To assess possible losses during solvent transfer steps after derivitization, we derivitized a set of samples containing 1 mg/L of the different acidic drugs and subjected them to three different treatments. In the first treatment, the extracts were blown to dryness with a gentle stream of nitrogen gas and resuspended in isooctane. In the second treatment, the samples were analyzed immediately after derivitization (no blowdown). In the third treatment, 250 µL of isooctane was added to the derivitized sample prior to nitrogen blowdown. These samples then were blown down until only the isooctane remained. Results indicated good recoveries for all samples and no significant differences between the three treatments (Figure 1). Similar experiments with volatile compounds, such as caffeine, showed losses of approximately 30% when samples were blown to dryness (data not shown). As a result of these experiments, we concluded that low recoveries of acidic drugs were not attributable to volatilization of the derivatives during blowdown.
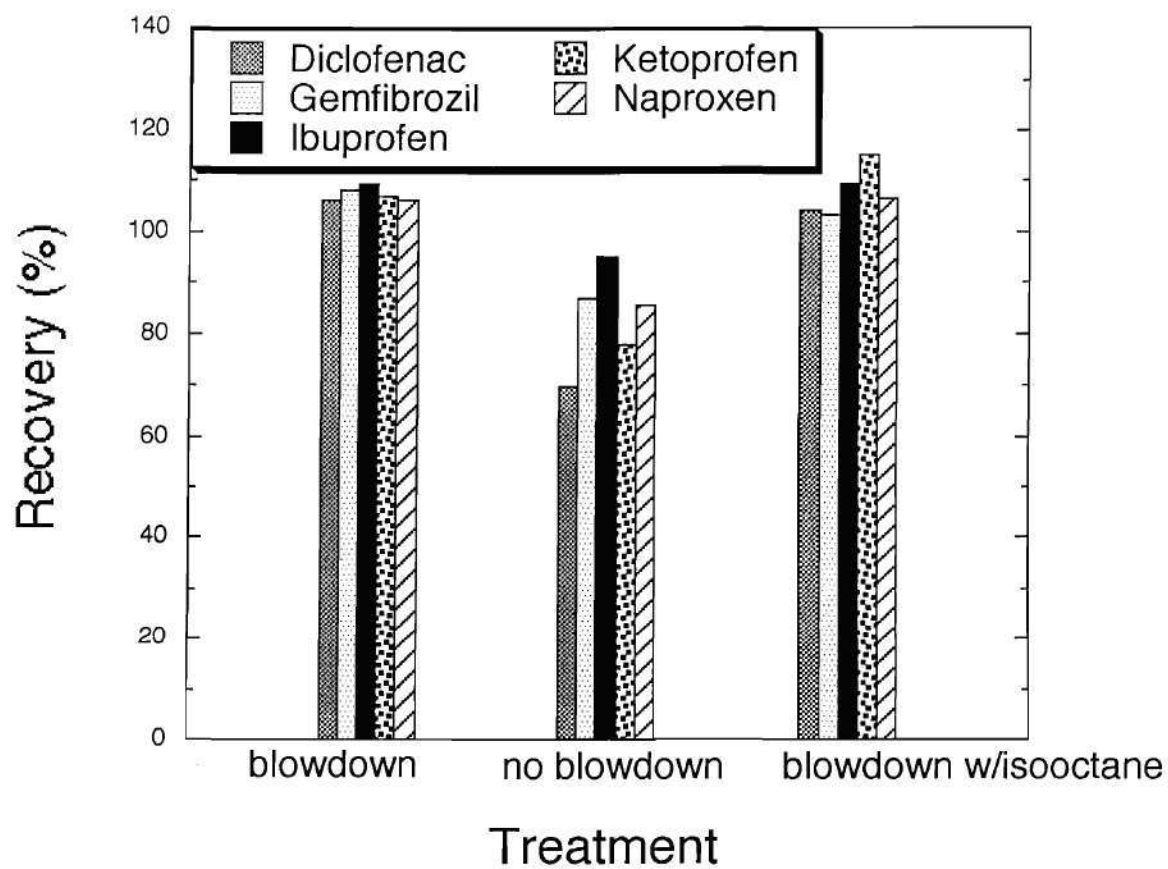
Figure 1: Effect of different blowdown methods on recovery of acidic drugs. Data are based on the average of duplicate experiments conducted with 1 mg/L of acidic drugs.

To assess the possibility that acidic drugs were lost when the methanolic extracts were evaporated and transferred to 1-mL volumetric flasks (prior to derivitization), we added acidic drugs to 10 mL of methanol and treated the sample exactly as we would treat the eluent from SPE. Results indicate recoveries of approximately 80% for all of the analytes (Figure 2). Therefore, we conclude that significant losses of acidic drugs did not occur during blowdown and solvent transfer of the methanolic extracts.
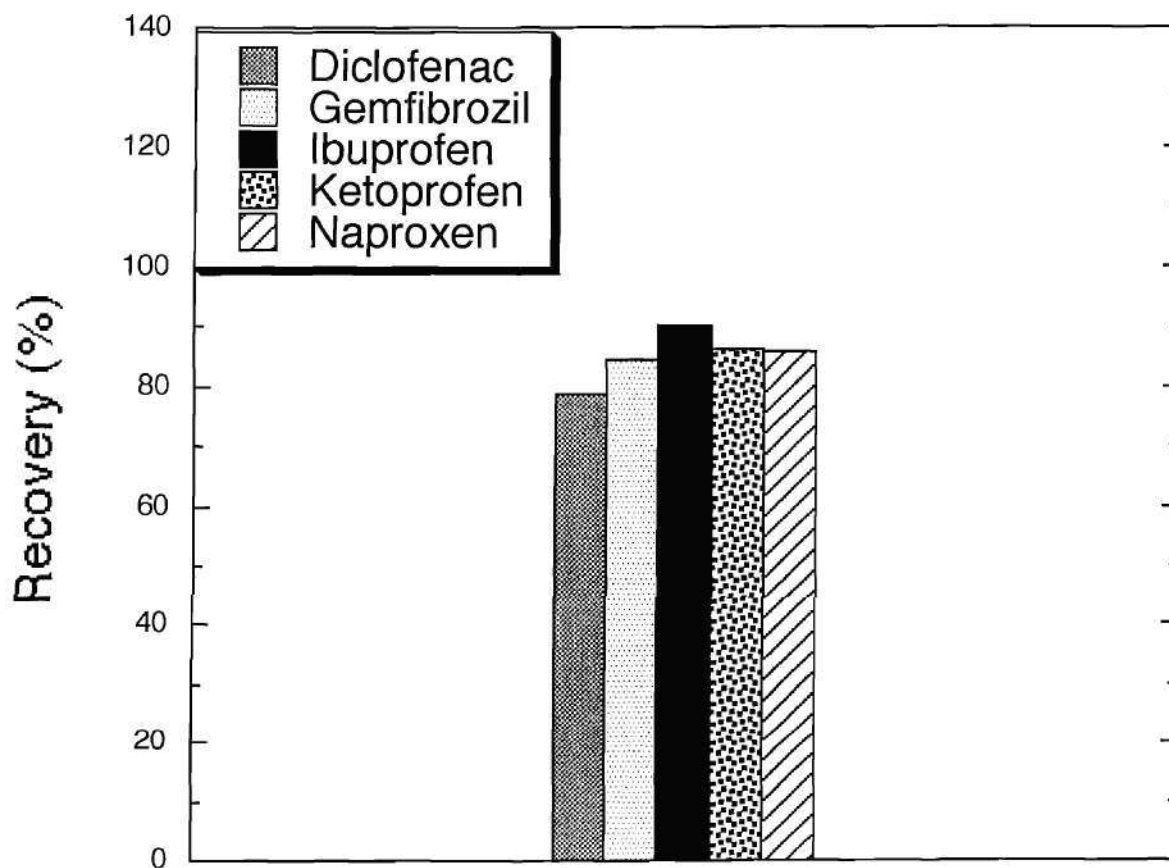

Figure 2: Recovery of acidic drugs from methanol stock solutions. The initial concentration of each compound was 100 μg/L. Results indicate average of duplicate extractions.

After determining that significant losses of acidic drugs were not resulting from transfer steps, solvent evaporation or derivitization, we considered the possibility that solid phase extraction was responsible for the relatively low recoveries observed in many samples. We suspected that poor recoveries of the acidic compounds were attributable to the use of a mixed solid phase consisting of C-18 and Lichrolut ENV. To test this hypothesis, we extracted three sets of samples: (1) mixed resin SPE consisting of C-18 and Lichrolut EN; (2) single-resin SPE method consisting of only Lichrolut EN; and, (3) single-resin SPE method consisting of only C-18. Results suggested that the single resin C-18 SPE method yielded superior results compared to the other two methods (Figure 3). Therefore, we concluded that the low and variable recoveries of acidic drugs were attributable to the Lichrolut EN.
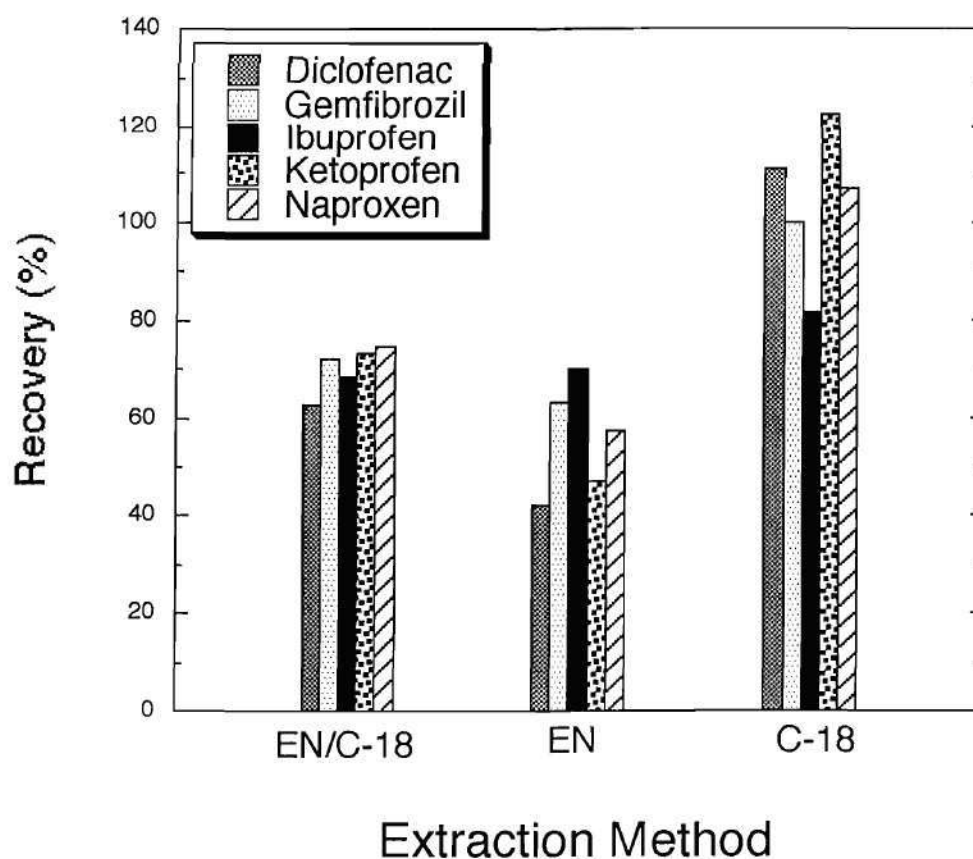

Figure 3: Recovery of acidic drugs subjected to extraction with different types of resins. EN/C-18 = mixed resin; EN = Lichrolut EN resin; C-18 = C-18 based resin.

During the present project period, we collected and analyzed spiked and unspiked samples from the West Central Basin Advanced Wastewater Treatment Plant, the Russian River, and the Sweetwater Groundwater Recharge Basin. The samples from the West Central Basin Facility were analyzed for acidic compounds using the mixed C-18/Lichrolut-EN SPE while the other two sets of samples were analyzed using the C-18 SPE only. Beta-blockers were extracted using the C-18 SPE method described previously. To assess recoveries, selected samples and deionized water blanks were amended with approximately 1,000 ng/L of each analyte.

Recoveries of acidic compounds were consistent with our hypothesis that the mixed-resin SPE material was responsible for the variable recoveries reported in the previous progress reports: recoveries of acidic compounds improved considerably after we switched to the single resin C-18 SPE columns. For example, if we were to adopt a QA/QC target of 60-120% for recovery of matrix spike samples, approximately 30% of the measurements of acidic drugs would have exhibited acceptable recoveries for the two-resin SPE. After changing to the single-resin SPE, 85% of the measurements would have been in the acceptable recovery range. Average recoveries are plotted for each of the three rounds of analyses in Figures 4 through 6 and are included in tabular form in Appendix A. The average percent error between duplicate spike recovery or unspiked samples was approximately 15%, after excluding those samples where unusually low recoveries were measured.
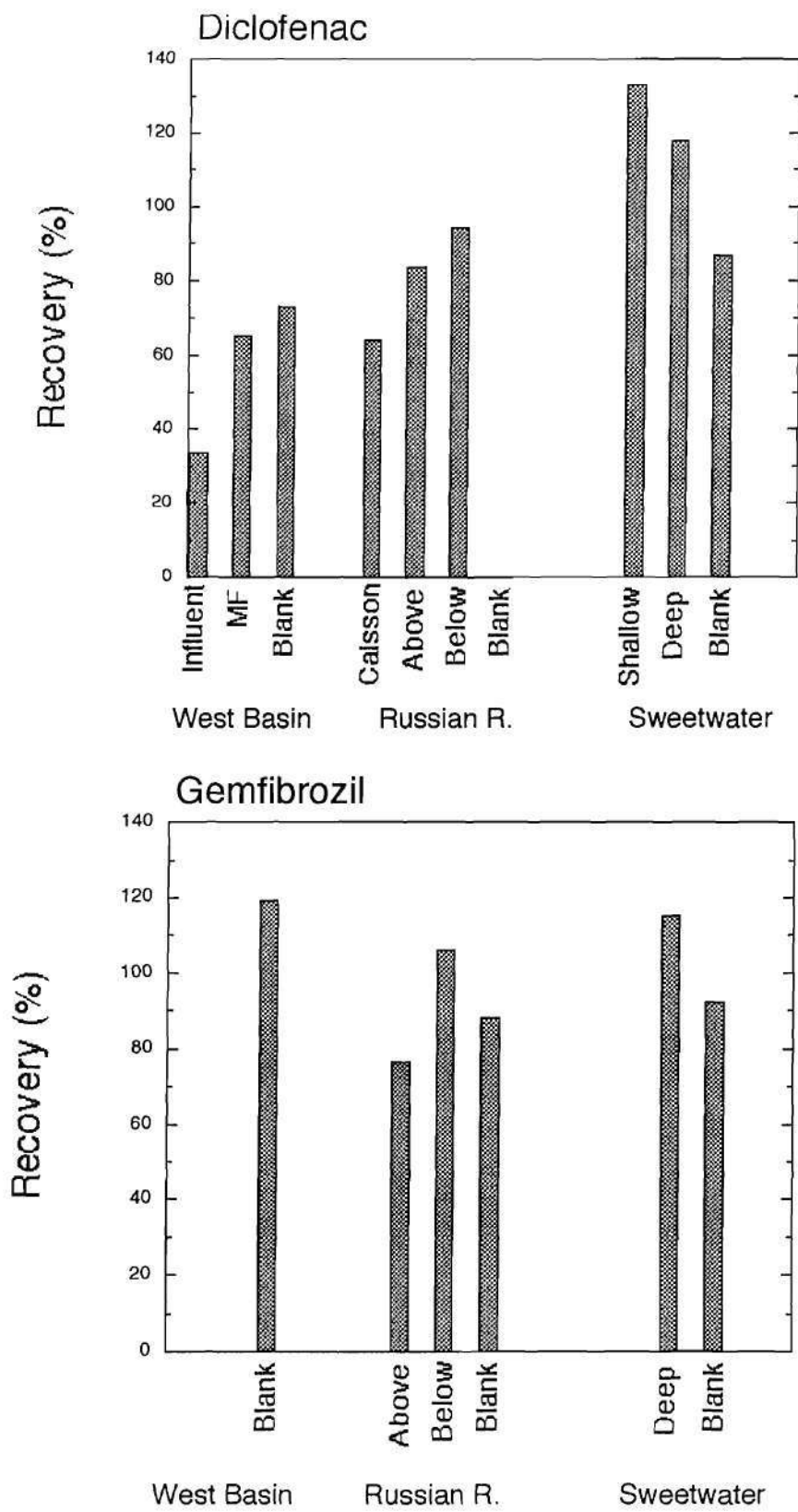
Figure 4: Recoveries of diclofenac and gemfibrozil measured during the third project period.
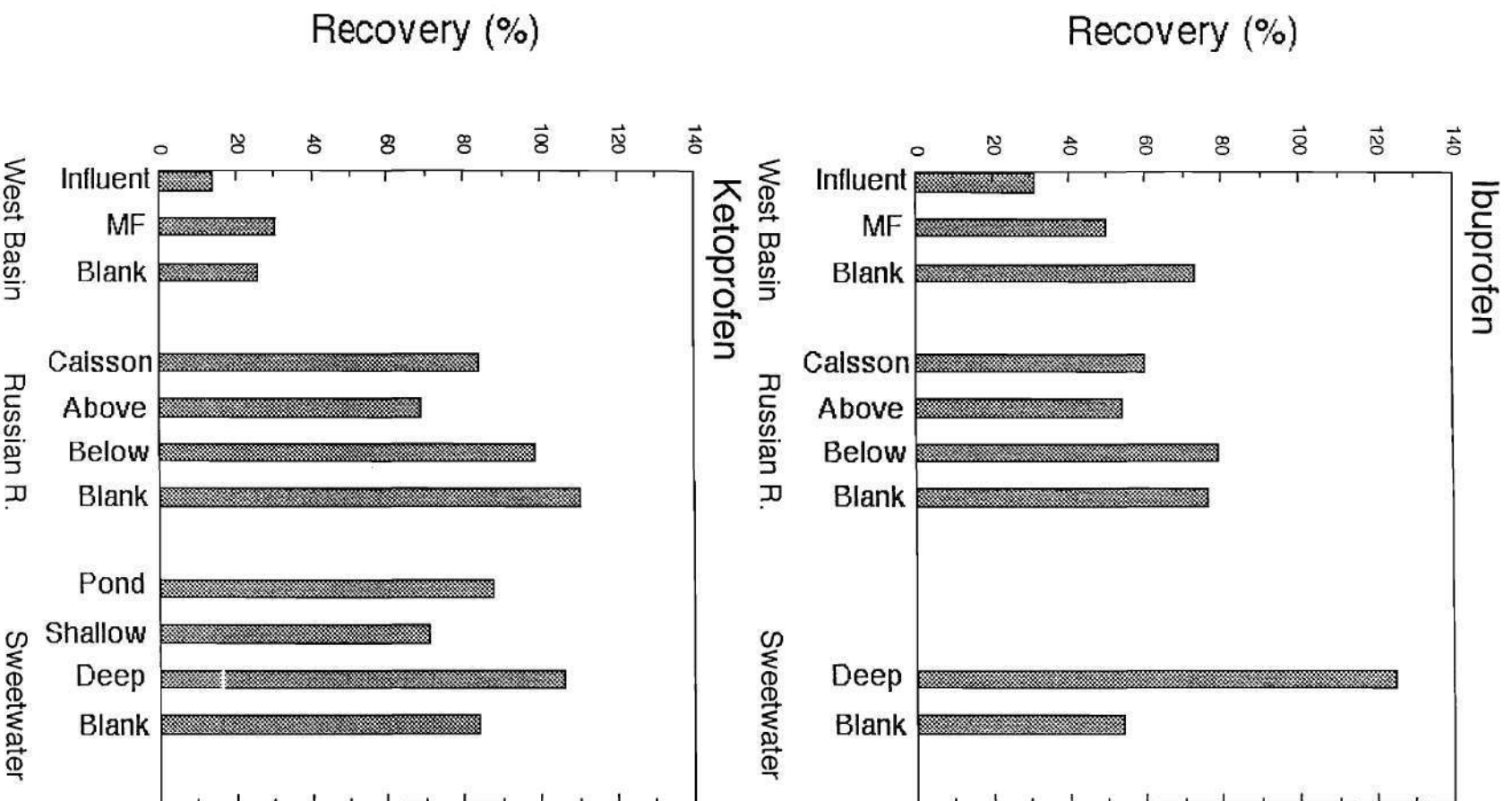
Figure 5: Recoveries of ibuprofen and ketoprofen measured during the third project period.

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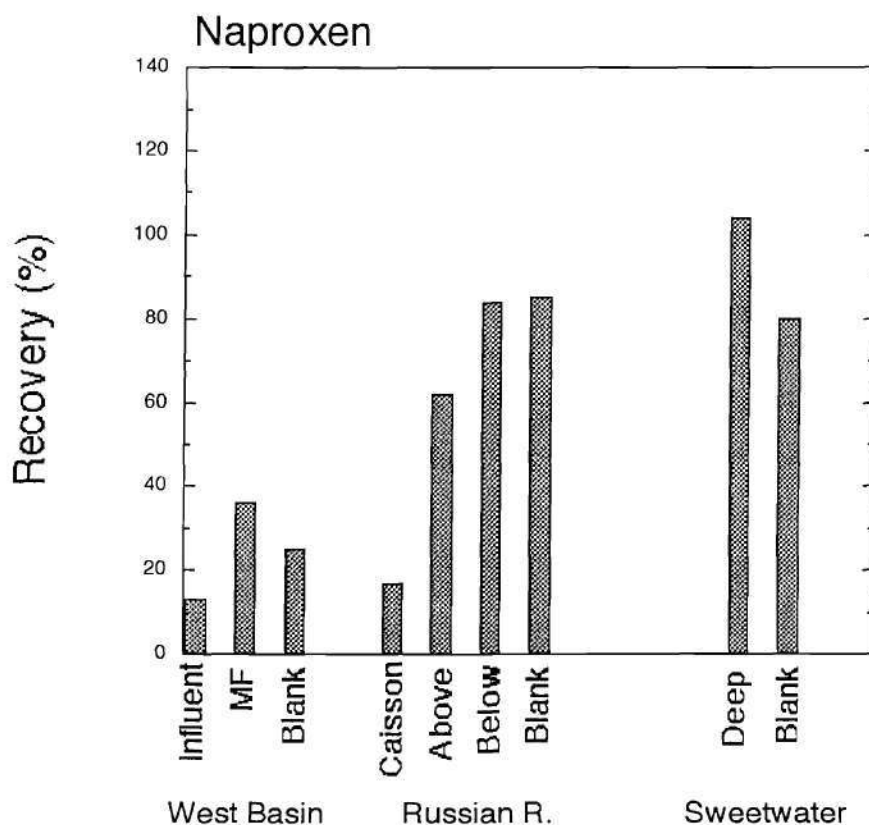
Naproxen

Figure 6: Recoveries of naproxen measured during the third project period.

The beta-blockers, propranolol and metaprolol were extracted using a C-18 SPE method. As a result, their recovery was not affected by the extraction problems encountered for the acidic drugs. We suspect that variability in the recovery efficiency of these compounds is attributable to the presence of trace amounts of water during derivitization. Typically, the recovery of these two compounds was consistent within a batch of samples. During the present project period, recoveries were between 56 and 106% in the samples from the West Basin Advanced WWTP and the Sweetwater groundwater recharge facility (Figure 7). Recoveries were considerably lower (average recovery of 25%) for the Russian River samples. We presume that the problems with the Russian River samples were related to incomplete derivitization in that batch of samples. The recovery of propranolol was not quantified for the West Basin samples because the use of radiolabeled propranolol as an internal standard precluded analysis of propranolol. This issue is discussed later in this section of the report.

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Figure 7: Recoveries of metaprolol and proranolol measured during the third project period.

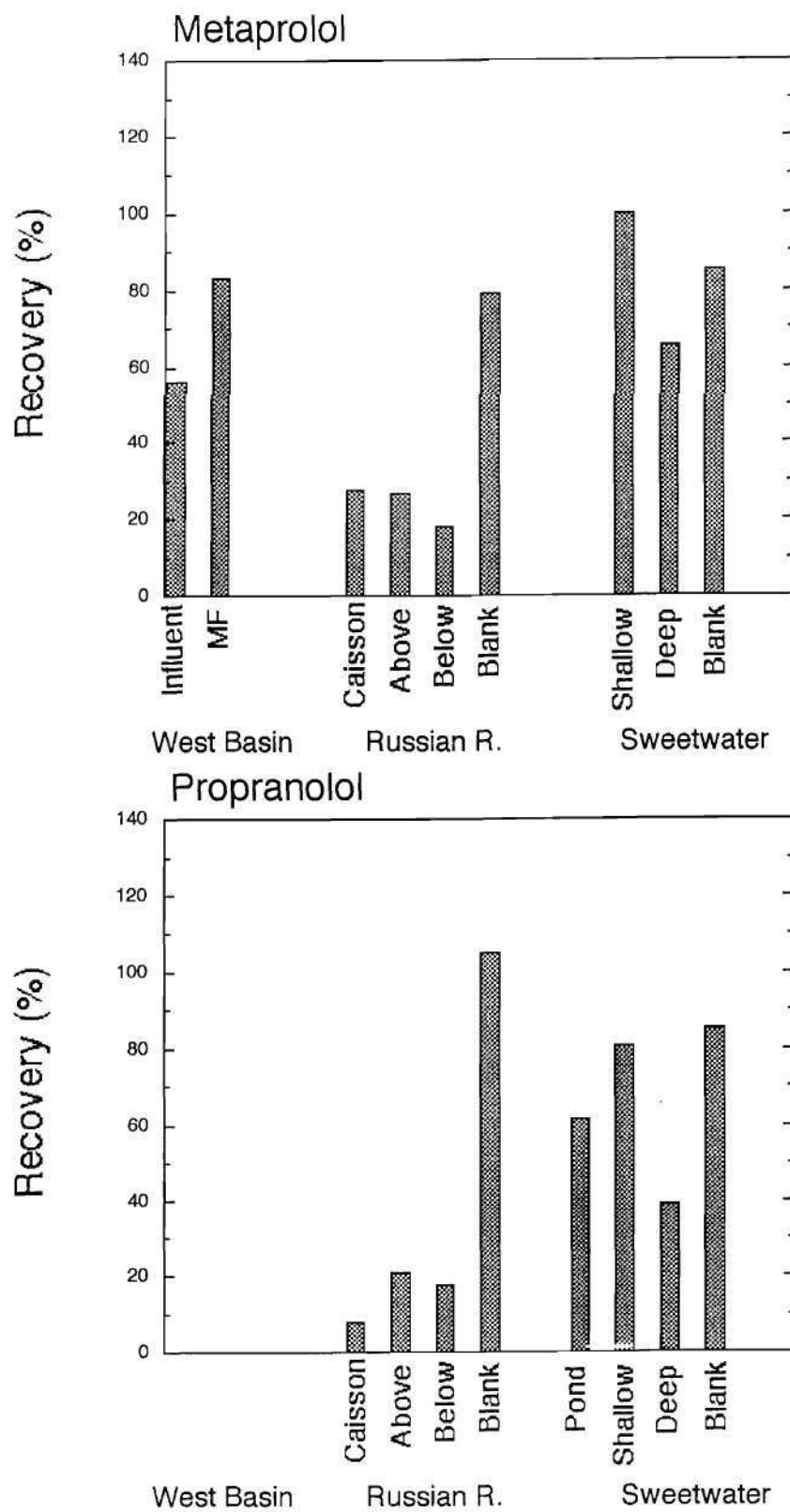During the initial screening of compounds, we identified a total of eleven compounds to be considered for inclusion in the occurrence survey. Three of these compounds were not analyzed during the second or third project periods because of analytical difficulties:

(1) Carbamazepine was not analyzed during this project period because it could not be analyzed successfully with our existing methods. We recently received a GC/MS/MS method from Dr. Heberer, and plan to implement it during the next project period.

(2) Nabumetone was not analyzed because it did not yield reproducible standard curves. We presume that these difficulties are related to the derivitization process.

(3) Nadolol was not analyzed because we are unable to obtain low detection limits needed for quantifying the compound in the aquatic environment. The relatively low sensitivity that we are able to achieve for nadolol is attributable to our inability to use tandem mass spectrometry because the mass difference between the parent and daughter ions are large and the ion trap is unable to capture both fragments. As a result, we can only use single ion monitoring, which lacks the sensitivity to detect the compound at concentrations likely to be present in the wastewater effluent.

We also had difficulty analyzing indometacine during this project period. Although we sometimes were able to construct good standard curves for the compound, we frequently encountered coeluting peaks and anomalous results for duplicate injections.

As a result of the difficulties described above, we plan to exclude indometacine, nabumetone and nadolol from the occurrence survey.

During the PAC's site visit, we discussed the possible use of internal standards to monitor for recovery efficiency. During the current project period, we evaluated the possible use of radiolabeled forms of caffeine, mecoprop (a herbicide that is not used in significant quantities in the United States) and propranolol. While all three compounds could be analyzed by GC/MS/MS, only mecoprop was satisfactory as an internal standard. Caffeine yielded poor peak shapes and was difficult to quantify accurately with our column, presumably because it is too polar for the GC column (i.e., DB-5). Radiolabeled propranolol could be analyzed with our analytical method. However, the form of the compound that we used was not very useful as an internal standard because the label was not located on the fragment used for tandem mass spectrometry. As a result, labeled propranolol could not be distinguished from the native

compound. We recently found a supplier who can provide propranol with a label at a position that is part of the fragment used for analysis. Therefore, we are hopeful that we will be able to use radiolabeled propranolol as an internal standard in future analyses.

Results from analysis of mecoprop suggest that it will be an acceptable internal standard. Mecoprop was added to samples from the West Basin Advanced WWTP and the Sweetwater groundwater recharge system and prior to extraction. Results indicate that the recovery of mecoprop was reasonably well correlated (i.e., $r^2=0.6$) with the recovery of other acidic compounds in the samples (Figure 8).
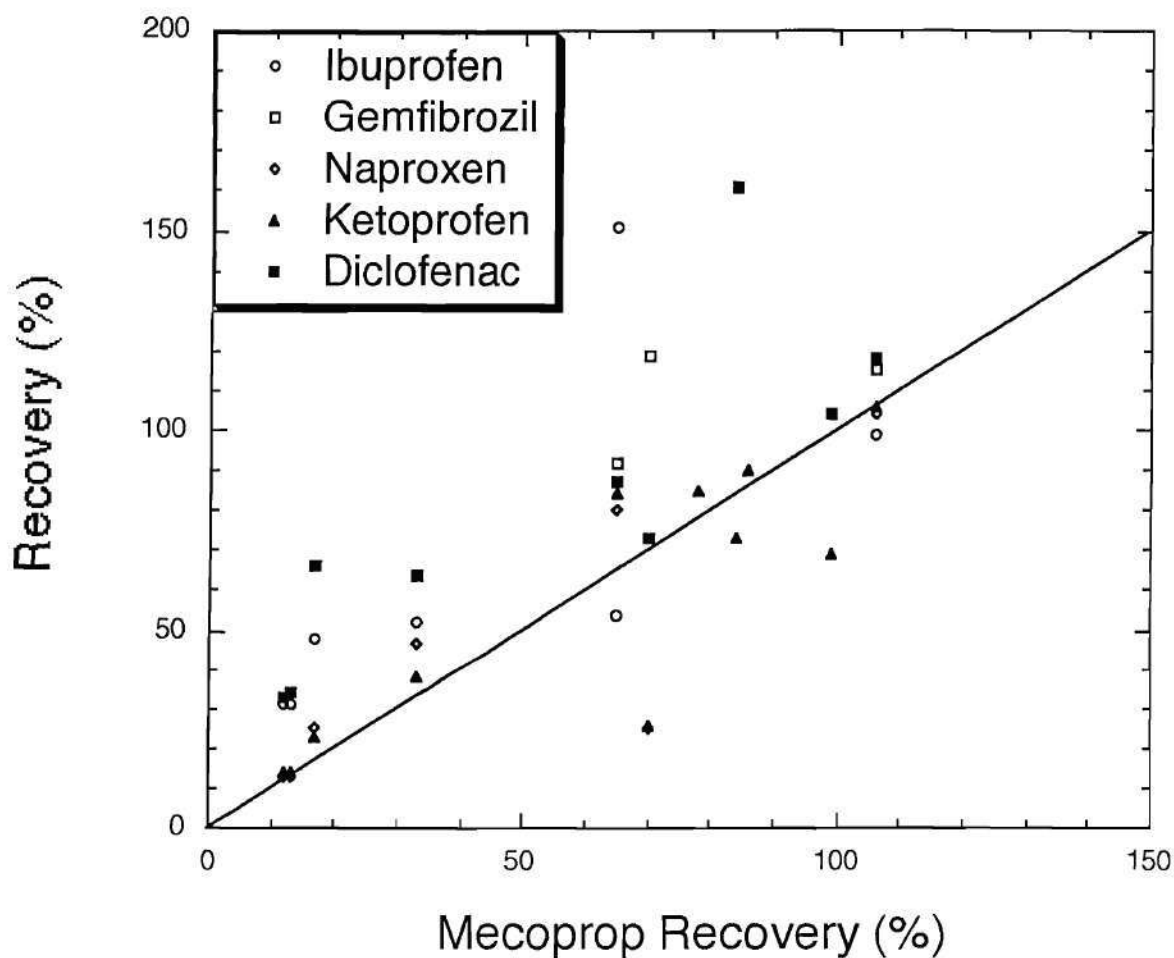

Figure 8: Relationship between the recovery of radiolabeled mecoprop and acidic drugs measured during the third project period. The line indicates a 1:1 relationship.

As discussed above, we have almost completed our method development activities. In preparation for the occurrence survey, we have prepared a draft QA/QC plan to share our approach with the PAC (Appendix B). During the next project period, we will complete the method development and respond to any comments on the QA/QC plan from the PAC.

During this project period we also conducted studies to evaluate the possible sorption of analytes to the PFE-lined containers used for sample collection. Studies were conducted by adding 1,000 ng/L of each pharmaceutical to PFE-lined sample containers and to glass sample containers to which 2 g/L of sodium chloride was added prior to extraction. Results indicated no significant losses of acidic drugs in either type of sample container (Figure 9). However, significant losses of metaprolol was observed. Unfortunately, the derivitization efficiency for metaprolol was low on this date, and the samples from the glass container only exhibited around 40% recovery. Repitition of this experiment on dates when recovery was better verified that significant losses of beta-blockers occurs through sorption (data not shown). The conditions used here likely overestimate the importance of sorption in environmental samples because other solutes in natural waters will compete for sorption sites. Nevertheless, we conclude that glass containers should be used for the occurrence survey.

In preparing for the recovery study, we also considered the possible losses of analytes after sample collection through reactions with residual chlorine. Preliminary studies in our laboratory have indicated that pharmaceuticals with amine functional groups (e.g., diclofenac, propranolol) react with hypochlorous acid. Because some of the wastewater effluents that we plan to study may have residual chlorine, we believe that transformation of compounds could occur after sample collection. During the next project period, we will investigate the use of sodium thiosulfate as a preservative for samples with chlorine residuals.
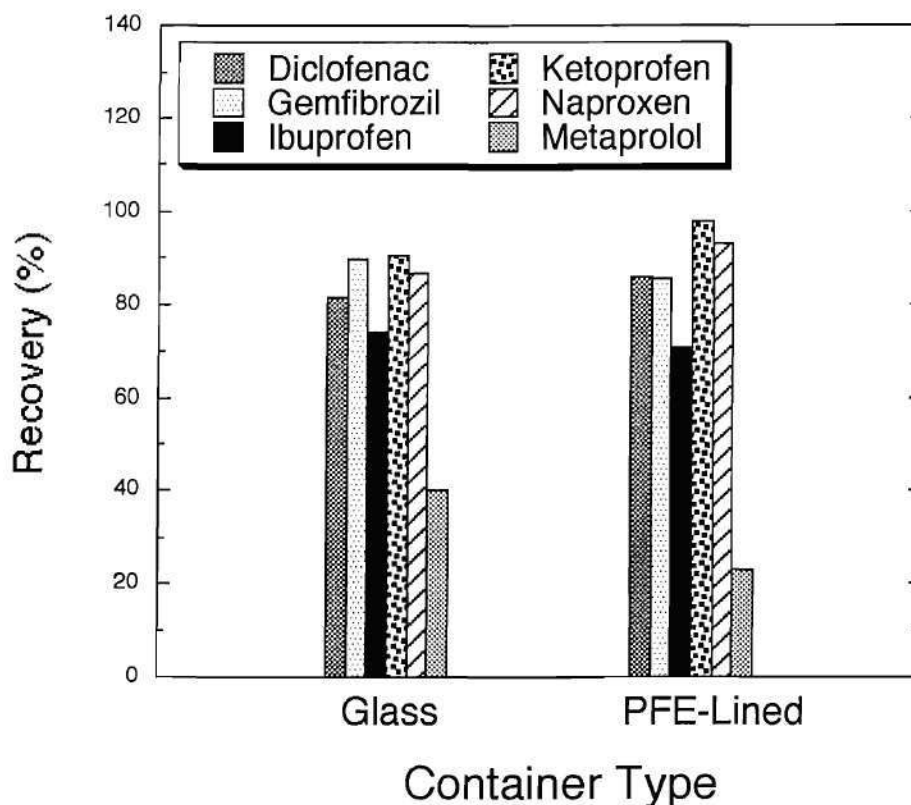
Figure 9: Effect of container type on recovery of acidic drugs and metaprolol. Results are based on the average of duplicate experiments.

Preliminary Results: During method development and testing, duplicate samples were analyzed from sites that may be included in the occurrence survey. These results are considered preliminary because method development is incomplete. However, the preliminary results will guide us in the design of the occurrence survey. During the first and second project periods we collected samples from a total of seven different candidate sites (i.e., four wastewater treatment plants, two engineered wetlands and one groundwater recharge site). During the current project period, we collected samples from the West Central Basin Advanced Wastewater Treatment Plant, the Russian River (which we consider to be a background site because it receives <5% of

its water from wastewater-derived sources) and the Sweetwater groundwater replenishment site. Results are included in Appendix A and are discussed under sub-task 3a.

Sub-Task 2B: Antibiotics

During the third project period, we continued method development for ciprofloxacin, sulfamethazine, and sulfamethoxazole antibiotics. Much of our efforts were devoted to the development of solid phase extraction (SPE) methods for measuring these antibiotics in wastewater effluent. Fine-tuning of the previously developed HPLC-FLD (fluorescence) and HPLC-UV methods was also conducted. Development of HPLC/MS methods for the three antibiotics was initiated near the end of this quarter; preliminary methods for sulfamethazine and sulfamethoxazole are described in this report. As mentioned in the previous report, we intend to use HPLC-FLD to quantify ciprofloxacin with confirmatory analysis by HPLC/MS. We consider HPLC-FLD to be a highly sensitive technique and combination of two independent analytical methods can provide the most accurate results. We will employ HPLC/MS to quantify sulfamethazine and sulfamethoxazole. The development of HPLC-UV methods for these antibiotics was necessary to evaluate the efficiency of SPE methods and was not intended as a quantification method for environmental samples.

To develop analytical methods, wastewater samples were collected from the F. Wayne Hill Wastewater Treatment Plant (FWH WWTP), which is located in the metropolitan Atlanta area. The FWH WWTP is a 12 MGD (to be expanded to 20 MGD in the near future) municipal wastewater facility quipped with multiple-zone activated sludge biological reactors, high lime clarification, declining rate filters, granular activated carbon filters, and ozone disinfection. During the third quarter of the project, grab samples of secondary (after activated sludge treatment) and final effluents were collected on three different dates. Wastewater samples from this plant are used for method development because of convenience of sampling. In addition, the results from conventional and advanced treatment processes may provide insights for removal of antibiotics by different treatment processes. Future method development will also employ samples collected from facilities in California and Arizona where analyses of other PhACs are being conducted.

In the following paragraphs, the progress made in method development during the third quarter is discussed. Details of the method development are described in the Appendix C.

Solid Phase Extraction

After testing various solid phase extraction methods, we established a SPE method using a combination of Oasis HLB cartridges (500 mg/6ml, hydrophilic-lipophilic balance, Waters Corporation, Milford, MA) and HPLC-SAX cartridges (500 mg/3mL, quaternary amine, Cl⁻ counterion, Supelco, Bellefonte, PA). The HPLC-SAX cartridge is stacked on top of the Oasis HLB cartridge using an adapter. Among all the SPE cartridges tested, the Oasis HLB cartridges yielded the highest recovery for all three antibiotics. The HPLC-SAX anion exchangers were used to extract highly negatively-charged dissolved organic matter but not the antibiotics, thus can be used to reduce the amount of dissolved organic matter extracted that may later interfere with analysis by HPLC-FLD and HPLC/MS.

Prior to extraction, acidification of wastewater (to a pH of 4.0 to 4.5) is necessary to maintain a neutral charge on the antibiotics. Results indicate that anion exchange cartridges reduce the amount of interfering organic matter in the concentrated extracts without retaining the antibiotics. The reduction in the amount of dissolved organic matter extracted alleviates much of the interference in the chromatograms; however, it is still not yet sufficient enough for some secondary effluent extracts as discussed in a later section regarding HPLC-FLD.

The spike recovery data from the method development are listed in Tables 2 and 3. In the secondary effluent, recoveries ranged between 45 to 59% for ciprofloxacin, 45 to 46% for sulfamethoxazole, and 48 to 62% for sulfamethazine. In the final effluent, recoveries ranged between 120 to 174% for ciprofloxacin, and 20 to 31% for the sulfonamides. Problems were encountered during the blowdown step for the final effluent samples and probably contributed to the observed wide range of recoveries. In general, the spike recoveries obtained in wastewater matrices are comparable to those in DI water matrices. These recoveries are considerably better than those obtained using other reverse-phase SPE cartridges (typically less then 20% of recoveries, see Appendix C for details of SPE methods). Compared to analysis by HPLC/UV, HPLC/MS analysis indicated lower recoveries (Table 3). We attribute these discrepancies to problems with HPLC/MS analysis, which are discussed in a later section of this report.

We also dedicated a considerable amount of effort during the third project period to development of a cation exchange SPE method for ciprofloxacin before turning our attention to the double-cartridge method described above. Extraction of fluoroquinolones from wastewater and surface water by cation-exchangers has been reported in the literature. Despite various

efforts in testing and modifying the methods, recoveries of spiked samples were generally less then 20% (see Table 2 and Appendix C). This is probably due to breakthrough that is difficult to minimize. Compared to the reverse-phase SPE methods, cation exchange extraction is more selective, less likely to lead to significant matrix interference in detection, and thus more suitable for detection method such as HPLC-FLD (this has been shown during our method development). We are currently conducting additional tests to evaluate cation exchange SPE (e.g., different flow conditions and cation exchangers). Based on these results, we will determine whether to continue or terminate pursuit of cation exchange SPE methods.

In addition to experiments with extraction and elution methods in solid phase extraction, we also assessed losses that occur during our blowdown steps. The results indicate that the blow down step may contribute to the observed fluctuation in recovery. We will continue establishing an improved technique to increase consistency and percentage in recovery.

Table 2: Results of method development for ciprofloxacin in wastewater samples.

Wastewater Sample Type	SPE Method	Spike Recovery % By HPLC/FLD	Conc. (ng/L) in WW, Corrected by Recov.
Secondary Effluent #1	A	11	90
Secondary Effluent #2	A	17	62
DI Water Spiked #1	A	12	-
DI Water Spiked #2	A	13	-
Secondary Effluent #1	C	59	39
Secondary Effluent #2	C	45	89
Final Effluent #1	C	174	10
Final Effluent #2	C	120	6
DI Water Spiked #1	C	0	-
DI Water Spiked #2	C	67	-

Where: A = Oasis-MCX cation exchange tubes, sample acidified to pH 2.0 to 2.5;
C = HPLC-SAX + Oasis HLB tubes, sample acidified to pH 4.0 to 4.5.

Table 3: Results of method development for sulfamethoxazole and sulfamethazine in wastewater samples.

Wastewater Sample Type	Sampling Date	SPE Method	Spike Recovery %		
			HPLC/UV (1)	HPLC/UV (2)	HPLC/MS
Sulfamethoxazole:					
Secondary Effluent #1	2/8/01	B	31	-	-
Secondary Effluent #2	2/8/01	B	34	-	-
DI Water Spiked #1	2/8/01	B	72	-	-
DI Water Spiked #2	2/8/01	B	70	-	-
Secondary Effluent #1	3/23/01	C	45	45	0
Secondary Effluent #2	3/23/01	C	45	46	17
Final Effluent #1	3/23/01	C	26	27	13
Final Effluent #2	3/23/01	C	20	20	12
DI Water Spiked #1	3/23/01	C	47	-	-
DI Water Spiked #2	3/23/01	C	0	-	-
Sulfamethazine:					
Secondary Effluent #1	2/8/01	B	83	-	-
Secondary Effluent #2	2/8/01	B	61	-	-
DI Water Spiked #1	2/8/01	B	80	-	-
DI Water Spiked #2	2/8/01	B	77	-	-
Secondary Effluent #1	3/23/01	C	62	52	43
Secondary Effluent #2	3/23/01	C	60	48	30
Final Effluent #1	3/23/01	C	31	27	37
Final Effluent #2	3/23/01	C	25	18	13
DI Water Spiked #1	3/23/01	C	47	-	-
DI Water Spiked #2	3/23/01	C	54	-	-

Where: B = Oasis-HLB tubes only; sample acidified to pH 4.0 to 4.5; C = HPLC-SAX + Oasis HLB tubes, sample acidified to pH 4.0 to 4.5. Note that analyses by HPLC-UV (2) and HPLC-MS were conducted simultaneously on a HPLC/UV/MS system.

Analysis by HPLC-FLD

In the last quarter, problems were encountered concerning the stability and recoveries of ciprofloxacin from standards, deionized water, and wastewater samples. These problems were resolved through acidification of the samples to a pH of less than 3.0. Ciprofloxacin appears to be more stable at a lower pH and thus has allowed the standards and samples to be stable for longer periods of time. The other problem seen last quarter was carryover of ciprofloxacin in the HPLC system. This problem was eliminated with a new column, consistent needle washing with methanol, and an updated gradient program (see Appendix C). The new method shows a smaller standard deviation and consistently yields a linear calibration curve ($r^2 > 0.995$).

Concentrated wastewater extracts from the double-cartridge SPE method were analyzed by the updated HPLC-FLD method. Based on the retention time and fluorescence spectrum,

ciprofloxacin can be identified in the wastewater qualitatively. However, quantification is uncertain. As shown in Figures 10 and 11, the elevated and noisy baselines (caused by co-extracted dissolved organic matter) in secondary effluent extracts significantly interfere with quantification and may lead to inaccurate results. This problem is less pronounced in the final effluent extracts; the final effluent has considerably less dissolved organic matter compared to the secondary effluent. We conducted quantification on these results to assess the likely concentration rage of ciprofloxacin in wastewater. The preliminary calculation yielded ciprofloxacin concentrations at approximately 70 ng/L and 8 ng/L in secondary and final effluent respectively (Table 2).

Our investigation shows that the new extraction method (HPLC-SAX plus Oasis HLB cartridges) improves recoveries significantly, but may result in highly complex sample extracts that are not suitable for fluorescence analysis, particularly when the analyzed wastewater has high organic matter content. The improved recovery can be attributed to the higher affinity of Oasis HLB cartridges to the antibiotics. However, the high affinity of Oasis HLB cartridges to dissolved organic matter in general also leads to greater matrix interference. Although the HPLC-SAX anion exchangers reduce the amount of dissolved organics extracted, the matrix interference in secondary effluents still cannot be eliminated. A HPLC/MS method for ciprofloxacin is currently under development and may overcome the interference problem. We will also utilize proper internal standards such as related fluoroquinolones in future analysis to assess matrix effect on quantitation of ciprofloxacin.

We recently began assessing three other fluoroquinolones (ofloxacin, norfloxacin and enrofloxacin) for their potential use as internal standards and any interference with ciprofloxacin quantification. Ofloxacin and norfloxacin could potentially exist in wastewater because they are also used for human disease treatment, however they are likely to be present at considerably lower concentrations than ciprofloxacin (i.e., they were not among the top 200 prescription drugs in 1999). Enrofloxacin is used in poultry industry; its presence in some surface water is possible but generally not expected in municipal wastewater. Enrofloxacin can serve as an internal standard after its absence in municipal wastewater has been confirmed. Fluoroquinolone antibiotics currently not used in the U.S. will also be good internal standards. Obtaining such fluoroquinolone compounds is more difficult and we will continue to explore the possibilities of obtaining appropriate compounds. Our preliminary results indicate that ofloxacin and

norfloxacin do not interfere with the analysis of ciprofloxacin. We will investigate enrofloxacin in the next quarter, and the results will be discussed in the following report.

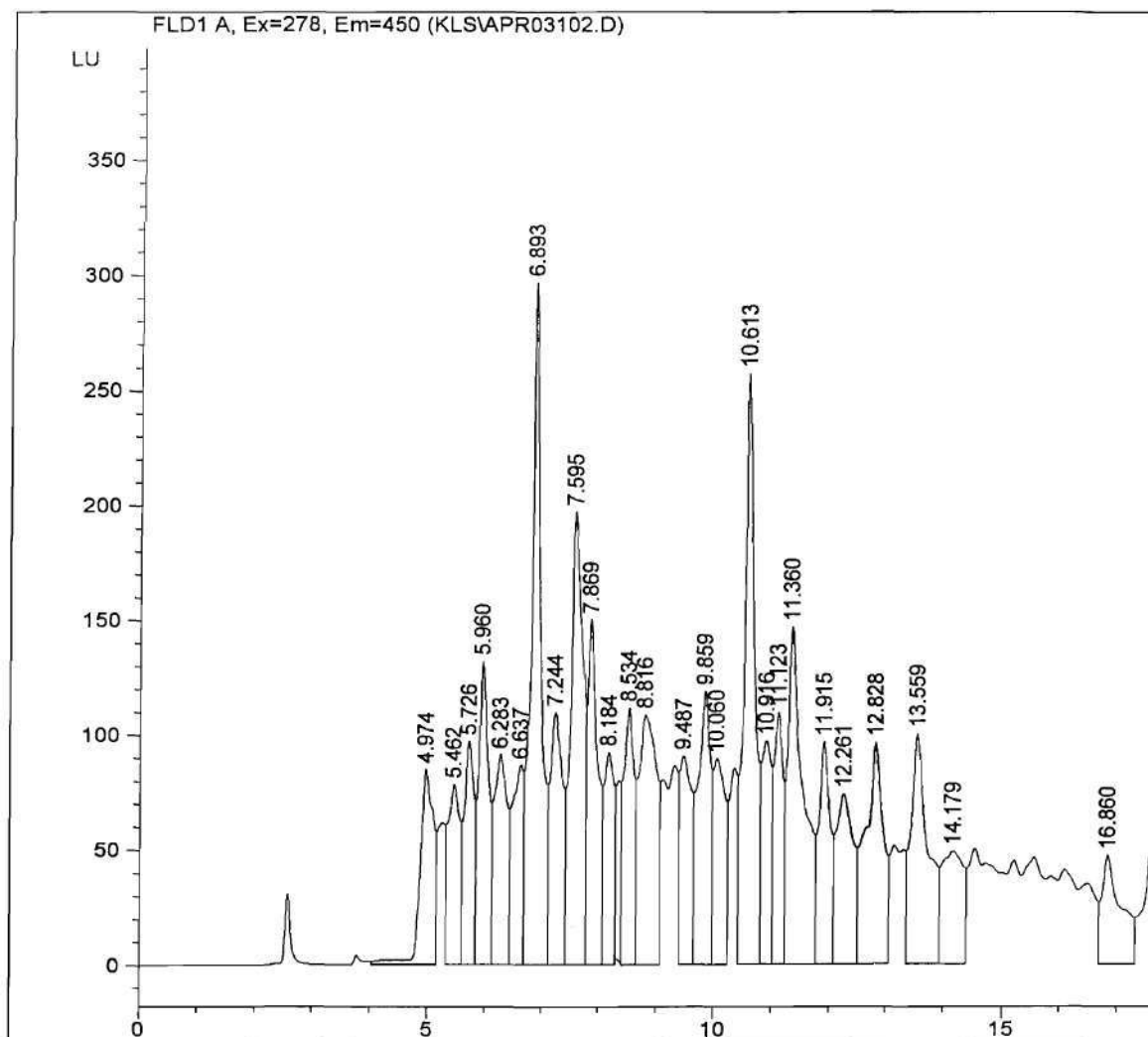Figure 10: HPLC/Fluorescence chromatogram for ciprofloxacin (11.123 min) in secondary wastewater effluent extract.
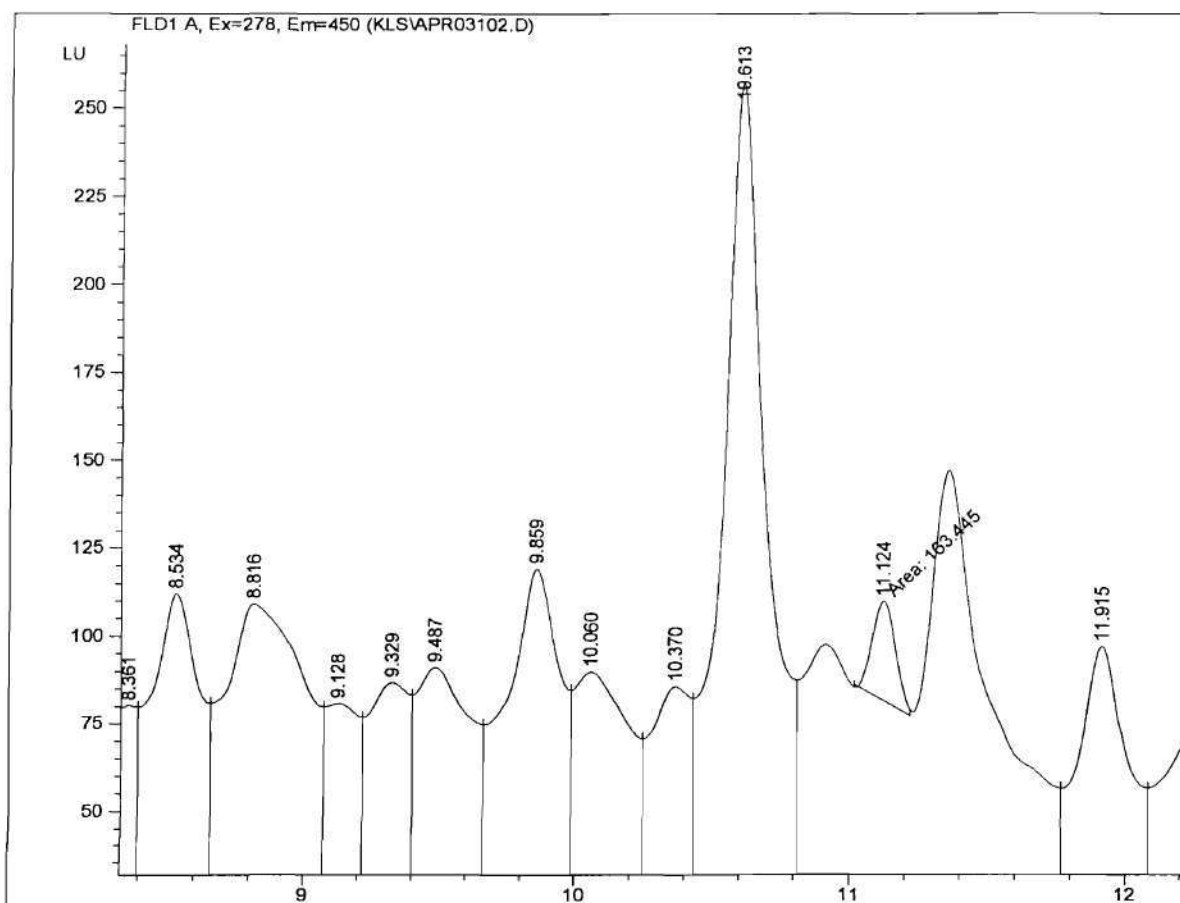
Figure 11: HPLC/Fluorescence chromatogram for ciprofloxacin (11.124 min) in secondary wastewater effluent extract, zoom view. The peak area corresponds to a concentration of approx. 46 µg/L (note that the wastewater extract has a concentration factor of 1000 and an observed average recovery of 52%).

A preliminary electrospray HPLC/MS method with good sensitivity has been developed for sulfamethoxazole and sulfamethazine. Both scan and selected ion monitoring (SIM) data collection methods were used. Details of the preliminary HPLC/MS methods are described in Appendix C.

Figure 13 illustrates the SIM results of sulfamethoxazole and sulfamethazine molecular ions (254 m/z and 279 m/z respectively) in both unspiked and spiked secondary effluent extracts. The preliminary results suggest that sulfamethoxazole may be present in the secondary wastewater effluent while sulfamethazine is not. This 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 preliminary analysis did not show significant amount of either sulfonamide in the final effluent (i.e., after GAC and ozone).

For the same wastewater extract, MS analysis yielded lower recoveries compared to UV analysis (Table 3). This is probably caused by matrix interference in antibiotic ionization; the higher amount of interfering compounds present, the lower the effectiveness in ionizing the antibiotic molecules. The matrix interference should be proportional to the organic matter content of the samples. This seems to be consistent with the observed results that this discrepancy is smaller in the final effluent extracts than in the secondary effluent extracts (Table 3). We plan to analyze the spiked DI water extracts to assess this effect. It also appears that sulfamethoxazole is more susceptible to matrix interference than sulfamethazine. During the next project period, we will evaluate appropriate related sulfonamides as potential internal standards in the future analysis to assess the effect of matrices on analysis and quantification. We will also continue to improve this HPLC/MS method. For example, we plan to improve the HPLC gradient program to better separate interfering compounds from antibiotics, and to conduct more analysis to assess the occurrence of these two sulfonamides.

Time (minutes)

Time (minutes)
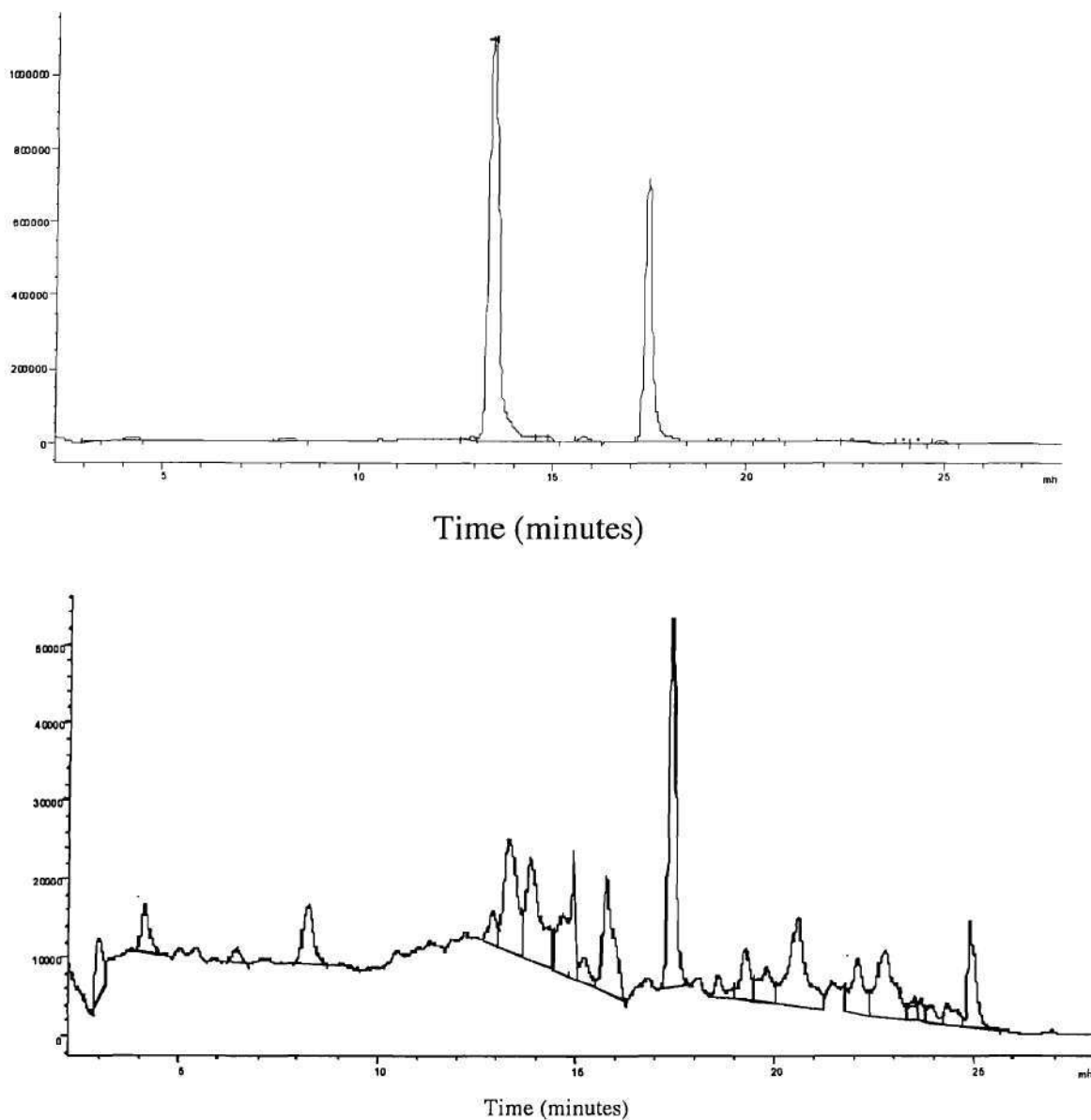
Figure 12. HPLC/MS analysis for sulfamethazine (~13.4 min) and sulfamethoxazole (~17.4 min) in secondary wastewater effluent extracts. Top: spiked sample; bottom: unspiked sample. Electrospray ionization with SIM (279 m/z and 254 m/z ions). Concentrations of sulfamethazine and sulfamethoxazole in the spiked sample are approx. 6.0 and 3.5 mg/L respectively. In the unspiked sample, the sulfamethoxazole concentration is approximately 60 µg/L (note the wastewater extract has a concentration factor of 1000).

TASK 3: OCCURRENCE SURVEY

Sub-Task 3A: Site Selection

As part of the site selection process, preliminary samples have been collected from sites that we are considering for inclusion in the occurrence survey. A brief description of the sites sampled during this project period and interpretation of the preliminary data are included below:

Municipal Wastewater Treatment Plants: The West Central Basin Municipal Water District operates an advanced wastewater treatment plant in Los Angeles. The treatment plant subjects secondary wastewater effluent from the Hyperion Municipal Wastewater Treatment Plant to microfiltration, reverse osmosis, decarbonation and disinfection with ultraviolet light and chlorine. The water is then injected into the local groundwater aquifer. During the current project period, samples were collected at the influent of the advanced treatment plant, and after microfiltration, reverse osmosis, decarbonation and ultraviolet disinfection. In addition, a background sample of water delivered to the plant from the Metropolitan Water District supply also was analyzed. Results indicate acidic drugs and beta-blockers were present in the plant's influent at concentrations comparable to those measured in other secondary effluent samples during the first two project periods (Figure 13). Concentrations of pharmaceuticals detected after microfiltration were nearly identical to those detected in the influent. After reverse osmosis, concentrations of all compounds were below the limit of quantification (i.e., 10 ng/L). No drugs were detected in the sample collected from the MWD water supply.

Groundwater Recharge Systems: Samples will be collected from groundwater infiltration systems to assess the removal of compounds during recharge. During the second project period, we analyzed samples from the Sweetwater groundwater recharge site, which is located in Tuscon, Arizona. Samples were collected from a basin that receives secondary wastewater effluent and a groundwater well downgradient of the basin screened at a depth of approximately 30 meters. Low concentrations (<5% of the concentrations detected in the basin) of pharmaceuticals were detected in the well. During the current project period, we collected another set of samples from the Sweetwater facility. During this round, we also collected samples from a shallow groundwater well located between the basin and the deep groundwater
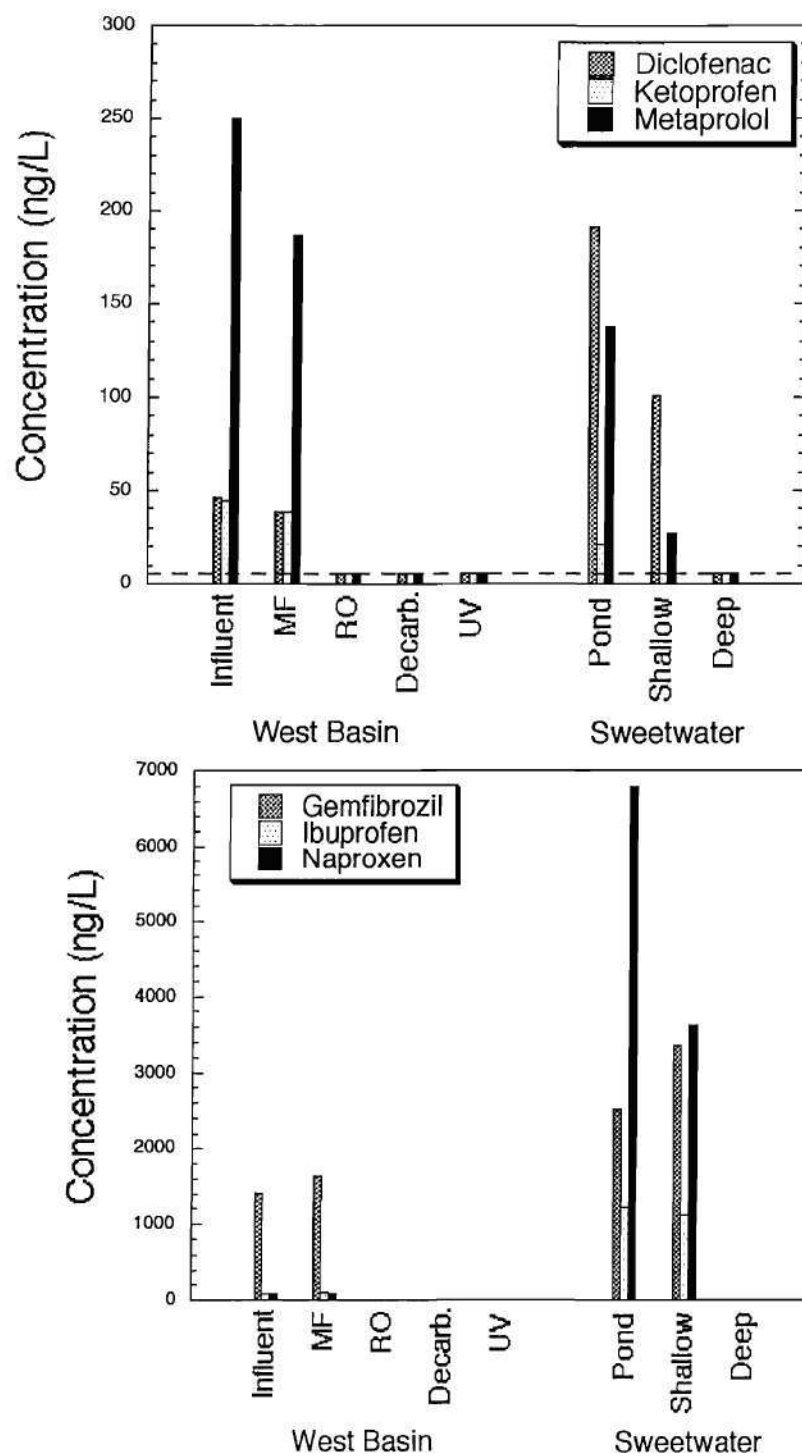
31

Figure 13: Concentrations of acidic drugs and beta blockers measured during method development activities. In the top figure, the dashed line indicates the method detection limit. In the bottom figure, data below the method detection limit are not depicted.

well. Results indicate that the basin contained concentrations of pharmaceuticals comparable to those detected in other secondary wastewater effluent samples (Figure 13). Concentrations of pharmaceuticals in the shallow well were comparable to, or slightly lower than, those detected in the basin. Concentrations of all pharmaceuticals were below the detection limit in the deep well.

During the occurrence survey we plan to collect samples from conventional and advanced wastewater treatment plants, engineered wetlands, groundwater recharge sites and surface waters. The emphasis of our survey will be locations where intentional or unintentional water recycling is practiced. During the first phase of the occurrence survey, we plan to collect samples from representative sites in each of the aforementioned categories. Table 4 lists locations where we plan to collect samples. It includes several sites that we have not yet sampled. If we are unable to obtain samples from these sites, similar sites will be substituted with sites exhibiting similar features. It is our intention to include more sites in the occurrence survey; however, we hesitate to plan for sampling at additional locations until we are satisfied with results from the sites listed in Table 4. After completing the first phase, we will discuss our results with the PAC and propose additional sampling at these sites and/or additional locations.

Sub-Task 3B: Sample Collection and Analysis

No activities related to this sub-task were conducted during this project period.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task were conducted during this project period.

Table 4: Sites to be included in the initial phase of the occurrence survey.

Location	Category[1]	Sample locations[2]
Dublin/San Ramon WWTP	WWTP (2° AS, HOCl)	2° Treatment, Disinfection
Mt. View WWTP	WWTP (Trickling filter, UV)	2° Treatment, Disinfection
Sweetwater WWTP	WWTP (2° AS, HOCl)	2° Treatment, Disinfection
San Jose/Santa Clara WWTP	WWTP (2° AS, BNR, HOCl)	2° Treatment, BNR, Disinfection
Hyperion WWTP[3]	WWTP (2° AS, HOCl)	2° Treatment, Disinfection
Dublin/San Ramon AWWTP	AWWTP (MF, RO, UV, HOCl)	MF, UV, RO, HOCl
West Basin AWWTP	AWWTP (MF, RO, UV, HOCl)	MF, UV, RO, HOCl
OCWD Pilot Plant	AWWTP (MF, RO, UV, HOCl)	MF, UV, RO, HOCl
Witchita Falls (TX) Pilot Plant	AWWTP	MF, UV, RO, HOCl
Mt. View Wetlands	Engineered Wetlands	Influent, middle, effluent
Prado Wetlands	Engineered Wetlands	Influent, middle, effluent
Rio Hondo Spreading Basins	Groundwater Recharge	Pond, shallow well, deep well
Sweetwater Recharge Facility	Groundwater Recharge	Pond, shallow well, deep well
Sacramento Delta	Background	
MWD Water	Background	

Notes:

[1] WWTP = conventional municipal wastewater treatment plant; 2° AS = secondary activated sludge; UV = ultraviolet disinfection; HOCl = chlorine disinfection; BNR = biological nutrient removal; AWWTP = advanced wastewater treatment plant; MF = microfiltration; RO = reverse osmosis. OCWD = Orange County (CA) Water District.

[2] Samples will be collected after each of the unit processes or locations listed.

[3] Influent to the West Basin AWWTP.

PLANS FOR NEXT PERIOD

The following section describes research planned during the next project period. In addition, plans for the remainder of the project are described at the end of each section. A revised schedule for the project is presented in Appendix D.

Task 1: Literature Review

Sub-Task 1A: Drugs Used in Human Therapy

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Sub-Task 1B: Drugs Used in Animal Husbandry

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Task 2: Analytical Method Development and Testing

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

During the next project period, we will complete studies associated with method development and respond to any comments from the PAC on our QA/QC plan (Appendix B). Planned activities are summarized below:

We will evaluate the use of a different form of radiolabeled propranolol as an internal standard for the beta-blockers. Method development will consist of spike recovery studies similar to those performed for mecoprop. We believe that this form of the compound will be useful as an internal standard. If not, we will attempt to identify an alternative with properties similar to those of the beta-blockers.

We will conduct studies to evaluate the efficacy of sodium thiosulfate as a preservative for samples containing residual oxidants. These studies will consist of addition of 0.2 mM sodium hypochlorite to secondary effluent samples spiked with compounds of interest in the presence and absence of sodium thiosulfate.

We will attempt to use the analytical method for carbamazepine developed by Dr. Heberer to analyze samples collected from one or more candidate sites.

Sub-Task 2B: Antibiotics

During the next project period we plan to continue method development and improvement. The planned tasks include improvement of SPE recoveries, use of internal standards to assess recoveries and matrix effects and improvement of the HPLC/MS methods. In addition to using wastewater samples from the FWH WWTP, we also plan to analyze wastewater samples from sites listed in Table 4. Our research plans are summarized below:

Improvement in SPE methods: The HPLC-SAX plus Oasis HLB double-cartridge SPE method appears to yield reasonably good recoveries. Our future efforts will focus on minimizing sample loss during the blowdown step and increasing the recovery efficiency. We will evaluate the feasibility of using a cation exchange SPE method for ciprofloxacin. The cation exchange SPE method is likely to succeed with a smaller extraction volume and very slow flow rate. If these attempts do not considerably enhance the recovery, we will only rely on the developed double-cartridge SPE method. HPLC/MS will then be the primary technique for analyzing ciprofloxacin, particularly in more complex wastewater matrices.

Use of internal standards: The use of internal standards structurally related to our target antibiotics will provide useful information in evaluating the analytical methods, and is also important for QA/QC in occurrence survey. We will continue our investigation on the three related fluoroquinolones (ofloxacin, norfloxacin and enrofloxacin) for their potential use as internal standards. Appropriate sulfonamides related to sulfamethoxazole and sulfamethazine will also be assessed for their use as internal standards. Various sulfonamide antibiotics are available (see Table 5 in the second progress report).

The use of these antibiotics in either humans or agricultural food animals will be taken into consideration in selecting internal standards. In general, antibiotics used in food animals are unlikely to be present in municipal wastewater effluent because of their different routes of entering the environment. For this reason, enrofloxacin may be an appropriate internal standard in analyzing municipal wastewater after its absence has been confirmed. Antibiotics used in

humans but at low use frequency may be appropriate internal standard in surface water samples since their concentration in environmental water is likely to be negligible (this will also need to be tested). In addition, we will explore the possibility of using other fluoroquinolones and sulfonamide antibiotics, particularly those that are used in other countries but not used in the U.S. currently. Although our preliminary experience indicates that obtaining such chemicals is more difficult, we will initiate more contact to explore such possibilities.

Development and improvement in HPLC/MS methods: A new HPLC/MS method for ciprofloxacin will be developed. We will continue to improve the HPLC/MS method for sulfamethoxazole and sulfamethazine. The preliminary results indicate the need to improve the HPLC gradient program to better separate antibiotics from interfering compounds. Non-linearity between concentration and response was observed in MS detection. The deviation from linearity increases as antibiotic concentration increases. We will carefully assess quantification accuracy by establishing more appropriate calibration ranges and by utilization of internal standards.

Task 3: Occurrence Survey

Sub-Task 3A: Site Selection

We will confirm the participation of sites listed in Table 4 and prepare a schedule for sample collection and analysis.

Sub-Task 3B: Sample Collection and Analysis

After completing method development and responding to any comments from the PAC about our QA/QC plan, we will begin to collect samples from the sites listed in Table 4. If no major problems are encountered during method development, we plan to complete one round of analysis of samples from at least five of the sites. If progress is better than anticipated, we will attempt to collect samples from additional sites.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task are planned during this project period. After completion of the occurrence survey, data will be evaluated to identify trends meriting further

study. Data will be compared with expectations based on physical/chemical properties of the compounds as well as results reported by other researchers.

APPENDIX A: Summary of Data for Acidic Drugs and Beta-Blockers during the Third Project Period

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Diclofenac	West Central Basin-Influent	2/21/01	46, 48	34%, 33%
	West Central Basin-Microfiltration	2/21/01	42, 35	66%, 64%
	West Central Basin-Reverse Osmosis	2/21/01	<10, <10	
	West Central Basin-Decarbonation	2/21/01	<10, <10	
	West Central Basin-UV Disinfection	2/21/01	<10, <10	
	West Central Basin-MWD Water	2/21/01	<10, <10	
	Blank	2/21/01	22	73%
	Russian River-Caisson Site	4/9/01	<10, <10	
	Russian River Upstream Site	4/9/01	<10, <10	93%, 35%
	Russian River-Downstream Site	4/9/01	<10, <10	76%, 91%
	Blank	4/9/01	<10	94%
	Sweetwater-Pond	4/13/01	160, 220	
	Sweetwater-Shallow Well	4/13/01	100, 100	160%, 100%
	Sweetwater-Deep Well	4/13/01	<10, <10	118%
	Blank	4/13/01	<10	87%
Gemfibrozil	West Central Basin-Influent	2/21/01	1500, 1300	
	West Central Basin-Microfiltration	2/21/01	1500, 1800	
	West Central Basin-Reverse Osmosis	2/21/01	<10, <10	
	West Central Basin-Decarbonation	2/21/01	<10, <10	
	West Central Basin-UV Disinfection	2/21/01	<10, <10	
	West Central Basin-MWD Water	2/21/01	<10, <10	
	Blank	2/21/01	14	119%
	Russian River-Caisson Site	4/9/01	<10, <10	
	Russian River Upstream Site	4/9/01	<10, <10	112%, 41%
	Russian River-Downstream Site	4/9/01	<10, <10	106%, 106%
	Blank	4/9/01	<10	88%
	Sweetwater-Pond	4/13/01	2400, 2500	
	Sweetwater-Shallow Well	4/13/01	3400, 3200	
	Sweetwater-Deep Well	4/13/01	<10, <10	115%
	Blank	4/13/01	<10	92%
Ibuprofen	West Central Basin-Influent	2/21/01	82, 82	31%, 31%
	West Central Basin-Microfiltration	2/21/01	88, 110	48%, 52%
	West Central Basin-Reverse Osmosis	2/21/01	<10, <10	
	West Central Basin-Decarbonation	2/21/01	<10, <10	
	West Central Basin-UV Disinfection	2/21/01	<10, <10	
	West Central Basin-MWD Water	2/21/01	<10, <10	
	Blank	2/21/01	<10, <10	73%
	Russian River-Caisson Site	4/9/01	<10, <10	57%, 63%
	Russian River Upstream Site	4/9/01	<10, <10	75%, 33%
	Russian River-Downstream Site	4/9/01	<10, <10	75%, 83%
	Blank	4/9/01	<10, <10	76%
	Sweetwater-Pond	4/13/01	1100, 1300	
	Sweetwater-Shallow Well	4/13/01	1200, 1000	
	Sweetwater-Deep Well	4/13/01	<10, <10	99%, 150%
	Blank	4/13/01	<10	54%

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Ketoprofen	West Central Basin-Influent	2/21/01	40, 50	14%, 44%
	West Central Basin-Microfiltration	2/21/01	42, 35	23%, 38%
	West Central Basin-Reverse Osmosis	2/21/01	<10, <10	
	West Central Basin-Decarbonation	2/21/01	<10, <10	
	West Central Basin-UV Disinfection	2/21/01	<10, <10	
	West Central Basin-MWD Water	2/21/01	<10, <10	
	Blank	2/21/01	<10	26%
	Russian River-Caisson Site	4/9/01	<10, <10	70%, 98%
	Russian River Upstream Site	4/9/01	<10, <10	107%, 31%
	Russian River-Downstream Site	4/9/01	<10, <10	95%, 100%
	Blank	4/9/01	<10	110%
	Sweetwater-Pond	4/13/01	16, 27	85%, 98%
	Sweetwater-Shallow Well	4/13/01	<10, <10	73%, 69%
	Sweetwater-Deep Well	4/13/01	<10, <10	106%
	Blank	4/13/01	<10	84%
Naproxen	West Central Basin-Influent	2/21/01	70, 92	13%, 13%
	West Central Basin-Microfiltration	2/21/01	89, 89	25%, 47%
	West Central Basin-Reverse Osmosis	2/21/01	<10, <10	
	West Central Basin-Decarbonation	2/21/01	<10, <10	
	West Central Basin-UV Disinfection	2/21/01	<10, <10	
	West Central Basin-MWD Water	2/21/01	<10, <10	
	Blank	2/21/01	17	25%
	Russian River-Caisson Site	4/9/01	<10, <10	17%, 17%
	Russian River Upstream Site	4/9/01	<10, <10	94%, 30%
	Russian River-Downstream Site	4/9/01	<10, <10	86%, 82%
	Blank	4/9/01	<10	85%
	Sweetwater-Pond	4/13/01	5600, 8000	
	Sweetwater-Shallow Well	4/13/01	3400, 3800	
	Sweetwater-Deep Well	4/13/01	<10, <10	104%
	Blank	4/13/01	<10	80%

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Metaprolol	West Central Basin-Influent	2/21/01	250, 250	56%
	West Central Basin-Microfiltration	2/21/01	220, 160	83%
	West Central Basin-Reverse Osmosis	2/21/01	<10, <10	
	West Central Basin-Decarbonation	2/21/01	<10, <10	
	West Central Basin-UV Disinfection	2/21/01	<10, <10	
	West Central Basin-MWD Water	2/21/01	<10, <10	
	Blank	2/21/01	<10, <10	
	Russian River-Caisson Site	4/9/01	<10, <10	14%, 41%
	Russian River Upstream Site	4/9/01	<10, <10	29%, 24%
	Russian River-Downstream Site	4/9/01	<10	24%, 12%
	Blank	4/9/01	<10	79%
	Sweetwater-Pond	4/13/01	71, 200	
	Sweetwater-Shallow Well	4/13/01	31, 23	100%
	Sweetwater-Deep Well	4/13/01	<10, <10	50%, 81%
	Blank	4/13/01	<10	85%
Propranolol	Russian River-Caisson Site	4/9/01	<10, <10	4%, 12%
	Russian River Upstream Site	4/9/01	<10, <10	14%, 28%
	Russian River-Downstream Site	4/9/01	<10	24%, 11%
	Blank	4/9/01	<10	105%
	Sweetwater-Pond	4/13/01	<10, <10	76%, 47%
	Sweetwater-Shallow Well	4/13/01	<10, <10	73%, 88%
	Sweetwater-Deep Well	4/13/01	<10, <10	38%, 41%
	Blank	4/13/01	<10	85%

APPENDIX B: QA/QC Plan for Drugs Used in Human Therapy (Excluding Antibiotics)

Sample Collection: Grab samples will be collected in 1-L glass bottles with Teflon-lined screw caps. Each bottle will be kept in an individual polyethylene bag. Prior to sampling, bottles will be cleaned in our laboratory with Micro brand laboratory detergent, rinsed with water followed by methanol and deionized water between each analysis. Bottles will be shipped to participants in coolers with blue ice packs.

For samples collected from wastewater treatment plants or water treatment plants using chlorine for disinfection, $Na_2S_2O_3$ will be added to the samples bottle as a preservative. Each set of samples will be shipped with a field blank, which will be analyzed with the samples. Samples will be collected by field personnel who are familiar with trace organic sampling protocols. Field personnel will wear polyethylene gloves when handling bottles and will be instructed to minimize the amount of time that the bottle is kept uncapped outside of the cooler.

Sampling times, locations and personnel will be recorded on a log sheet that will accompany each set of samples. Each sample will be given a unique sequential sample identification number as indicated on the log sheet. To prevent bias, sample numbers will not provide any indication of sample locations. Samples will be shipped in the cooler via overnight mail. Upon arrival at UC Berkeley, samples and log sheets will be visually inspected and transferred to a 5°C storage area. Samples will extracted as soon as practical and within no more than 72 hours after arrival.

Sample Extraction and Analysis: Each set of ten samples will be analyzed in a batch that contains appropriate QA/QC standards. The following samples will be included with each set of samples.

(1) Field blank (1 L of deionized water that travels to and from the field site);

(2) Matrix recovery sample (1 sample from the site spiked with each analyte at a concentration greater than 20 times that expected in the sample);

(3) Duplicate sample;

(4) Auxiliary standard consisting of a mixture of the derivatized analytes, as prepared by a third party in our laboratory.

All samples will be amended with an internal standard (radiolabeled mecroprop for the acidic drugs and radiolabeled propranolol for the beta-blockers) prior to extraction. After derivitization, samples will be diluted to 1 mL prior to addition of the secondary internal standard, hexachlorobenzene.

The run sequence will consist of five standards followed by a randomized mixture of the samples and QA/QC samples. The calibration curve will be checked every ten samples by running a blank and a reslope standard from the middle of the calibration curve. If the calibration standard disagrees with the standard curve by more than 25% the samples in the following section will be rerun.

Our target for recoveries will be 60-120%. For any sample or batch of samples in which these values are not obtained, we will rerun all of the samples or repeat the analysis. If acceptable recoveries are not obtained, we will report the data with permanent qualifiers.

APPENDIX C: Analytical Methods for Antibiotics

Sources of chemicals and preparation of antibiotic standard solutions were described in the second progress report Appendix B.

Collection of Samples

The samples were collected in 2 liter PFE-lined bottles that had been washed with Alconox detergent, methanol and deionized water. The collected samples will be kept in a cooler with ice and transported back to the laboratory within 2 hours. The samples were immediately filtered with a 0.5 μm glass fiber filter and stored at 4°C until extraction, which occurred within seven days.

QA/QC in Method Development

For each sample, at least a total of four independent analyses were made; two unspiked duplicates and two spiked duplicates. For each analysis, the sample underwent acidification, solid phase extraction and analysis by one to two different detection methods. In addition, a blank consisting of 1 L of deionized water and a spiked blank were also included on each date in which extractions were performed. The standard curve used to quantify ciprofloxacin consisted of six points in the range of 5 μg/L to 500 μg/L. The standard curve used to quantify sulfamethazine and sulfamethoxazole consisted of 0.1 mg/L to 20 mg/L. All the standards were prepared in DI water matrices.

Solid Phase Extraction of Antibiotics

Current Method: To analyze samples for ciprofloxacin, sulfamethazine and sulfamethoxazole, 1 L of wastewater was extracted. Such extraction volume is considered necessary for unspiked wastewater samples to be analyzed by the selected analytical methods. Prior to solid phase extraction, wastewater samples were acidified to a pH of 4.0 to 4.5 with phosphoric acid. For spiked recovery analyses, 200 ng/L of ciprofloxacin and 20 μg/L sulfamethazine and sulfamethoxazole were added. The higher spike concentration for the two sulfonamides is necessary for assessing recovery by HPLC/UV detection. The spike recovery for ciprofloxacin was determined by HPLC/FLD.

Extraction was performed with a 500 mg/6 mL Oasis HLB (hydrophilic-lipophilic balance) cartridge (Waters Corporation, Milford, MA) and a 500 mg/3 mL HPLC-SAX (quaternary amine, Cl⁻ counterion) cartridge (Supelcean, Supelco, Bellefonte, PA). The HPLC-SAX SPE tube was stacked on top of the HLB cartridge using an adapter. This configuration was chosen in order to reduce the interference of the wastewater organics in the chromatograms. Before being stacked together, the cartridges were conditioned with 10 mL of methanol and 10 mL of deionized water that had been adjusted to a pH of 4.0 to 4.5 with phosphoric acid.

The sample was then pulled through the stacked cartridges under a vacuum at a flow rate of less than 5 mL per minute using a solid phase extraction apparatus (Visiprep, Supelco, Bellefonte, PA). After the entire sample had passed through the cartridges, the cartridges were dried by pulling air through it for approximately five minutes. Wastewater organics were then eluted with 10 mL of acidified methanol (5 % 1 M phosphoric acid, 95 % methanol). Elution was conducted separately for the Oasis HLB and HPLC-SAX cartridges. The collected eluents were blown down to dryness under a gentle stream of nitrogen gas and in a water bath of 37 °C. The dried extracts were then redissolved in 800 μL deionized water and 200 μL methanol.

The SPE method described above was determined to have the best spike recovery performance after testing various conditions including several different acids, acidification pHs and elution solvent combinations. Analysis by HPLC/FLD and HPLC/UV indicate that the antibiotics were not retained by the anion exchange cartridges. The double-cartridge SPE method reduces the amount of interfering compounds in the concentrated wastewater extracts.

Other Methods Tested: Other cartridges were tested thoroughly with ciprofloxacin samples and determined to be less efficient than the HLB cartridges. Cartridges tested include 500 mg HPLC-18 (octadecyl, endcapped), ENVI-18 (octadecyl, endcapped), HPLC-SCX (aliphatic sulfonic acid, Na⁺ counterion), HPLC-Ph (phenyl) cartridges (Visiprep, Supelco, Bellefonte, PA) and Oasis MCX (mixed-mode polymeric sorbent) cartridges (Waters corporation, Milford, MA). For the cation exchange cartridges (HPLC-SCX and Oasis MCX), samples were acidified to a pH of 2.0 to 2.5 with phosphoric acid. Cartridges were conditioned with 10 mL of methanol followed by 10 mL of acidified deionized water at a pH of 2.0 to 2.5. Samples were eluted with 5-10 mL of 0.5 M NH₄Cl in methanol and then blown down gently as described above. For the other reverse-phase tubes tested, the extraction was performed similarly to the method described

other reverse-phase tubes tested, the extraction was performed similarly to the method described for HLB tubes, except that elution was performed with 5-10 mL of methanol. The range of recoveries found for each tube is listed in Table C-1.

Table C-1: Range of spike recoveries for SPE tubes for antibtioics.

Antibiotic	SPE Tube	Range of Recoveries	Mean Recovery
Ciprofloxacin	HPLC-18	5% to 32%	20%
	HPLC-Ph	~5%	5%
	ENVI-18	< 5%	< 5%
	HPLC-SCX	24% to 80%	30%
	Oasis-MCX	7% to 18 %	10%
	Oasis-HLB	35% to 74%	42%
Sulfamethoxazole	ENVI-18	10 - 28 %	20%
Sulfamethazine	ENVI-18	10 - 24 %	20%

Several modified approaches were also tested on the cation exchange cartridges including reducing the flow rate, reducing wastewater volumes for extraction and various condition/elution solutions. Despite those efforts, spike recoveries were not improved significantly.

The ENVI-18 cartridges were previously used for extracting sulfamethoxazole and sulfamethazine from wastewater. Wastewater samples were acidified to a pH of 2.5 with trifluoroacetic acid. The ENVI-18 cartridges were conditioned with 6 mL of methanol and 6 mL of deionized water acidified to a pH of 2.5 with trifluoroacetic acid. Elution was performed with a solution consisting of 90 % deionized water and 10 % of the acidified water. The highest recovery for spiked samples was only 28 % (Table C-1).

HPLC Analysis

Analysis was performed using a reversed-phase HPLC system (1100, Aligent Technology, Santa Clara, CA) with a quaternary pump, an autosampler, a diode-array UV/Vis detector and a multiple-wavelength fluorescence detector. A 250 mm Eclipse XDB-C18 column (4.6 mm, 5 μm particles, Aligent Technology) was used for sulfamethazine and sulfamethoxazole. A 250 mm Zorbax RX-C18 column (4.6 mm, 5 μm particles, Aligent Technology) was used for ciprofloxacin and related fluoroquinolones. Column temperature was maintained at 25 °C and a flow rate of 1.0 mL/min was employed. Injection volumes were 100 μL.

For ciprofloxacin, the mobile phases include a solution containing 20 mM phosphoric acid and 20 mM sodium phosphate, monobasic (eluent A, pH ≈ 2.4), HPLC-grade acetonitrile (eluent B) and HPLC-grade methanol (eluent C). The mobile phase begins with 0.5 minute isocratic 98% A (2% B), followed by a gradient decrease to 90%A in 0.5 minute, then a gradient decrease to 75% A in 9 minutes, followed by 5 minutes isocratic 75% A. The mobile phase is then switched to 15% A, and the column was flushed under these conditions for 5 minutes. The mobile phase then shifts to isocratic 100% C for 10 minutes to flush the column thoroughly and finally switches back to 98% A and 2% B for the final 6 minutes of the run. Detection of ciprofloxacin was conducted with the UV detector at 278 nm, and with the fluorescence detector at an excitation wavelength of 278 nm and an emission wavelength of 450 nm. UV and FLD spectra were collected for wastewater analyses to ensure correct identification of ciprofloxacin. The FLD detection limit for ciprofloxacin is approx. 5 μg/L in wastewater matrices. Carryover problem was minimized by this updated method. Acidified standards (to a pH of less than 3.0) were employed, yielding consistently linear calibration curves ($r^2 > 0.995$) and a smaller standard deviation (0.1889) than neutral pH standards.

For sulfamethazine and sulfamethoxazole, the mobile phases include a solution containing 6.5 mM trifluoroacetic acid (eluent A, pH~2.5) and HPLC-grade acetonitrile (eluent B). The conditions for HPLC were as follows: 2 minutes isocratic 3% B followed by a gradient increase to 33.8% B in 28 minutes. The column is then flushed with 64. 6% B for 2 minutes followed by a 3 minute equilibration time for 3% B before the next injection. The retention time for the sulfamethazine and sulfamethoxazole were approximately 17 min and 25 min respectively. Detection of both sulfonamides was at 260 and 270 nm. The UV detection yielded consistently linear calibration curves for both sulfonamides with R^2 value greater than 0.98. The detection limit of UV detection is approx. 1 μg/L for sulfonamide standards. However, due to severe interference from other dissolved organics, UV detection is not sensitive enough to detect sulfamethazine and sulfamethoxazole in unspiked wastewater samples.

HPLC/MS Analysis: Sulfamethazine and Sulfamethoxazole

Methods were developed on a HPLC/UV/MS system (Hewlett-Packard, Series 100 MSD G1946A, Palo Alto, CA) for sulfamethazine and sulfamethoxazole. A 150 mm Zorbax SB-C18 column was used (2.1 mm, 5 μm particles, Agilent Technology). The employed flow rate was

0.2 ml/min with an injection volume of 20 µL. The column temperature was maintained at 30°C. The mobile phases include a solution containing 0.2% acetic acid and 10% acetonitrile (eluent A) and HPLC-grade acetonitrile (eluent B). The conditions for HPLC were as follows: 2 minutes isocratic 100% A followed by a gradient increase to 33.8% B in 16 minutes. The column is then flushed with 64.0% B for 4 minutes. Sulfamethazine and sulfamethoxazole were detected by the UV detector at 265 nm and retention time approx. 14 min and 17 min respectively. For each sample analysis, both UV and MS spectra were collected.

MS analysis was conducted using electrospray ionization at positive ion mode. The employed MS conditions are summarized in Table AB.2. In addition, the fragmentation voltage used was 70 eV. Figure C-1 and C-2 show the typical mass spectrum of sulfamethoxazole and sulfamethazine respectively. Both scan and single ion monitoring analyses were conducted. The scan parameter had a mass range of 250 to 300 m/z. SIM was conducted for the molecular ions of sulfamethoxazole and sulfamethazine (254 m/z and 279 m/z).

The calibration curves by MS analysis yielded reasonably good linearity for sulfamethazine with a R^2 of 0.94. Non-linearity in calibration curves was more pronounced for sulfamethoxazole with a R^2 of 0.84. At high sulfamethoxazole concentrations, MS response was lower than expected resulting in deviation from linear relationship.

Table C-2. Operation parameters of HPLC/MS

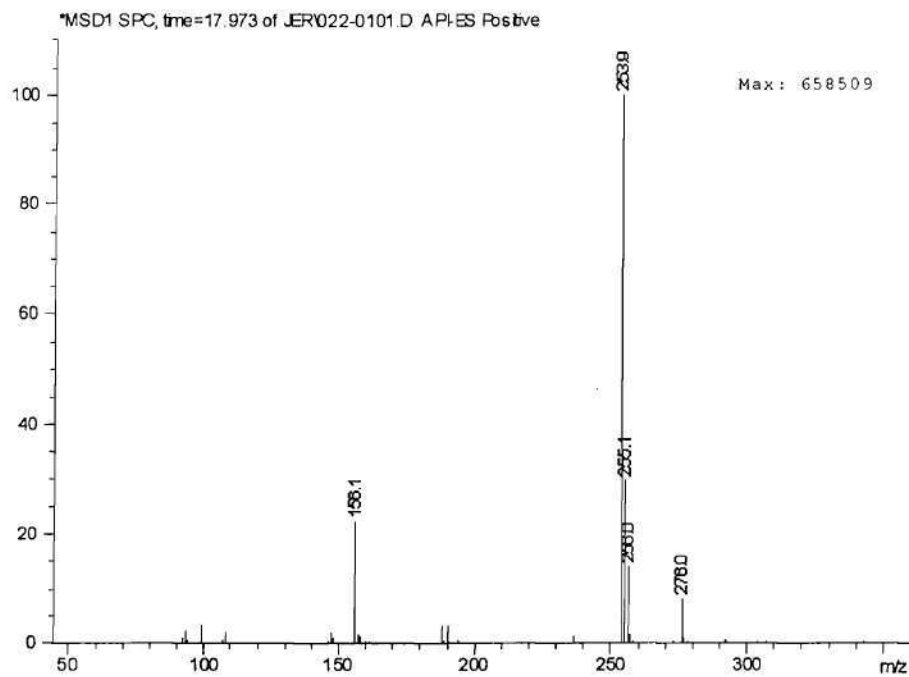| Parameter	Condition
Ionization Mode	electrospray
Polarity	positive
Electron Multiplier Voltage (V)	2748
Nitrogen Drying Gas Flow Rate (L/min)	10
Nebulizer Pressure (psi)	55
Gas Temperature (°C)	325
Spray Chamber Capillary Voltage (V)	4000

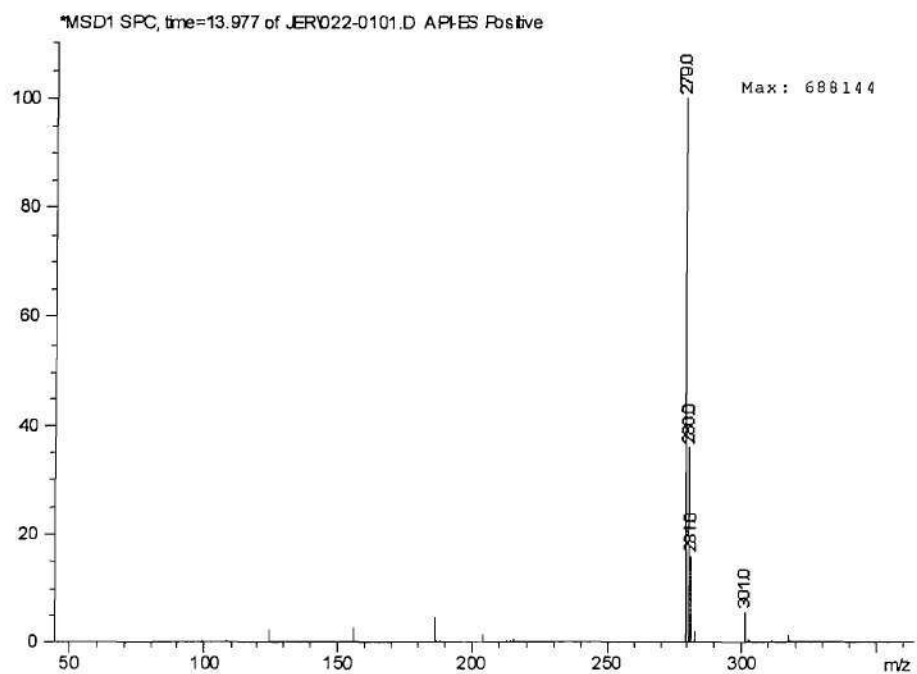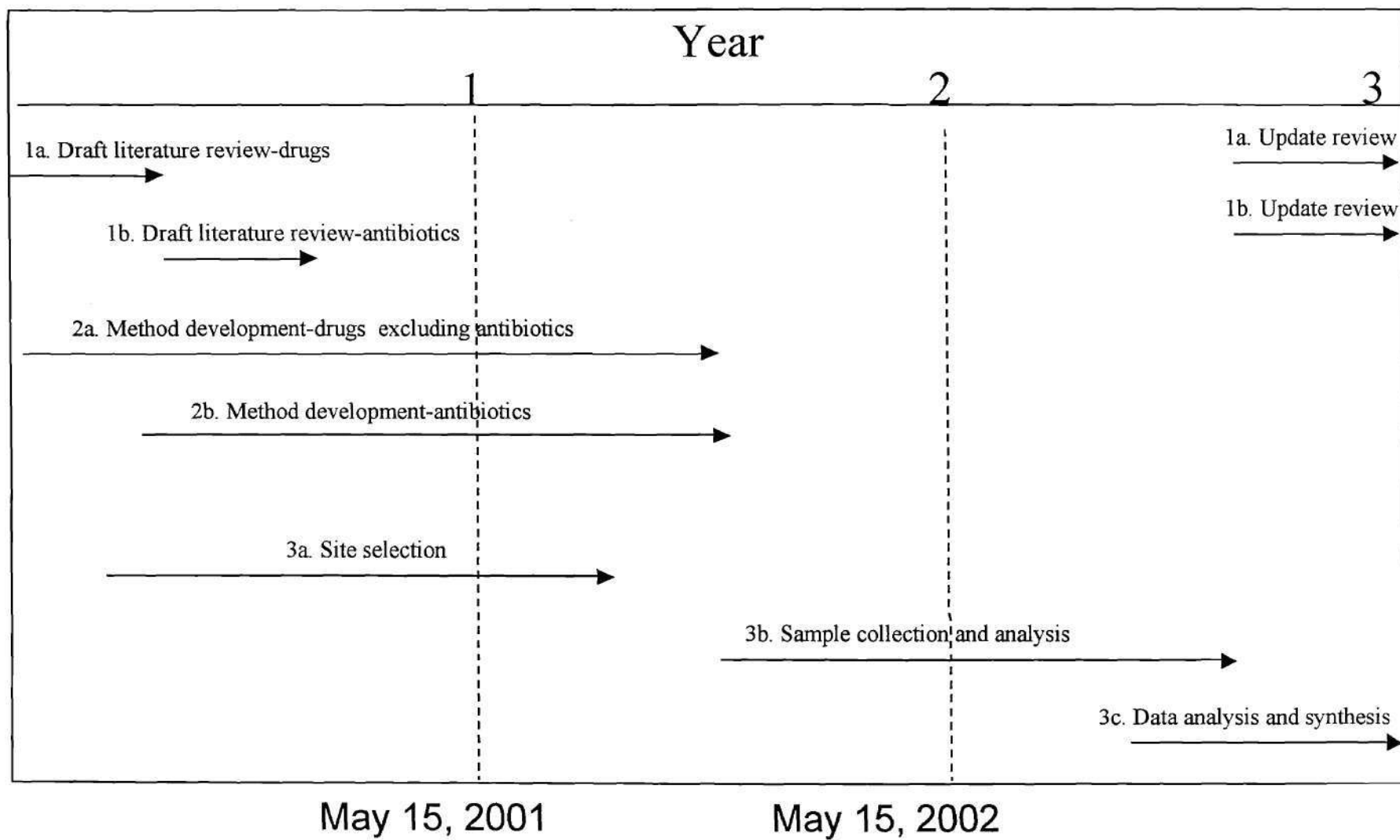Figure C-1: Mass spectrum for sulfamethoxazole in DI Water.

Figure C-2: Mass spectrum for sulfamethazine in DI Water.

Appendix D: Revised Schedule

Year

| | 1 | 2 | 3 |

1a. Draft literature review-drugs

1a. Update review

1b. Draft literature review-antibiotics

1b. Update review

2a. Method development-drugs excluding antibiotics

2b. Method development-antibiotics

3a. Site selection

3b. Sample collection and analysis

3c. Data analysis and synthesis

May 15, 2001   May 15, 2002

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Comment 1: The second periodic report submitted by David Sedlak is comprehensive and includes most of the suggestions made by the PAC at the meeting in Berkeley in November 2000. The change in the quality on the report is remarkable. The research team is commended on assimilating constructive criticism in a collegial manner and producing a much-improved report. Overall, the research team is doing a great job and the PAC found this report very informative.

Response: None required

Comment 2:
QA/QC plan
At a couple of locations in the report, the PI indicates that "differences (regarding loss of PhACs in treatment processes) merit further examination". For example, on page 33 in the top paragraph: These statements require further clarification, as the emphasis for this survey should be "occurrence", not impact of treatment processes. Given the tradeoff of more occurrence data versus more data on the effectiveness of treatment, I would opt for occurrence data. In any event, the number of samples to be collected for different objectives should be defined in the QA plan.

Response: We agree that detailed analysis of attenuation mechanisms is not merited at this stage. However, we also believe that analysis of the efficacy of treatment processes is an important part of the occurrence survey and will provide information that will be valuable in guiding future research. For example, we have analyzed samples from advanced wastewater treatment plants after different unit processes. Our preliminary data suggest that microfiltration has little effect on concentrations of pharmaceuticals while reverse osmosis is very effective. For a relatively small investment of effort, the analysis of several additional samples has provided valuable information that can be used in the future to guide decisions about appropriate treatment systems for these compounds. Therefore, we will limit our sampling to before and after unit processes as indicated in this progress report.

Comment 3: The PI indicates that a QA/QC plan will be provided in the next report. This may be acceptable; however, the team should not go too much further in expending monies on "occurrence" samples until a complete QA plan has been submitted and approved. The QA plan should include both analytical and field sampling information.

Response: A draft QA/QC plan is included in this report (Appendix B). We will respond to PAC comments on the plan prior to initiating the occurrence survey. If necessary, we will hold a conference call to discuss any issues requiring immediate attention.

Comment 4:
Calculation:
The calculations that have been made for the use and occurrence of antibiotics seem to be very rough, somehow, they are sufficient for the selection of the target analytes. To get more reliable

data on the occurrence of the antibiotics in the environment several of the presumptions or postulations that were made need to be verified (e.g. 50/50 application for the individual antibiotics recommended for the different species, p.8; or (table3, p11) assuming 80% of unmetabolized excretion and 0% (com: is this realistic?) of treatment removal).

Response: Based upon information in several recently obtained reports, the assumed 50 % use frequency for each antibiotic in feed in our original estimation is too high. The more appropriate use frequency for each antibiotic in feed is as following: around 10-20 % for antibiotics used in cattle, around 5-10 % for antibiotics used in swine (with the exception of bacitracin, which is used at approx. 50% frequency). The frequency of each antibiotic compound used in poultry is more difficult to determine because several mixtures (each contain 3 to 4 antibiotics) are commonly used and no data is available on the use patterns of these mixtures.

Metabolism of antibiotics in humans is much better studied than that in animals. However, several references suggest that up to 80% or higher of the antibiotics given to animals are released in their unmetabolized form. The degradation of antibiotics between after being released from the animals to waste storage in the CAFOs is essentially unknown at present. The employed assumptions in our estimation were based upon the worst scenario consideration.

These modifications will be included in the final report.

Comment 5: Page 2, p. 2 "five candidate compounds" Animal or human?

Response: Three compounds are human antibiotics, two are used mainly from husbandry.

Comment 6: Page 2, p. 3 "Preliminary results indicate the presence of PhACs in secondary wastewater effluent ..." Any variation between sites?

Response: We believe that it is premature to comment on variation between sites. However, it appears that different treatment technologies (e.g., nitrification compared to secondary treatment) may have an impact on concentrations of pharmaceuticals in wastewater effluents. We plan to sample effluent from different types of plants during the occurrence survey (Table 4).

Comment 7: Page 8 – table-Is there a difference in consumption of cattle for slaughter vs. dairy?

Response: There is some difference in antibiotic use between cattle and dairy. This difference was not accounted for in our estimation due to two reasons: (1) the number of animals obtained from USDA statistics did not differentiate dairy from cattle; and (2) the number of cattle in the U.S. is considerably larger than the number of dairy cows.

Comment 8: Are the records from the antibiotic producers as to quantities produced each year for each product?

Response: We are unaware of the availability of such information for the United States.

Comment 9: page 9   "only 4 antibiotics" should read "only 5 antibiotics"

Response: The change will be made in the final report.

Comment 10: Page 11 – p.1 "80% of antibiotics" Is this substantiated?

Response: see response to comment 4.

Comment 11: Table 5-page 14. Nice table; nice summary of info.

Response: None required.

Comment 12: Table 7-page 17.   At some point (not necessarily for this report), it would be nice to have specific environmental fate data. In any event, there should be some definition of what is meant by high, moderate or slow degradation rates. The HSDB (National Library of Medicine web site) might be a good source of info, as the fate information when lacking is often provided based on structure/function relationships and model estimates.

Response: In the final report, we will provide additional description and explanation for these comparisons.

Comment 13: Page 18, 3rd para. Compounds were eliminated from indicator list if not detected in water. However, these detections were from European sources. Are there compounds that would have been judged worthy of study had a different criterion been selected?

Response: We employed this elimination criterion mostly from the perspective of antibiotic class rather than individual antibiotic compound.   Therefore such elimination is a reliable approach in considering the likelihood of persistence of certain antibiotic class. The β-lactams (amoxicillin and penicillin) were eliminated from the indicator list by this criterion.   Both compounds are popular β-lactams in both U.S. and the Europe, although β-lactams are used more extensively in both human medicine and animal production in the U.S. Other information on β-lactam transformation also indicates their likely short-lives in the environment.

Comment 14: Page 21,2nd paragraph: All analytes can be analyzed by HPLC with UV -> However, this cannot be true because two of the selected antibiotics (acithromycin and tylosin) do not contain chromophore moieties. Is that correct?

Response: HPLC/MS will be a more appropriate detection method than UV for these compounds.

Comment 15: Page 27: Another research group also came to know this problem (decreased sensitivity for high mass fragments) using the Finnegan GCQ: Actually, two errors may be responsible for this effect. 1. a spring underneath the ion trap may be damaged or 2. A tuning error: for the auto tuning the GCQ software sometimes (e.g. when the ion source or the lenses are contaminated) sets the tuning range automatically to lower masses, neglecting the higher mass range. Unfortunately, there is only a short hint displayed during the tuning procedure (!),

thus, if you don't know this effect you will not recognize the problem which results in low sensitivity for higher masses (often they are totally missing in the mass spectra, when you're doing MS/MS this may be fatal).

Response: After numerous visits from Finnigan and replacement of several parts on the ion trap, we have solved the problem associated with low sensitivity of the high-mass fragments. We appreciate the suggestion from the PAC member and will keep it in mind should we ever encounter similar problems.

Comment 16: Page 27, 2nd paragraph: (Although these recoveries...) the resulting values may only be called semi-quantative.

Response: We agree with the comment. In the draft QA/QC plan we propose the use of a permanent qualifier for any data that do not meet our target QA/QC criteria.

Comment 17: Page 28 – p. 3 "...serve as useful screening tools."
Would you look at these first?

Response: (We believe that this comment refers to paragraph 2 of page 21) We plan to focus on HPLC/MS methods and have begun method development.

Comment 18: para. 4 – "sulfamethazine and sulfamethoxazole."
Aren't these in the same class and come out anyway if either one is looked at? Wouldn't it be more advantageous to look at one or the other and one from a different class?

Response: The two sulfonamide antibiotics can be simultaneously analyzed using the same method and thus require minimum additional experimental efforts. In addition, analysis of the two sulfonamides may provide information on the source of these compounds because sulfamethoxazole is used primarily in human medicine while sulfamethazine is used primarily in livestock.

Two antibiotics among the indicator list belong to the macrolide class. As mentioned in the second progress report, the literature review indicates that extraction methods for macrolides are considerably different from those used for fluoroquinolones and sulfonamides due to their much higher polarity. Analysis of all three classes of antibiotics will complicate the analytical procedures, resulting in longer run times. Therefore, we focused our method development for ciprofloxacin, sulfamethoxazole and sulfamethazine.

Comment 19: Figures 4-8 should be redeveloped to be more reader-friendly for the final report. It is difficult to see the x axis.

Response: The format of these figures has been modified. We hope that the PAC finds the revised version to be satisfactory.

Comment 20: Page 33 and continuing on page 38 regarding Sub-Task 3A. If possible, it would probably be beneficial for the research team to reevaluate the Mt. View River sample with dilution before and then after the wastewater treatment process. This information would be

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useful in determining the impact(s) the wastewater treatment process will have on the removal of these compounds. It would be important to demonstrate whether treatment processes are effectively removing these compounds. However, as noted on page 1 this should not be done at the expenses of occurrence studies that are a priority of this project.

Response: The Mt. View samples are collected from an engineered treatment wetland that receives no other sources of water. Therefore, dilution is negligible between the treatment plant and the wetland. It is worth noting that in a separate study we observed variation in the concentrations of hormones of approximately 50% in the effluent of this treatment plant.

Comment 20: Page 34, 2nd para. There is a statement that concentrations decreased during passage through, in this case a wetland. The PI suggests that synoptic sampling will be employed during the occurrence survey. It was mentioned at the meeting and also in previous comments that locations, frequencies of sampling, duration, methods, objectives all need to be defined before the research team goes much further with sampling. The mechanism for this is a QA/QC plan.

Response: On the basis of this and the following comment, we have decided to limit ourselves to grab samples during the occurrence survey. We decided against composite sampling because the logistical complications associated with sample preservation did not merit the effort. For example, 24-hour composite samplers typically collect unfiltered samples and would therefore require a biocide to minimize biotransformation. Furthermore, the engineered wetlands and groundwater infiltration systems provide ample opportunities for mixing and we do not expect large variations of concentrations with relatively short time periods. In situations where we are sampling from before and after a unit process that exhibits relatively little mixing, such as microfiltration systems in advanced wastewater treatment plants, we will use synoptic sampling techniques based on estimates of residence times in the treatment process.

Comment 21: Page 37, 3rd para. Here is mentioning of 24 hr composite sampling. Again, there is a need for a QA/QC plan. Given some of the other uncertainties, it is not clear that the team wants to get into this (24 hour composites) at this time.

Response: See above.

Comment 22: Appendix A, p.43-44: The concentration values shown in the table (e.g. 147 or 41 ppt) may pretend an analytical accuracy that has not been demonstrated and that may never be achievable.

Response: In light of the variability in duplicate samples (i.e., typically within 20%), we believe that two significant figures are merited. We will report all data in subsequent reports to reflect this decision.

Comment 23: Appendix 1, the spike recoveries over 100% are not explained. Recoveries ~ 120% or so can be explained by method or matrix variation. However, recoveries over 120% need some kind of explanation.

Response: As indicated in the draft QA/QC plan, our target for spike recoveries is 60-120%. Any runs in which data are beyond the target range will be will be repeated or qualified. In the current project period, only 2 of 95 recovery analyses exhibited recoveries above 120%.

Comment 24: Page 47, Schedule. Please insert specific dates in the schedule. Where do you think you are at in terms of the progress report?

Response: Dates have been included in the revised schedule (Appendix D). We believe that we are on target with respect to our goals of beginning the occurrence survey during the first half of Year 2.

OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Fourth Progress Report

September 15, 2001

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SUMMARY

During the fourth project period, we continued to focus our attention on method development in association with the occurrence survey. Method performance and reproducibility were evaluated by analysis of deionized water and environmental samples before and after amendment with target analytes.

For the drugs other than antibiotics, our results indicate that method development and testing is nearly complete. Analysis of seventeen environmental samples demonstrate that mecoprop is an appropriate internal standard for acidic drugs and that recoveries between 60 and 120% could be achieved routinely. Analysis of samples amended with beta-blockers indicated reproducible recoveries of 40 to 60%. Although this is below our QA/QC target for recoveries, we believe that the use of the internal standard, deoxyepinephrine, may help us to account for matrix effects. Attempts to analyze samples for the anti-epileptic drug, carbamazepine, suggested significant losses during sample extraction and elution. After testing of an internal standard for the beta-blockers, method development and testing for acidic drugs and beta-blockers will be complete.

For the antibiotics, improvements in sample extraction and chromatography have greatly improved accuracy and precision. To identify steps where antibiotic losses occur, we focused our attention on blowdown steps, sample containers and chromatographic separation. Evaluation of the method using spike recoveries indicated that recoveries could be improved by changing containers used for sample collection and extract blowdown. Modification of LC/MS conditions solved many of the problems caused by co-eluting organic matter. After incorporating these modifications in our analyses, we obtained recoveries above 80% for deionized water and GAC effluents and recoveries above 60% for secondary effluent samples. Analysis of samples from two wastewater treatment plants indicated the presence of antibiotics in secondary wastewater effluent. After incorporating internal standards into the LC/MS methods, we will have completed our method development activities.

CONTENTS

FIGURES

PROGRESS THIS PERIOD

TASK 1: LITERATURE REVIEW

The objective of this task is to identify compounds to be studied during the occurrence survey. The selection criteria for compounds in the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and availability of suitable analytical methods. Progress on the literature review was presented in the first and second progress reports. The literature review has been completed and will be updated prior to preparation of the final report. No additional progress related to task one was made during the current project period.

Sub-Task 1A: Drugs Used in Human Therapy

In the first progress report, we reviewed data on prescription drugs likely to be present in wastewater effluent in the United States. Using data on the number of prescriptions for the top 200 drugs and information on prescription formulations and metabolism, we estimated ranges of concentrations of 136 drugs expected to be present in wastewater effluent. In addition, we reviewed published occurrence data to identify other compounds of interest that did not appear among the common prescription drugs. A total of eleven drugs were identified for further consideration.

During the current project period no activity was conducted in association with this sub-task.

Sub-Task 1B: Drugs Used in Animal Husbandry

In the second progress report, we reviewed data on antibiotics used in livestock both therapeutically to treat diseases and sub-therapeutically as feed additives to promote growth. We focused our review on antibiotics used in animal feeding operations, because these sources could result in concentrated discharges of antibiotics. We also reviewed fate and transport properties and available analytical methods. On the basis of these results and our review of antibiotics used in human therapy (sub-task 1a), we identified five antibiotics that merit further consideration.

During the current project period no activity was conducted in association with this sub-task.

TASK 2: ANALYTICAL METHOD DEVELOPMENT AND TESTING

The objective of this task is to identify and test analytical methods that will be used during the occurrence survey. As part of the task, analytical methods for extraction, cleanup and derivitization will be tested by adding drugs to deionized water and to samples collected from sites being considered for inclusion in the occurrence survey. Analysis of antibiotics is included as a separate task from the other pharmaceutically active compounds (PhACs) because the analytical methods for antibiotics (e.g., HPLC/MS) are fundamentally different from the gas chromatography methods used for the other compounds.

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the first progress report, we described our preliminary efforts to develop suitable analytical methods for eleven compounds identified as part of the literature survey. We used solid phase extraction followed by derivitization and GC/MS/MS to quantify pharmaceuticals in deionized water and in samples collected from several of the candidate sampling sites. To quantify recoveries and matrix effects, all samples also were analyzed after addition of known quantities of analytes.

During the second project period, we focused our attention on improving the accuracy and precision of the analytical methods by analyzing samples from three sites being considered for inclusion in the occurrence survey. As a result of analytical difficulties, we did not analyze three of the compounds (i.e., carbamazepine, nabumetone and nadolol) during the second project period. Analysis of duplicate samples indicated that, with the exception of two beta-blockers, results were reproducible (i.e., we observed an average of 26% error between duplicate samples). Analysis of blank samples indicated that sample carryover and cross contamination were not significant problems. Analysis of spike recovery samples indicated variable recoveries, with values as low as 30% for some analytes. All of the drugs were detected in one or more of the unspiked samples.

During the third project period, we continued to improve the analytical methods by identifying steps where analytes were lost during analysis. For the acidic drugs, we changed the solid phase extraction technique and added radiolabeled mecoprop as an internal standard. For the beta-blockers, we increased the time of the drying step to improve the efficiency of

derivitization. We also eliminated the use of PFE-lined containers, which resulted in losses of beta-blockers during storage. A QA/QC plan also was submitted to the PAC.

During the fourth project period, we attempted to resolve the remaining issues associated with the analytical methods. Attempts to use radiolabeled propranolol as an internal standard for the beta-blockers failed because the labeled compound could not be discriminated from the unlabeled compounds. Alternative surrogates for beta-blockers could be derivitized and analyzed, but were too polar to be retained during solid phase extraction. We also evaluated the variability in method performance for acidic drugs and beta-blockers by analyzing a total of 18 samples from two surface waters and an advanced wastewater treatment plant. These surrogates will be added to samples after elution from SPE columns. Finally, we tested a GC/MS/MS technique for analysis of carbamazepine. Our progress in each of these areas is described below.

Analytical Methods: During the third project period we investigated compounds that could be used as internal standards to monitor recoveries of acidic drugs and beta-blockers. Results of our analyses indicated that radiolabeled mecoprop is an acceptable internal standard for the acidic drugs. However, attempts to use radiolabeled propranolol as an internal standard failed because the compound was labeled on the aromatic ring and that portion of the molecule could not be used for MS/MS analysis. Near the end of the third project period, we identified a supplier for another form of radiolabeled propranolol which was labeled on the side chain. However, the manufacturer was unable to provide the compound because they did not have it in stock. Subsequent inquiries indicated that the company would sell us the compound if we were willing to purchase a relatively large quantity for a price of approximately $1,000. Because we wanted to test the compound before spending a large sum of money, we decided to pursue an alternative approach.

During this project period, we explored alternative means of including an internal standard. Initially, we attempted to use selected-ion monitoring (SIM) to discriminate between labeled and unlabeled forms of propranolol. However, the method would have reduced our sensitivity for propranolol and prevented analysis of the compound in wastewater effluent samples. After we determined that radiolabeled propranolol would not be useful as an internal standard, we investigated commercially available compounds with structures similar to that of the beta-blockers. We determined that epinephrine and deoxyepinephrine (Figure 1) might be
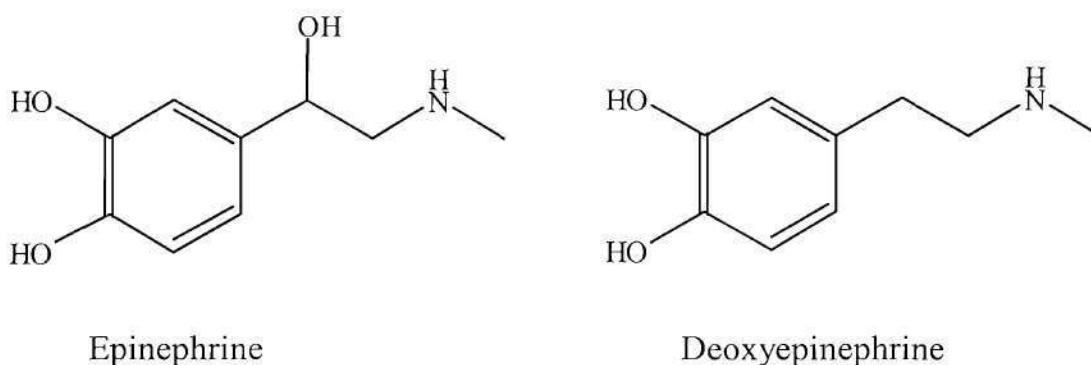
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Figure 1: Structural formulae of epinephrine and deoxyepinephrine.

acceptable internal standards because they have similar structures to the beta-blockers but are not excreted by humans in significant quantities. To test these compounds, we developed GC/MS/MS methods for the derivatives of both compounds. The sensitivity and linear range for both compounds were similar to that observed for the beta-blockers. However, attempts to extract the compounds from water failed, presumably because the compounds are more polar than the beta-blockers. Although it might be possible to synthesize a less polar compound with a structure similar to that of the beta-blockers, we do not believe that such an effort is merited. Furthermore, synthesis of internal standards would be an impediment to commercial laboratories trying to employ our methods. Therefore, we plan to add deoxyepinephrine to all samples after solid phase extraction. In this capacity, deoxyepinephrine will monitor losses of beta-blockers in all of the steps following extraction (i.e., solvent transfer steps, derivitization and analysis). Spike recovery studies performed with metoprolol and propranolol in a subset of samples will be used to evaluate losses that occur during solid phase extraction.

In addition to evaluating internal standards for beta-blockers, we also attempted to expand the number of analytes to be included in the occurrence survey by implementing a better analytical method for carbamazepine. During this project period we tested an analytical method for carbamazepine provided by Dr. Heberer. Although the standard curve for carbamazepine exhibited good sensitivity, there was evidence for nonlinear response at low concentrations (Figure 2). To evaluate the extraction efficiency for carbamazepine, we added the compound to deionized water at concentrations ranging from 35 to 1,200 ng/L and used solid phase extraction followed by derivitization and GC/MS/MS analysis. Results of our analyses indicated that
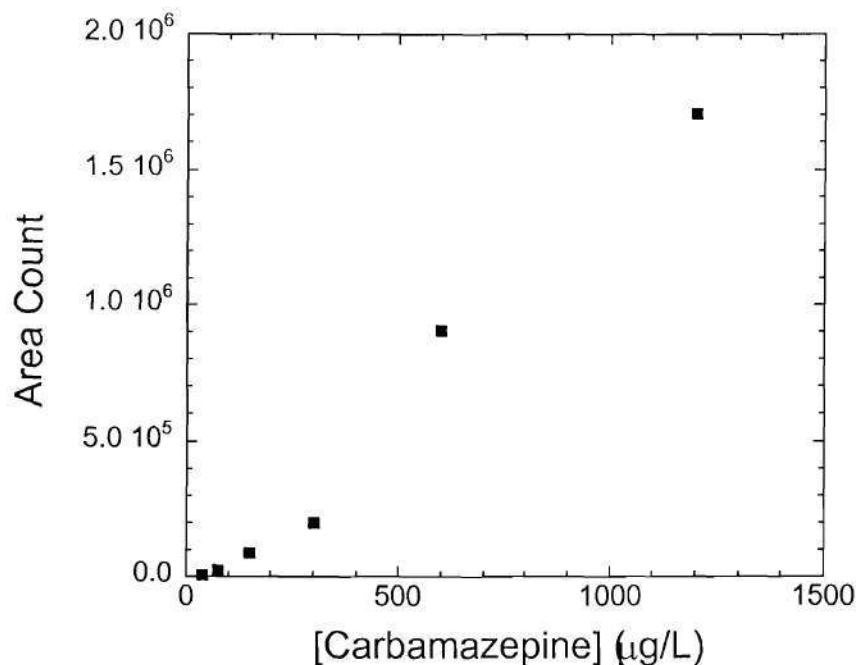
Figure 2: Standard curve for analysis of carbamazepine.

recoveries were affected by concentration: at concentrations above 250 ng/L recoveries were between 80 and 110% but at concentrations below 250 ng/L recoveries decreased to 15 to 50% (Figure 3). We hypothesize that the poor recoveries observed at low concentrations may be related to adsorption of the compound on the glass containers used to store the aqueous samples. We plan to repeat the experiment with freshly silanized glassware and additional solvent rinses of the glassware during the next project period. Any suggestions from the PAC, especially Dr. Heberer, on ways to improve the analytical method or to enhance recoveries of carbamazepine would be greatly appreciated.
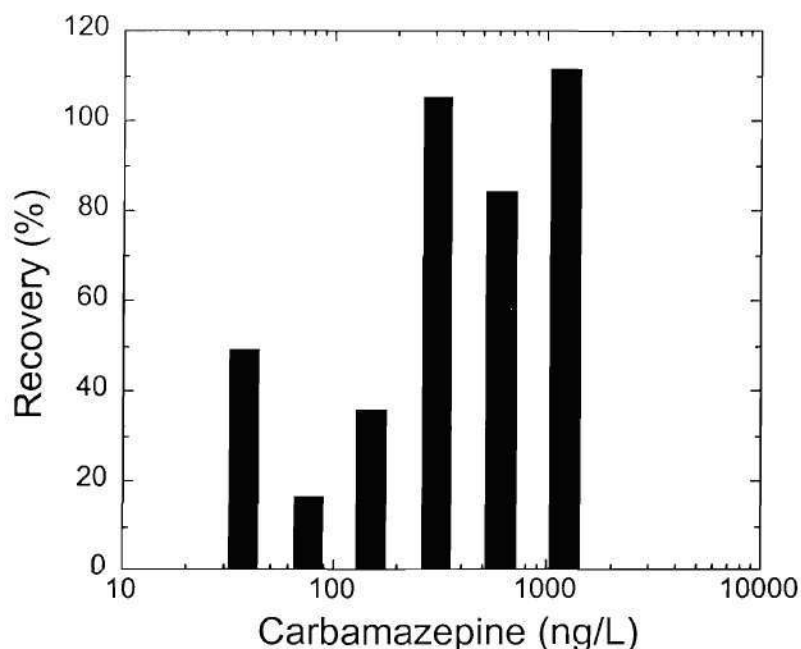
Figure 3: Recovery of aqueous carbamazepine as a function of concentration.

Quality Assurance/Quality Control (QA/QC): During the third project period we described the use of radiolabeled mecoprop as an internal standard for the acidic drugs. During fourth project period we collected additional data to verify the performance of the internal standard. Samples were collected from three locations in the Russian River (CA) on a monthly basis between May and August. Samples also were collected from an advanced wastewater treatment plant located in Texas and from the Delaware River, near Philadelphia. The Russian River samples were collected to evaluate potential matrix effects on analyte and internal standard recovery while the other two samples were collected as part of the occurrence survey. Descriptions of the sampling locations and our basis for selecting these locations are included under sub-task 3A (occurrence survey).

To evaluate analyte recovery and its correlation with the recovery of radiolabeled mecoprop, duplicate samples from the Russian River were analyzed. Samples also were analyzed after amending the samples with 1,000 ng/L of each of the acidic drugs. Recoveries of

radiolabeled mecoprop in the unamended samples are depicted in Figures 4 and 5. With the exception of one sample, recoveries for samples collected in the Russian River upstream and downstream of the City of Santa Rosa's wastewater discharge point all met our QA/QC recovery target of 60-120%. For samples collected at the sampling location referred to as Caisson, recoveries were typically lower than those observed at the other locations. For three of the Caisson samples, recoveries were below our QA/QC target of 60%. After analysis of these samples, we learned chlorine is added near the Caisson sampling site. As described later in this section, we presume that mecoprop and several other analytes degraded in the presence of chlorine. In the future, we plan to add sodium thiosulfate to samples collected from this location. Recoveries of mecoprop in samples from the Witchita Falls (TX) Advanced Wastewater Treatment Plant exhibited greater variability than those collected from the Russian River (Figure 5). We are suspicious of all data from the Witchita Falls AWWTP because we discovered that the diazomethane solution used to derivatize these samples had undergone a phase separation in the container. Since that time, we have monitored the diazomethane and plan to generate smaller quantities that will be replaced more rapidly. Both samples from the Delaware River exhibited recoveries of approximately 60%.
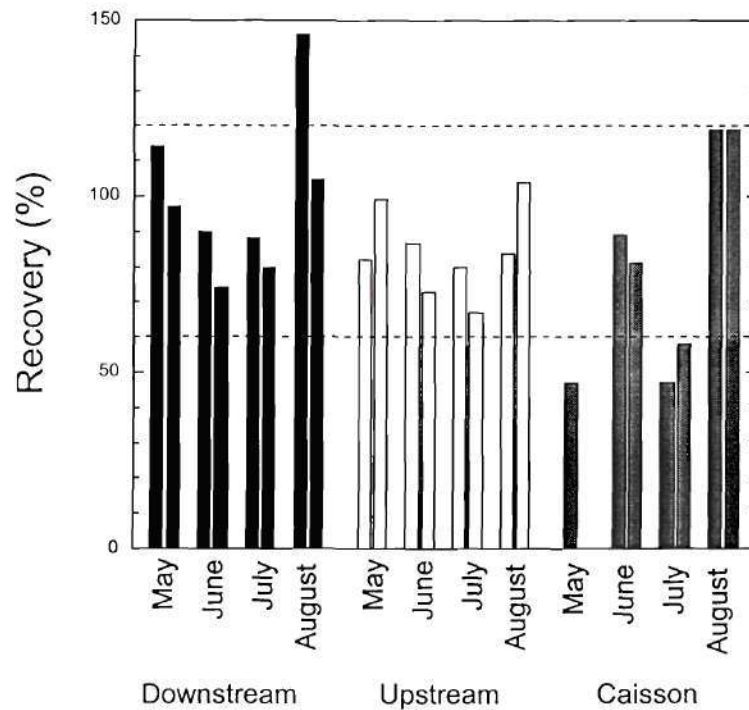
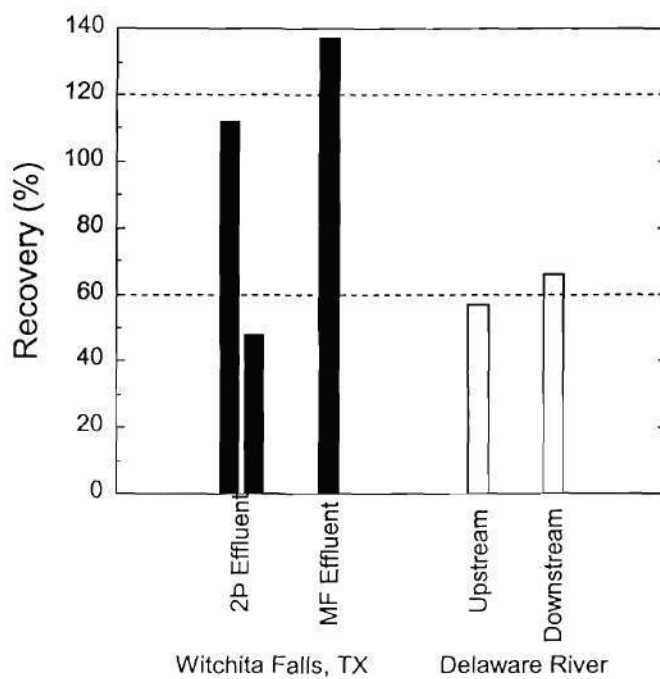Figure 4: Recoveries of radiolabeled mecoprop in samples collected from the Russian River.

Figure 5: Recoveries of radiolabeled mecoprop in samples from Texas and the Delaware River.

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Evaluation of data from the spike recovery samples further confirms the applicability of radiolabeled mecoprop as an internal standard (Figures 6-11). For the samples collected from the Russian River, recoveries measured in the upstream and downstream samples exhibit a similar pattern to that observed for mecoprop, with most recoveries falling within the target range of 60-120%. The spike recovery samples indicated inadequate recoveries for the July samples (third bar for the upstream and downstream samples) while the mecoprop data indicated recoveries around 80%. For the Caisson samples, ibuprofen and ketoprofen recoveries were consistent with those of mecoprop, but diclofenac, gemfibrozil and naproxen were not detected in any of the recovery samples. Indometacine was only detected in one of the recovery samples. Because the Caisson sample was chlorinated and the chlorine was not quenched prior to adding the recovery spike, we surmise that these four drugs are transformed by chlorine (i.e., $HOCl/OCl^-$). For the Witcihta Falls samples, for which we discount the data because of problems with the diazomethane, the spike recovery data were consistent with the mecoprop data (i.e., the use of mecoprop as an internal standard correctly identified problems with the derivative both analyses indicated low recoveries).

Recoveries of the beta-blockers were consistently lower than those of the acidic drugs. Most of the spike recovery samples for metoprolol and propranolol from the Russian River exhibited recoveries below the target values of 60% (Figures 12 and 13). The average recoveries for metoprolol and propranolol in the Russian River samples were 43% and 35%, respectively. Although this represents an improvement over the previous project period when recoveries of beta-blockers ranged from 20-30% in samples from the Russian River sites, it is still lower than our target. For the samples from the two other sites recoveries were better, especially for metoprolol. We suspect that the lower recoveries in the Russian River samples could be attributable to a matrix effect. However, we cannot think of any practical way to further improve the recoveries of the beta-blockers. Therefore, we propose to use the protocol as it stands and to qualify any data below the recovery threshold.

Figure 6: Recoveries of diclofenac measured during the fourth project period.

Figure 7: Recoveries of gemfibrozil measured during the fourth project period.

Figure 8: Recoveries of ibuprofen measured during the fourth project period.

Figure 9: Recoveries of indometacine measured during the fourth project period.

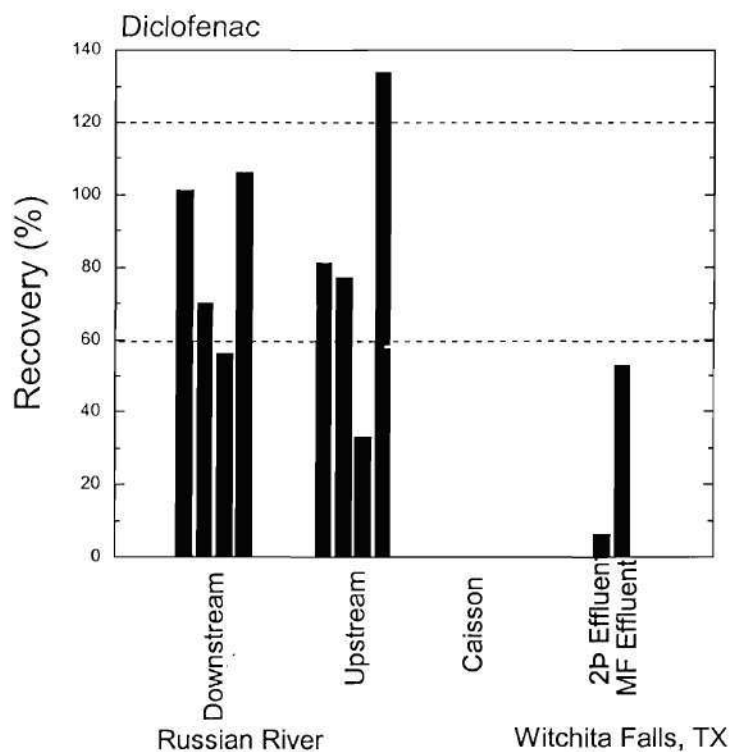Figure 10: Recoveries of ketoprofen measured during the fourth project period.
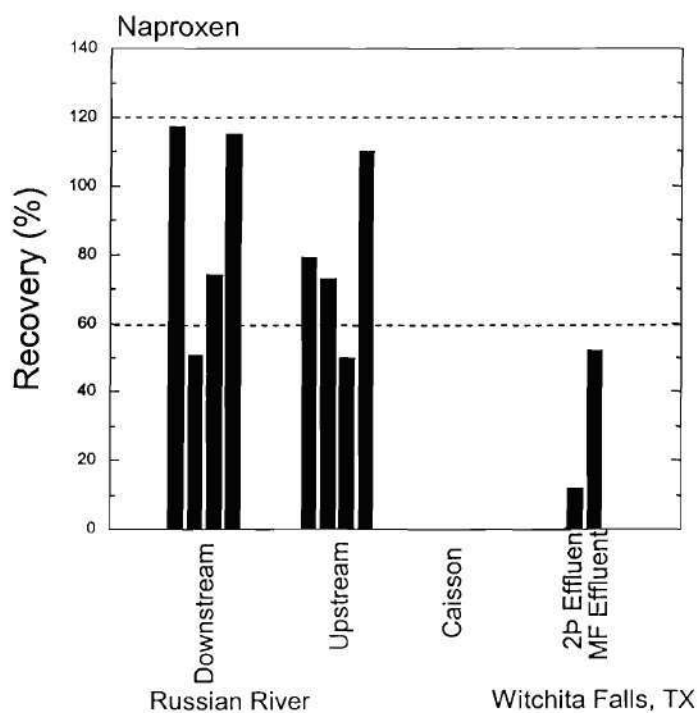
Figure 11: Recoveries of naproxen measured during the fourth project period.

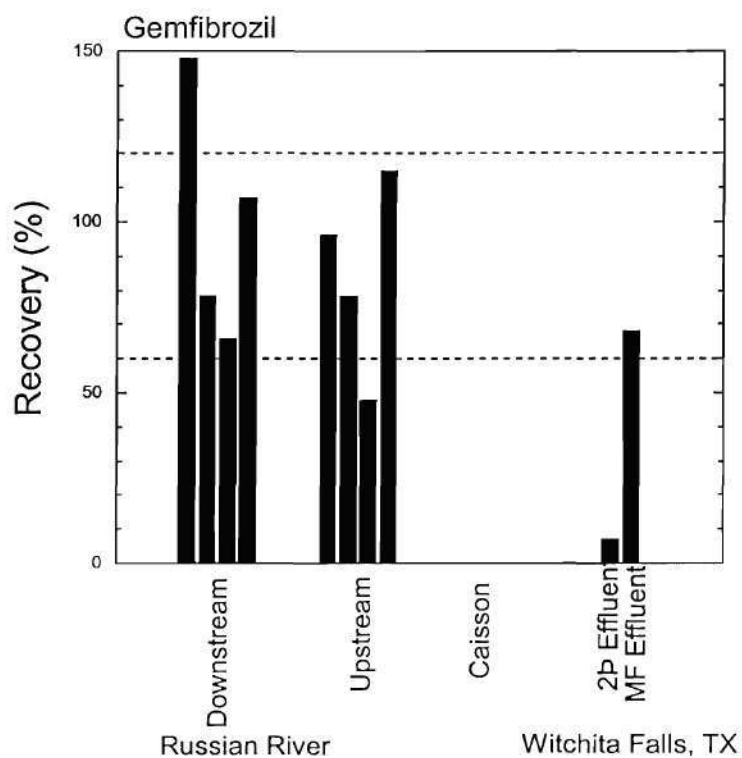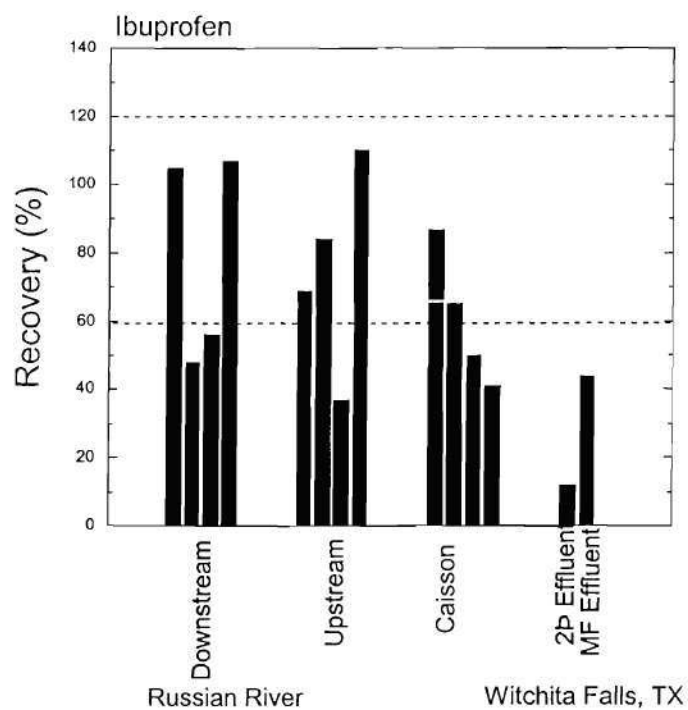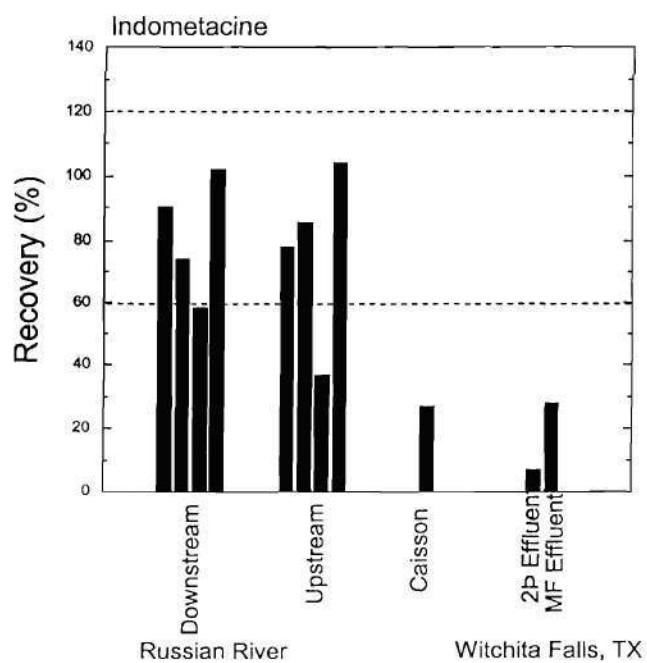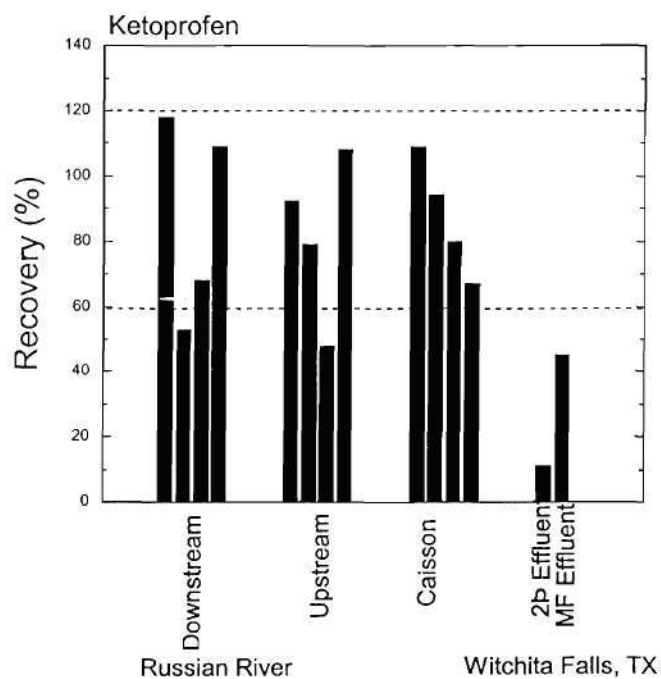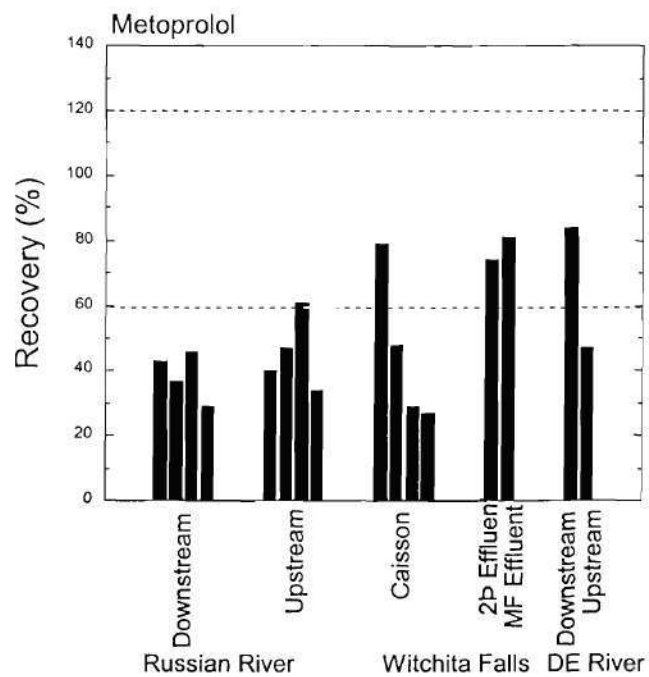Figure 12: Recoveries of metoprolol measured during the fourth project period.
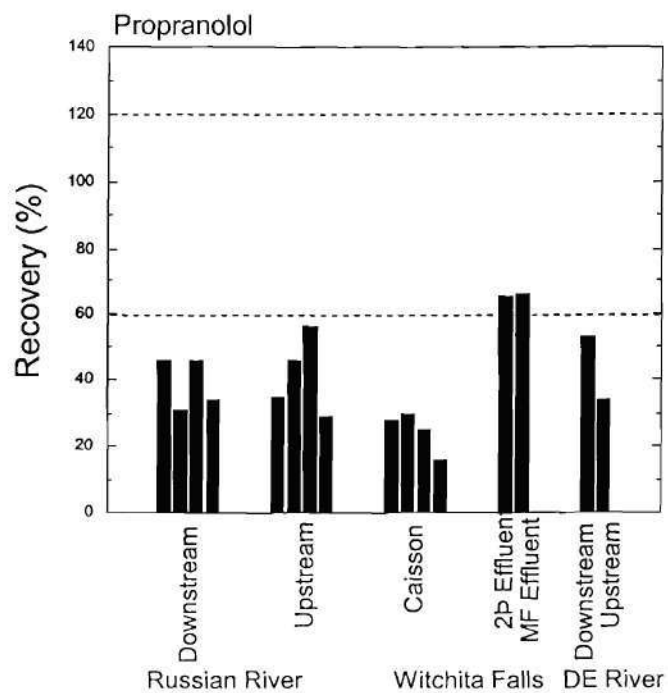
Figure 13: Recoveries of propranolol measured during the fourth project period.

Sub-Task 2B: Antibiotics

Our analysis of antibiotics has focused on fluoroquinolone and sulfonamide antibiotics. Ciprofloxacin, sulfamethoxazole and sulfamethazine are the selected target analytes of occurrence analysis. In the second and third progress reports, we described our preliminary efforts to develop suitable analytical methods for these compounds. A dual-cartridge solid phase extraction (SPE) method was developed to extract antibiotics from water samples. Analysis of antibiotics was conducted by LC/MS and LC/FLD (fluorescence detection).

During the fourth project period, we continued to improve the analytical methods. To address the low and variable recoveries, we performed a series of experiments designed to identify the steps where analytes were lost and modified the analytical steps to minimize losses of analytes. We improved the LC/MS method for sulfonamides and fluoroquinolones considerably. To improve the LC/MS method, we have modified the compositions and gradient program for the mobile phases to minimize potential matrix influence on signal suppression. We also evaluated the use of several internal standards in the analytical methods to improve accuracy. Furthermore, we evaluated the sorption of analytes onto glass and PFE-lined sampling containers. We also conducted several more experiments to evaluate the suitability of cation-exchange SPE method for extracting fluoroquinolones. As a result, the recoveries for antibiotics have improved considerably with the modified analytical methods. Our progress in these areas is described below.

Antibiotics Extraction: The dual-cartridge SPE

The dual-cartridge (an anion exchanger and a HLB cartridge) SPE method developed for antibiotic extraction was described in the Appendix C of the third progress report. The anion exchanger serves to reduce the amount of interfering organic matter in the concentrated extracts and the HLB cartridge serves to extract the antibiotic analytes. We modified this method by using a larger anion exchanger (500mg/6ml, Jones Chromatography) to improve the flow rate and thus shorten the extraction time. The modified method extracts antibiotics at a flow rate of approximately 6 ml/min, compared to a flow rate of approximately 2-3 ml/min in the previous setup. Analysis of the eluent from anion exchangers confirmed that the anion exchanger extracts much of the dissolved organic matter in sample but does not retain appreciable amounts of antibiotics. We also ensure sufficient drying time for the cartridge by pulling air through it prior

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to methanol elution, thereby minimizing water residue in the SPE cartridge and improving analyte elution efficacy.

During the previous project period, we suspected that losses of analytes during the blow-down step were one of the primary causes of fluctuation in extraction efficacy. In this project period, we performed experiments to establish a protocol that minimizes analyte losses during the blowdown. The experiments were conducted by blowing down 10 ml of methanol spiked with 250 ng/L to 100 μg/L of antibiotics to dryness and redissolving in 1 ml of methanol/water mixture. The experiments were performed in various high-density polyethylene and glass containers. Analyte losses during blowdown were less significant for sulfonamides than for fluoroquinolones (Figure 14). Among the tested containers, high-density polyethylene conical tubes yielded the best and most consistent recoveries for ciprofloxacin and have been used for all subsequent experiments in SPE eluent collection and blowdown.
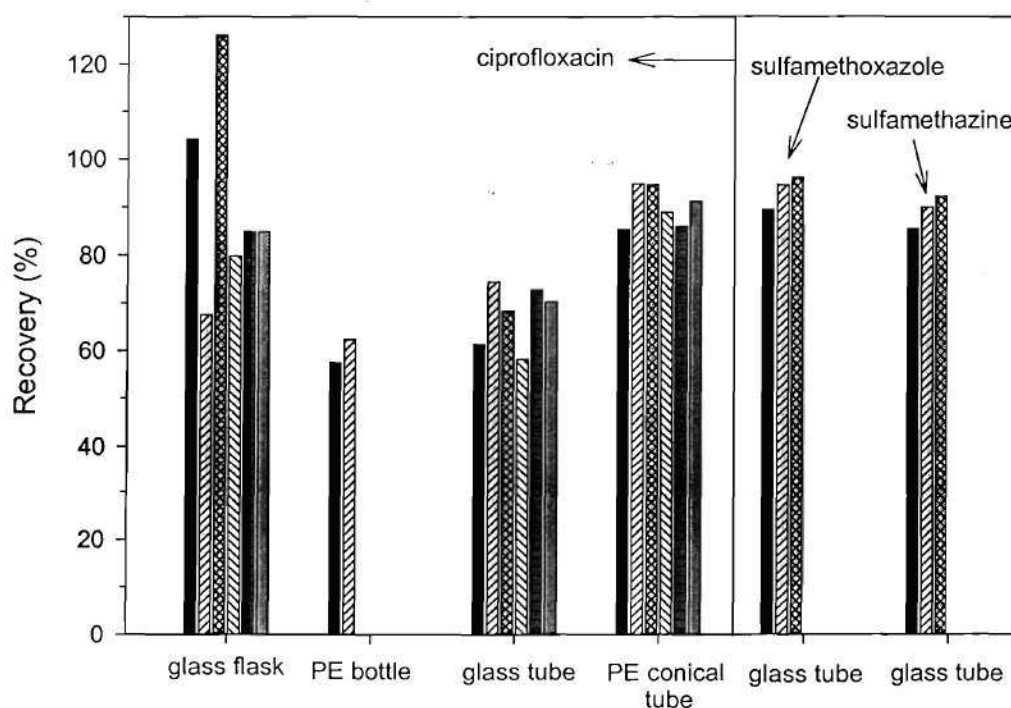
Figure 14: Recoveries of antibiotics in various containers during blowdown. Tested containers: borosilicate volumetric flasks (25 ml), polyethylene bottles (100 ml), borosilicate test tubes, and high-density polyethylene conical tubes.

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Our experiments also indicate that fluoroquinolones remain stable for a longer period in acidic solutions. Therefore, to minimize compound losses prior to solid phase extraction, we acidified wastewater samples with phosphoric acid immediately after the samples were filtered with 0.5 μm glass-fiber filters. In all cases, extraction of samples was completed within two days of collection. In addition, the analytes were redissolved after blowdown in an acidified water and methanol mixture (800 μl/200 μl) and were transferred to amber vials to minimize breakdown of antibiotics prior to analysis by LC/MS. In cases in which samples could not be immediately analyzed by LC/MS, the vials were stored at 0 °C until analysis.

Analysis by LC/MS

After solid phase extraction, antibiotics are analyzed using a single quadruple LC/MS (Hewlett-Packard, Series 100 MSD G1946A) with electrospray ionization at positive ion mode using selected-ion monitoring (SIM). The results of LC/MS analysis in previous experiments indicate that sample matrix affects the analysis by suppressing analyte signals and the matrix effects increase as the complexity of the sample matrix increase. Since the matrix effects are the result of co-eluting interfering compounds that affect the ionization of antibiotics, we conducted studies to minimize the matrix effects by achieving the best possible chromatographic separation that yields the least amounts of co-eluting compounds. In addition, we performed studies to improve the sensitivity and precision of the LC/MS analysis by: (1) modifying the gradient and compositions of mobile phases; (2) optimizing the mass spectrometer conditions; and (3) including internal standards in the analysis.

Selected-ion monitoring was conducted for the molecular ions to quantify antibiotics. In addition, the fragmentor voltage was optimized to yield at least two characteristic fragment ions. Selected ion monitoring was conducted on the molecular ions as well as fragment ions of the antibiotics. Identification of compounds was based upon retention time, m/z of molecular ion and characteristic fragment ions, as well as the relative abundance between ions. To avoid build-up of non-volatile matrix components on the spray tip that affects signal response during analyses, valve switches were used to send eluent to waste during the first 8 minutes and after 20 minutes of analysis.

Utilization of radiolabeled target antibiotics is desirable for the QA/QC of analysis. We were unable to find vendors for these radiolabeled chemicals during the fourth project period.

Instead, we evaluated structurally-related sulfonamides and fluoroquinolones as potential internal standards. For sulfamethoxazole and sulfamethazine, we tested sulfachlorpyridazine and sulfamerazine as internal standards. Sulfachlorpyridazine and sulfamerazine are veterinary therapeutic antibiotics and are not used at sub-therapeutic levels in livestock for growth promoting. These two sulfonamides are also not used in human therapy in the US. Therefore the presence of sulfachlorpyridazine and sulfamerazine in typical domestic wastewater effluent is unlikely, and their presence in surface water is likely to be low as well due to the nature of their occasional usage.

For ciprofloxacin, we selected enrofloxacin, norfloxacin and ofloxacin to be included into method development. Ofloxacin and norfloxacin are used in human therapy in the US, however at considerably less frequent prescription rate than ciprofloxacin (not among the top 200 prescription drugs in 1999). Enrofloxacin is used in poultry operations for therapeutic treatment and disease prevention. After their absence in the water samples has been confirmed, enrofloxacin can be used as an internal standard for municipal wastewater samples whereas ofloxacin or norfloxacin can be used as internal standard for surface water samples.

Our studies indicate that it is difficult to analyze all sulfonamide and fluoroquinolone compounds with the same method due to the high and very similar polarity among these compounds. The complicated charge behavior of fluoroquinolones also presents a unique challenge. Thus, our efforts of method development have focused on developing two separate LC/MS methods for the groups of sulfonamides and fluoroquinolones respectively.

During the fourth project period, we improved the LC/MS method for sulfonamides and fluoroquinolones. Details of the LC/MS method are described in the Appendix C of this progress report. After testing various mobile phase compositions, we conclude that the mobile phases of ammonia acetate (1 mM, pH 6.8) and acetonitrile yield the best results for sulfonamides, whereas 0.05% acetic acid and acetonitrile and higher nebulizer gas pressure yield the best results for fluoroquinolones on our system.

A fixed amount of internal standards were spiked into wastewater extracts after SPE and to the calibration standards (50 to 5000 μg/L at six levels in reagent water) to facilitate assessment of LC/MS performance. Sulfachlorpyridazine was less satisfactory as an internal standard than sulfamerazine due to its high tendency to fragment and very similar polarity to sulfamethazine. Therefore sulfamerazine was selected and used as the internal standard for

sulfonamides in all the later experiments. Enrofloxacin is less suitable than norfloxacin as an internal standard because it produces a fragment ion of the same m/z as the molecular ion of ciprofloxacin and exhibits very similar polarity to ciprofloxacin. Therefore, enrofloxacin has the potential to yield false positive results if it is not well separated from ciprofloxacin. Norfloxacin, on the contrary, can be easily separated from ciprofloxacin and does not produce similar important ions and will be used in future studies as an internal standard.

We assessed the effect of sample matrix on the signals of internal standards by comparing the signals of internal standards in wastewater extracts to those in reagent water at comparable concentrations. The signals of sulfamerazine in secondary wastewater extracts were about 20% lower than those in reagent water, and the signals of enrofloxacin in secondary wastewater extracts were about 30-40% lower than those in reagent water. Assessment of matrix effect on norfloxacin is currently underway. Considering that matrix effect to suppress (or increase) analyte signal is caused by co-eluting compounds and therefore the matrix effect on internal standards is not necessarily the same as that on the other antibiotic analytes since the other antibiotic analytes are eluted from the column at different retention times. We conducted similar tests to evaluate the matrix effect on sulfamethoxazole and sulfamethazine, and approximately 20% of signal suppression by the matrix of secondary wastewater extracts was observed, indicating that utilization of the internal standard sulfamerazine is an appropriate method to account for matrix effects in quantitation. Overall, the matrix effect for sulfonamides was reduced by the new LC/MS method, and the matrix effect in less complicated water samples was less than 10%. In the next project period, we plan to further assess the matrix effect on fluoroquinolones and improve analytical methods to reduce matrix effect when necessary.

The detection limits of the LC/MS methods were preliminarily assessed based upon a signal-to-noise ratio of at least five and were around 1 μg/L for all antibiotics in reagent water extracts (by a concentration factor of 1000), 11 μg/L for sulfamethoxazole, 17 μg/L for sulfamethazine, and 26 μg/L for ciprofloxacin in secondary wastewater extracts (by a concentration factor of 1000). With the concentration factor of 1000, the method detection limits are near 1 ng/L in reagent water, and 11-26 ng/L in wastewater matrices. In the next project period, we will determine the detection limits and quantification limits more rigorously with proper approaches that are commonly used in method validation.

The modifications on sample extraction, storage and LC/MS methods have yielded higher recoveries for antibiotics as shown in Figure 15-19. Overall the recoveries determined by LC/MS for sulfamethoxazole, sulfamethazine and ciprofloxacin were above 75% on average in reagent water, as well as in the less complicated matrix of the extracts from the GAC system of an advanced WWTP before and after ozonation. Among these samples, we observed an average of 14% error between duplicate samples for sulfamethoxazole and sulfamethazine, and an average of 24% error between duplicate samples for ciprofloxacin. In secondary effluent extracts, the recoveries were above 90% for sulfamethoxazole and sulfamethazine, and above 60% for ciprofloxacin once we began storing sample extracts in freezer prior to LC/MS analysis. Within the limited number of samples analyzed, we observed less than 15% of error between duplicate samples.
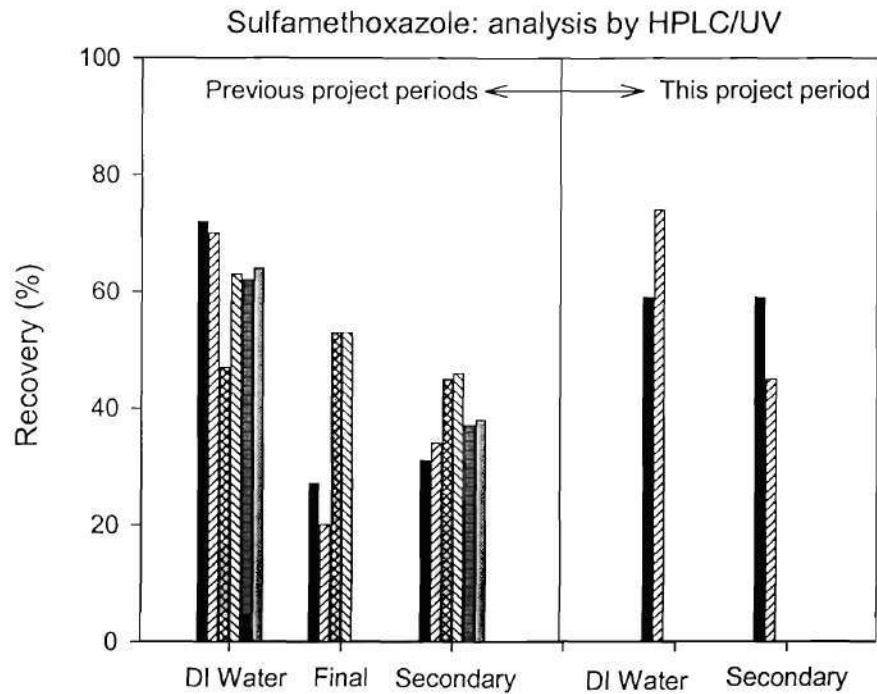
Figure 15: Recoveries of sulfamethoxazole determined by HPLC/UV at 260 nm. The spiked concentration of sulfamethoxazole ranged from 10-20 μg/L.
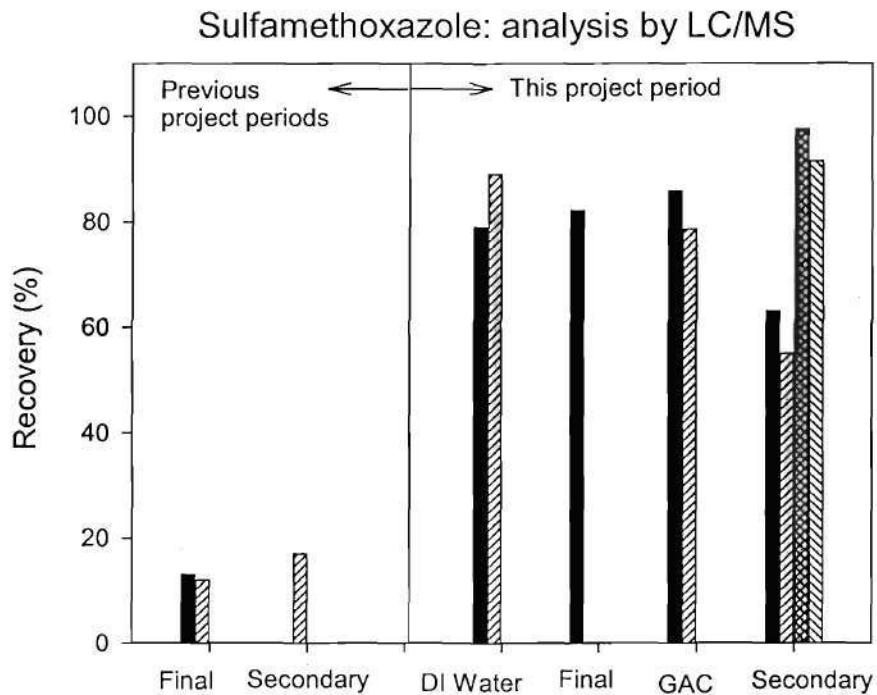
Figure 16: Recoveries of sulfamethoxazole determined by electrospray HPLC/MS at positive ion mode using selected-ion monitoring (SIM). The spiked concentration of sulfamethoxazole ranged from 1-10 μg/L.
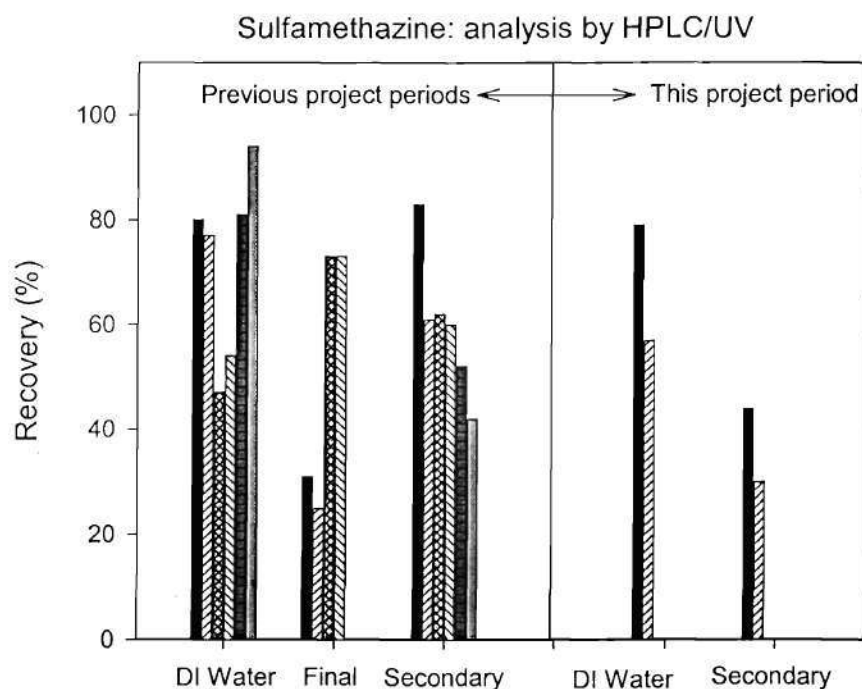
Figure 17: Recoveries of sulfamethazine determined by HPLC/UV at 260 nm. The spiked concentration of sulfamethoxazole ranged from 10-20 µg/L.
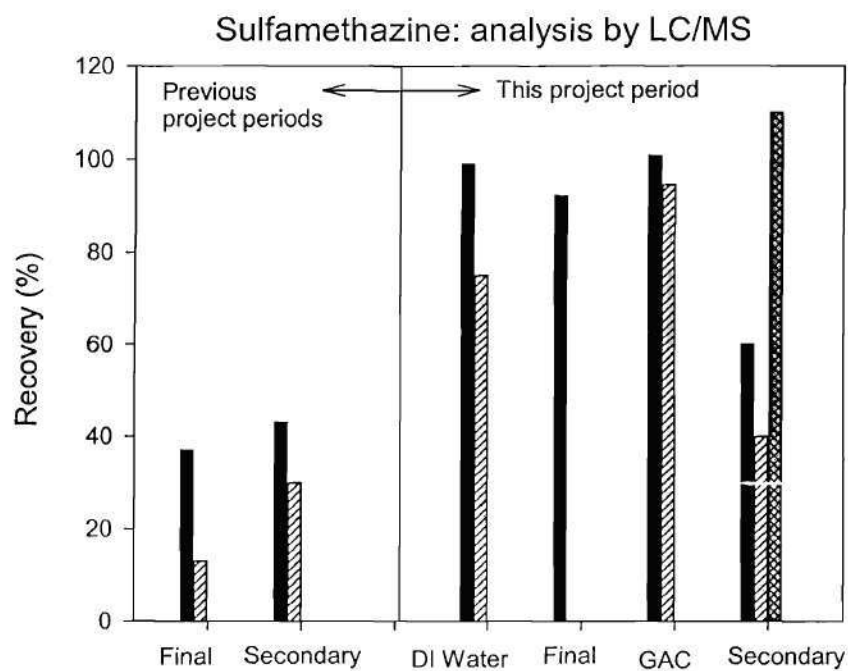
Figure 18: Recoveries of sulfamethazine determined by electrospray HPLC/MS at positive ion mode using selected-ion monitoring (SIM). The spiked concentration of sulfamethoxazole ranged from 1-10 µg/L.
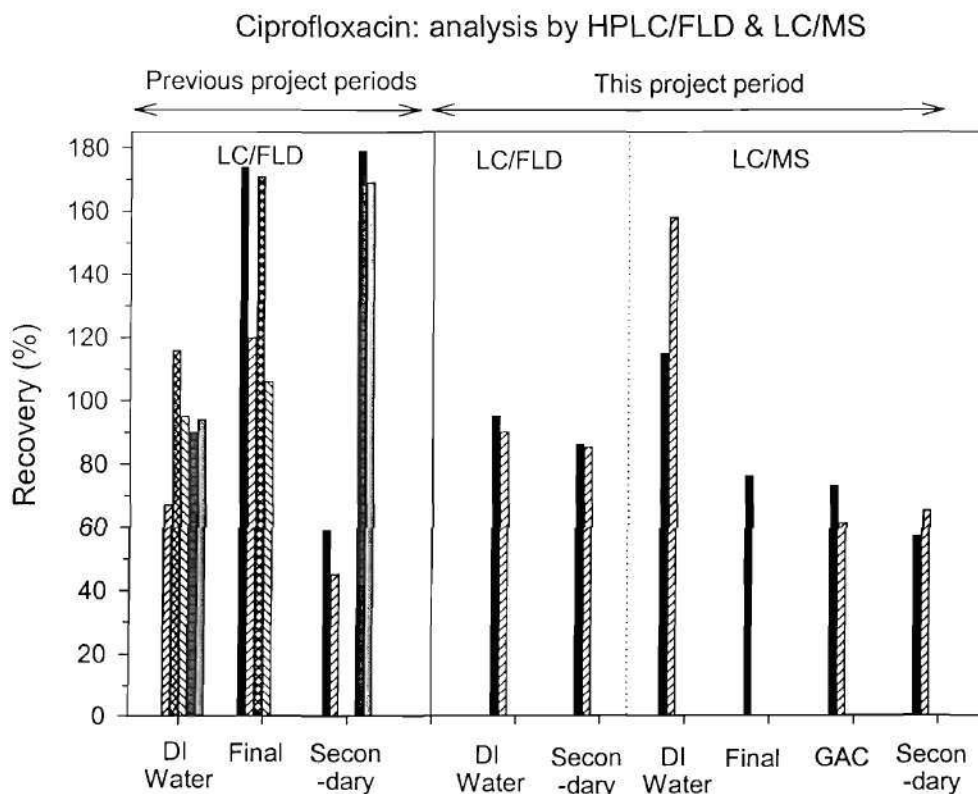
Figure 19: Recoveries of ciprofloxacin determined by HPLC/FLD at 278 nm excitation wavelength and 450 nm emission wavelength and by electrospray HPLC/MS at positive ion mode using selected-ion monitoring (SIM). The spiked concentration of ciprofloxacin ranged from 0.2-1.0 µg/L.

For the studies conducted during the fourth project period, wastewater samples were collected from the F. Wayne Hill Water Resources Center (FWH WRC) and the Clayton Wastewater Treatment Plant near Atlanta, Georgia. The FWH WRC is an advanced wastewater treatment plant and effluent samples of secondary, GAC and final (GAC and post-ozonation) were collected there. The Clayton WWTP has activated sludge biological treatment followed by UV disinfection; secondary effluent was collected from the Clayton WWTP. The results of studies indicated that sulfamethoxazole was present at around 250 µg/L in the secondary effluent of Clayton WWTP and was present at near the detection limit in the secondary effluent of FWH WRC. Ciprofloxacin was present in the secondary effluent of FWH WRC at concentrations near the detection limit. Sulfamethazine was below the detection limit in all the secondary effluent samples. In the effluent of advanced treatment (GAC and GAC/ozonation), no significant amounts of antibiotics were detected. These limited data were obtained from the most recent

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experiments after analytical methods have been improved. Since method development took significant amount of time and some of the samples had been in storage for some time, it is necessary to conduct more studies at these two plants in the next project period. We recently received samples of microfiltration influent, microfiltration effluent and reverse osmosis effluent from the West Basin Advanced Treatment Plant and will present results of analysis for these samples in the next project report.

Sorption of Antibiotics to Sample Containers

We evaluated the potential sorption of antibiotics (sulfamethoxazole, sulfamethazine, ciprofloxacin, enrofloxacin and norfloxacin) to the PFE-lined sampling bottles that have been used thus far, as well as to amber glass bottles. Sorption studies were conducted in two aqueous matrices (deionized water and secondary wastewater effluent) at neutral and acidic pH (pH ~4.5, acidified by phosphoric acid) respectively. Antibiotics were added to samples at 25-50 µg/L and the fortified solutions were stored in the PFE-lined or amber glass bottles at 4°C in the dark for at least 48 hours for the sorption to take place. LC/MS and HPLC/FLD analyses were conducted to evaluate any concentration changes of antibiotics due to sorption to the container walls.

As shown in Figure 20, the loss of sulfamethoxazole and sulfamethazine to the wall of either amber glass or PFE-lined bottles was negligible, less than 10% in DI water matrix and less than 3% in wastewater matrix. The loss of ciprofloxacin, enrofloxacin and norfloxacin to PFE-lined bottles was greater than to amber glass bottles. The loss ranged from 11 to 68% in PFE-lined bottles and 0 to 15% in amber glass bottles. In both containers, acidification of solution reduced the loss of fluoroquinolones. After acidification, the loss of fluoroquinolones to amber glass containers was less than 3% in either DI water or wastewater matrices. The results indicate that amber glass bottles are most appropriate for sample collection and storage and will be used in all studies in the future. Proper acidification of sample solutions will also be conducted to preserve the analytes.
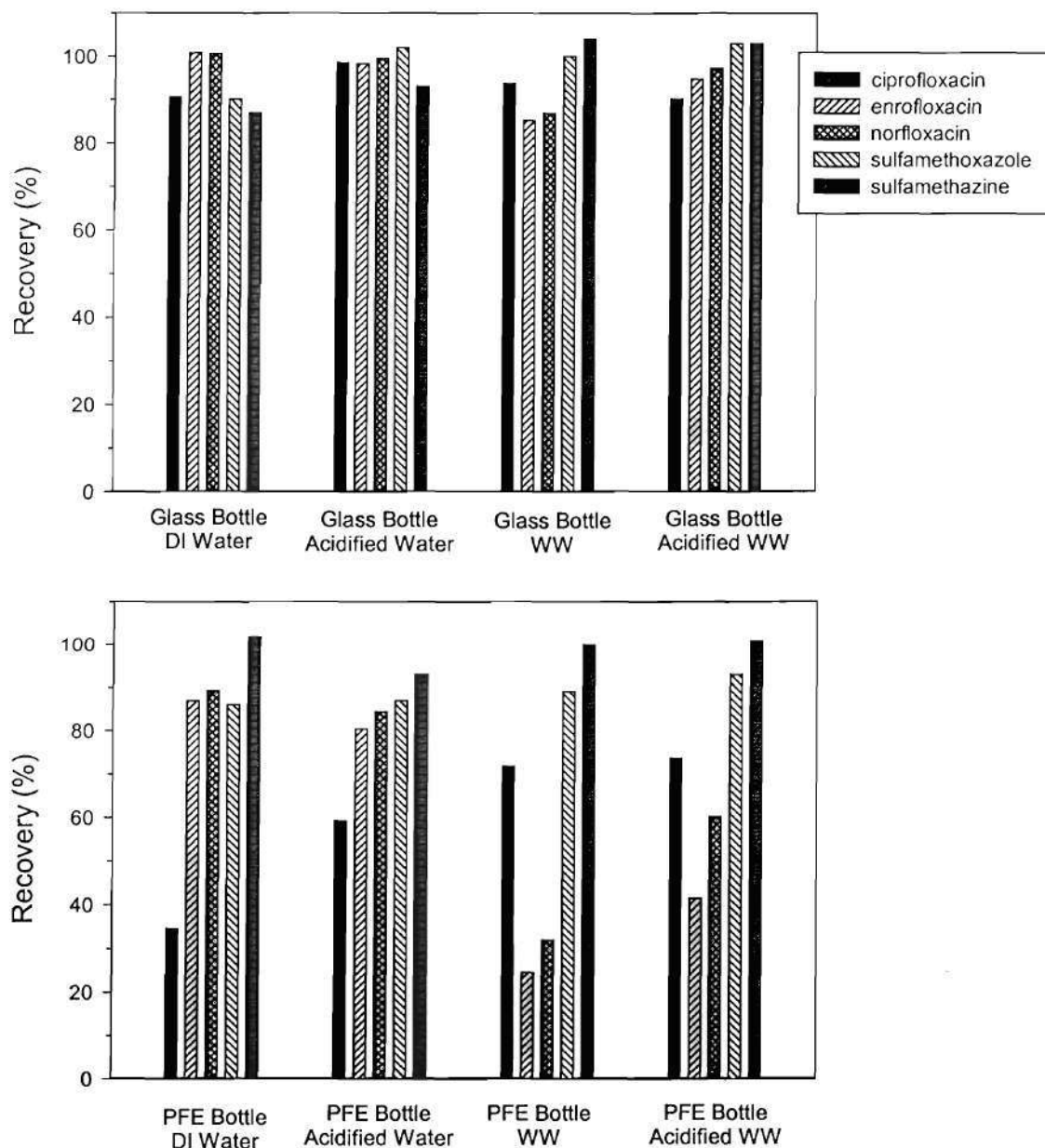
Figure 20: Loss of antibiotics in amber glass and PFE-line sample bottles. Samples were prepared in DI water and filtered secondary wastewater effluent at neutral and acidic pH respectively, and were stored at 4°C for at least 48 hours. The recovery represents the percentage of antibiotic concentration at 48 hours to the initial concentration.

Alternative Analytical Methods: Cation Exchange SPE and HPLC/FLD

In addition to the dual-cartridge SPE method, we conducted a few additional tests with the cation exchange SPE for ciprofloxacin. The objective of developing an effective cation exchange SPE followed by HPLC-FLD is to establish an alternative independent method that can be used to confirm the results by the dual-cartridge SPE followed by LC/MS analysis. The success of this alternative method is not critical for the occurrence survey of antibiotics as the dual-cartridge SPE and LC/MS method has proved to be effective in our recent studies, however, we think the potential applications of the cation exchange SPE merit a few more studies to explore its suitability.

We suspected that breakthrough was responsible for the poor recoveries of the cation exchange SPE. In addition, we discovered that one of the buffer solution used in extraction was not prepared correctly and might have resulted in some of the low recoveries seen previously. Furthermore, cation exchange resins from different manufacturers yield considerably different extraction efficacy based upon our experience and other studies. During the fourth project period, we conducted tests for cation exchange SPE using the high-density, mixed-phase cation exchanger (3M) that has been used in a previous study to extract fluoroquinolones (Golet et al., 2001). The method reported by Golet et al. (2001) was employed with small modifications. Studies were conducted to extract 50 to 400 ng/L of ciprofloxacin in 50 to 200 ml of reagent water at flow rate of approximately 1 ml/min. The recoveries of ciprofloxacin were over 90% for all the tests. These studies were conducted near the end of the fourth project period and further studies to use these cation-exchangers to extract fluoroquinolones in wastewater matrices are underway. Results from analysis of wastewater matrices will be reported in the next progress report.

TASK 3: OCCURRENCE SURVEY

Sub-Task 3A: Site Selection

In the previous progress report we provided a list of 15 sites that we planned to include in the occurrence survey. As part of the site selection process, preliminary samples were collected during the first three project periods from sites that we are considering for inclusion in the occurrence survey. During the fourth project period, we collected samples from one additional site that we are planning to include in the survey. In addition, we had the opportunity to collect a sample from another site that we are considering for inclusion in the occurrence survey. Brief descriptions of the sites sampled during this project period and interpretation of the preliminary data are included below:

Municipal Wastewater Treatment Plants: During the current project period we collected samples form an advanced wastewater treatment plant located in Witchita Falls, Texas. The plant subjects secondary effluent from an activated sludge treatment plant to microfiltration. Effluent from the advanced wastewater treatment plant is discharged to a lake that serves as a local water supply. The lake water, which also receives agricultural runoff, is subjected to reverse osmosis treatment prior to its use as a drinking water supply. During the current project period, we analyzed samples collected before and after microfiltration at the Witchita Falls AWWTP. As mentioned previously, we are uncertain of the validity of the data for the acidic drugs because our diazomethane underwent a phase separation. However, results from analysis of the beta-blockers met our QA/QC target with respect to recoveries. Concentrations of beta-blockers measured in the secondary effluent and microfiltration effluent samples (Appendix A) were similar to each other and slightly lower than concentrations measured in previous secondary effluent and microfiltration effluent samples (i.e., about 50 ng/L).

Surface Water Samples: During the current project period we collected samples from the Russian River and from the Delaware River, near Philadelphia. We did not include the Russian River in our initial list of sites for the occurrence survey. However, we were asked to analyze samples from the river by the Marin Municipal Water District (an AWWARF subscriber) as part of their planning analysis. During the third and fourth project periods., we used the samples from the

Russian River to test of QA/QC protocol. The Russian River receives a relatively small input of wastewater effluent from between the upstream and downstream sampling points. We have not detected pharmaceuticals in any of the samples collected during this or the previous project period. We plan to ask the agency for permission from the water agency to include the data in future publications related to this project.

During this project period we also analyzed samples from the Delaware River, near Philadelphia. We included these samples because in this section of the river a significant volume of wastewater effluent is discharged and the river is used downstream as a municipal water supply. In fact, this section of the river was been cited by the USEPA (Swayne et al. 1980) as one of the most important locations in which unintentional wastewater reuse could impact water quality. Furthermore, we had an opportunity to receive samples from the river as part of a monitoring program coordinated by Professor Jonathan Sharp, of the University of Delaware. During this quarter we analyzed a sample from a site near the outfall of the Southwest Wastewater Treatment Plant, which is located on the Delaware River near the point of entry of the Schuykill River. We analyzed a second sample collected near the intake of Philadelphia's Torresdale Water Treatment Plant, which is located upstream of the Southwest WWTP and approximately 30 km downstream of the Trenton (NJ) Wastewater Treatment Plant. These sites are referred to as downstream and upstream, respectivly. Samples collected at the upstream site contained less than 20 ng/L of all pharmaceuticals. At the downstream site, located near the wastewater discharge point, we detected 77 ng/L of gemfibrozil and 34 ng/L of naproxen. These data suggest that we might be able to assess the transport and transformation of these compounds by collecting samples at several additional locations above and below the outfall.

Sub-Task 3B: Sample Collection and Analysis

During the last month of this project period we finalized our QA/QC plan and evaluated method performance. We collected and extracted samples from the West Basin AWWTP (which also includes a samples from the Hyperion WWTP) and Orange County Water District's AWWTP in September. Samples are currently being analyzed with the modified QA/QC plan and results will be reported in the next progress report.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task were conducted during this project period.

PLANS FOR NEXT PERIOD

The following section describes research planned during the next project period. In addition, plans for the remainder of the project are described at the end of each section. A revised schedule for the project is presented in Appendix D.

Task 1: Literature Review

Sub-Task 1A: Drugs Used in Human Therapy

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Sub-Task 1B: Drugs Used in Animal Husbandry

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Task 2: Analytical Method Development and Testing

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the third project period we stated that we would evaluate the efficacy of sodium thiosulfate as a preservative for samples containing residual oxidants. However, we were unable to complete these activities during the fourth project period. These studies will consist of addition of 0.2 mM sodium hypochlorite to secondary effluent samples spiked with compounds of interest in the presence and absence of sodium thiosulfate.

As part of the analysis of samples for the occurrence survey, we also will measure the recoveries of deoxyepinephrine, the internal standard for the beta-blockers, and will compare it with recoveries measured in samples spiked with beta-blockers.

We also will repeat the recovery experiments with carbamazepine using freshly silanized glassware and samples from sites being considered for inclusion in the occurrence survey.

Sub-Task 2B: Antibiotics

During the next project period we plan to finish method development. The planned tasks include improvement of LC/MS method for fluoroquinolones, use of internal standards to assess matrix effect on LC/MS analysis and SPE recoveries, and development of cation-exchange SPE followed by HPLC/FLD for fluoroquinolones in wastewater samples.

Wastewater samples will be collected from the two local facilities FWH WRC and Clayton WWTP as well as several other sites that have been included in this project. The objective of these studies in the next project period is to evaluate the performance of the developed analytical methods in a variety of sample matrices. The results also serve as an initial screening for the occurrence of antibiotics in these samples.

Task 3: Occurrence Survey

Sub-Task 3A: Site Selection

We plan to include those sites specified in progress report 3. Additional sites will be included if time permits. No further activity is planned in association with this task.

Sub-Task 3B: Sample Collection and Analysis

Samples collected from sites listed in progress report 3 will be analyzed for acidic drugs and beta-blockers using the modified QA/QC plan included in Appendix B. We hope to analyze samples from these sites for carbamazepine and the antibiotics after completion of the QA/QC activities. These activities may not occur during the next project period.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task are planned during this project period. After completion of the occurrence survey, data will be evaluated to identify trends meriting further study. Data will be compared with expectations based on physical/chemical properties of the compounds as well as results reported by other researchers.

REFERENCES

Swayne M.D., Boone G.H., Bauer, D., Lee, J.S. 1980. *Wastewater in Receiving Waters at Water Supply Abstraction Points*. EPA-600/2-80-044.

Golet E. M., Alder A. C. Hartmann A., Ternes T. A.; Giger, W. 2001."Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection". *Anal. Chem.*, 73, 3632-3638.

APPENDIX A: Summary of Data for Acidic Drugs and Beta-Blockers

during the Third Project Period

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Diclofenac	Marin Upstream	5/14/01	<10, <10	81%
	Marin Downstream	5/14/01	<10, <10	101%
	Marin Caisson	5/14/01	<10	0%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		20%
	Witchita Fallas, TX Secondary	6/1/01	38, 14	6%
	Witchita Fallas, TX Microfiltration	6/1/01	72, 83	53%
	Witchita Fallas, TX Blank	6/1/01	63	
	Witchita Fallas, TX DI Spike	6/1/01		114%
	Marin Upstream	6/11/01	<10, <10	77% .
	Marin Downstream	6/11/01	<10, <10	70%
	Marin Caisson	6/11/01	<10, <10	0%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	33%
	Marin Downstream	7/11/01	<10, <10	56%
	Marin Caisson	7/11/01	<10, <10	0%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		0%
	Delaware River near Drinking Water Intake	7/19/01	<10	
	Delaware River near Wastewater Outfall	7/19/01	<10	
	Blank	7/19/01	<10	
	DI Spike	7/19/01		59%
	Marin Upstream	8/13/01	<10, <10	134%
	Marin Downstream	8/13/01	<10, <10	106%
	Marin Caisson	8/13/01	<10, <10	0%
	Blank	8/13/01	<10	
	DI Spike	8/13/01		94%
Gemfibrozil	Marin Upstream	5/14/01	<10, <10	96%
	Marin Downstream	5/14/01	<10, <10	148%
	Marin Caisson	5/14/01	<10	0%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		27%
	Witchita Fallas, TX Secondary	6/1/01	103, 31	7%
	Witchita Fallas, TX Microfiltration	6/1/01	157, <10	68%
	Witchita Fallas, TX Blank	6/1/01	<10	
	Witchita Fallas, TX DI Spike	6/1/01		118%
	Marin Upstream	6/11/01	<10, <10	101%
	Marin Downstream	6/11/01	<10, <10	78%
	Marin Caisson	6/11/01	<10, <10	0%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	48%
	Marin Downstream	7/11/01	<10, <10	66%
	Marin Caisson	7/11/01	<10, <10	0%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		68%
	Delaware River near Drinking Water Intake	7/19/01	16	
	Delaware River near Wastewater Outfall	7/19/01	77	
	Blank	7/19/01	<10	

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Gemfibrozil	DI Spike	7/19/01		90%
	Marin Upstream	8/13/01	<10, <10	115%
	Marin Downstream	8/13/01	<10, <10	107%
	Marin Caisson	8/13/01	<10, <10	0%
	Blank	8/13/01	<10	
	DI Spike	8/13/01		84%
Ibuprofen	Marin Upstream	5/14/01	<10, <10	69%
	Marin Downstream	5/14/01	<10, <10	105%
	Marin Caisson	5/14/01	<10	87%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		22%
	Witchita Fallas, TX Secondary	6/1/01	17, <10	12%
	Witchita Fallas, TX Microfiltration	6/1/01	25, <10	44%
	Witchita Fallas, TX Blank	6/1/01	<10	
	Witchita Fallas, TX DI Spike	6/1/01		54%
	Marin Upstream	6/11/01	<10, <10	84%
	Marin Downstream	6/11/01	<10, <10	48%
	Marin Caisson	6/11/01	<10, <10	65%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	37%
	Marin Downstream	7/11/01	<10, <10	56%
	Marin Caisson	7/11/01	<10, <10	50%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		24%
	Delaware River near Drinking Water Intake	7/19/01	16	
	Delaware River near Wastewater Outfall	7/19/01	16	
	Blank	7/19/01	<10	
	DI Spike	7/19/01		4%
	Marin Upstream	8/13/01	<10, <10	110%
	Marin Downstream	8/13/01	<10, <10	108%
	Marin Caisson	8/13/01	<10, <10	41%
	Blank	8/13/01	<10	
	DI Spike	8/13/01		77%
Indometacine	Marin Upstream	5/14/01	18, <10	78%
	Marin Downstream	5/14/01	21, <10	90%
	Marin Caisson	5/14/01	<10	0%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		21%
	Witchita Fallas, TX Secondary	6/1/01	67, 53	7%
	Witchita Fallas, TX Microfiltration	6/1/01	119, <10	28%
	Witchita Fallas, TX Blank	6/1/01	139	
	Witchita Fallas, TX DI Spike	6/1/01		118%
	Marin Upstream	6/11/01	<10, <10	85%
	Marin Downstream	6/11/01	<10, <10	74%
	Marin Caisson	6/11/01	<10	27%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	37%
	Marin Downstream	7/11/01	<10, <10	58%
	Marin Caisson	7/11/01	<10, <10	0%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		72%

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Indometacine	Delaware River near Drinking Water Intake	7/19/01	<10	
	Delaware River near Wastewater Outfall	7/19/01	<10	
	Blank	7/19/01	<10	
	DI Spike	7/19/01		75%
	Marin Upstream	8/13/01	<10, <10	104%
	Marin Downstream	8/13/01	<10, <10	102%
	Marin Caisson	8/13/01	<10, <10	0%
	Blank	8/13/01	<10	
	DI Spike	8/13/01		90%
Ketoprofen	Marin Upstream	5/14/01	<10, <10	92%
	Marin Downstream	5/14/01	<10, <10	118%
	Marin Caisson	5/14/01	<10	109%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		19%
	Witchita Fallas, TX Secondary	6/1/01	89, 76	11%
	Witchita Fallas, TX Microfiltration	6/1/01	108, ND	45%
	Witchita Fallas, TX Blank	6/1/01	85	
	Witchita Fallas, TX DI Spike	6/1/01		127%
	Marin Upstream	6/11/01	<10, <10	79%
	Marin Downstream	6/11/01	<10, <10	53%
	Marin Caisson	6/11/01	<10, <10	94%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	48%
	Marin Downstream	7/11/01	<10, <10	68%
	Marin Caisson	7/11/01	<10, <10	80%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		75%
	Delaware River near Drinking Water Intake	7/19/01	<10	
	Delaware River near Wastewater Outfall	7/19/01	<10	
	Blank	7/19/01	<10	
	DI Spike	7/19/01		80%
	Marin Upstream	8/13/01	<10, <10	108%
	Marin Downstream	8/13/01	<10, <10	109%
	Marin Caisson	8/13/01	<10, <10	67%
	Blank	8/13/01	<10	
	DI Spike	8/13/01		111%
Mecoprop	Marin Upstream	5/14/01	82%, 99%	88%
	Marin Downstream	5/14/01	114%, 97%	108%
	Marin Caisson	5/14/01	47%	54%
	Blank	5/14/01		
	DI Spike	5/14/01		59%
	Witchita Fallas, TX Secondary	6/1/01	112%, 48%	0%
	Witchita Fallas, TX Microfiltration	6/1/01	137%, 0%	32%
	Witchita Fallas, TX Blank	6/1/01		
	Witchita Fallas, TX DI Spike	6/1/01		54%
	Marin Upstream	6/11/01	87%, 73%	66%
	Marin Downstream	6/11/01	90%, 74%	0%
	Marin Caisson	6/11/01	89%, 81%	85%
	Blank	6/11/01		
	Marin Upstream	7/11/01	80%, 67%	74%
	Marin Downstream	7/11/01	88%, 80%	95%

Note: Mecoprop data indicate recoveries for the radiolabeled mecopropo internal standard.

Compound	Location	Date	Concentration (ppt)	Spike Recovery
Mecoprop	Marin Caisson	7/11/01	47%, 58%	91%
	Blank	7/11/01		
	DI Spike	7/11/01		67%
	Delaware River near Drinking Water Intake	7/19/01	66%	
	Delaware River near Wastewater Outfall	7/19/01	57%	
	Blank	7/19/01		
	DI Spike	7/19/01	48%	
	Marin Upstream	8/13/01	84%, 104%	206%
	Marin Downstream	8/13/01	146%. 105%	193%
	Marin Caisson	8/13/01	119%, 119%	0%
	Blank	8/13/01		
	DI Spike	8/13/01		105%
Naproxen	Marin Upstream	5/14/01	<10, <10	79%
	Marin Downstream	5/14/01	<10, <10	117%
	Marin Caisson	5/14/01	<10	0%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		17%
	Witchita Fallas, TX Secondary	6/1/01	180, 43	12%
	Witchita Fallas, TX Microfiltration	6/1/01	226, 39	52%
	Witchita Fallas, TX Blank	6/1/01	52	
	Witchita Fallas, TX DI Spike	6/1/01		124%
	Marin Upstream	6/11/01	<10, <10	73%
	Marin Downstream	6/11/01	<10, <10	51%
	Marin Caisson	6/11/01	<10, <10	0%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	50%
	Marin Downstream	7/11/01	<10, <10	74%
	Marin Caisson	7/11/01	<10, <10	0%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		55%
	Delaware River near Drinking Water Intake	7/19/01	17	
	Delaware River near Wastewater Outfall	7/19/01	34	
	Blank	7/19/01	<10	
	DI Spike	7/19/01		95%
	Marin Upstream	8/13/01	<10, <10	110%
	Marin Downstream	8/13/01	<10, <10	115%
	Marin Caisson	8/13/01	<10, <10	0%
	Blank	8/13/01	<10	
	DI Spike	8/13/01		113%

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Compound	Location	Date	Concentration (ppt)	Spike Recovery
Metoprolol	Marin Upstream	5/14/01	<10, <10	40%
	Marin Downstream	5/14/01	<10, <10	43%
	Marin Caisson	5/14/01	<10	79%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		57%
	Witchita Fallas, TX Secondary	6/1/01	26, 88	74%
	Witchita Fallas, TX Microfiltration	6/1/01	84, 91	81%
	Witchita Fallas, TX Blank	6/1/01	<10	
	Witchita Fallas, TX DI Spike	6/1/01		64%
	Marin Upstream	6/11/01	<10, <10	47%
	Marin Downstream	6/11/01	<10, <10	37%
	Marin Caisson	6/11/01	<10, <10	48%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	61%
	Marin Downstream	7/11/01	<10, <10	46%
	Marin Caisson	7/11/01	<10, <10	29%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		75%
	Delaware River near Drinking Water Intake	7/19/01	<10, <10	47%
	Delaware River near Wastewater Outfall	7/19/01	12, <10	84%
	Blank	7/19/01	<10	
	DI Spike	7/19/01		21%
	Marin Upstream	8/13/01	<10, <10	34%
	Marin Downstream	8/13/01	<10, <10	29%
	Marin Caisson	8/13/01	<10, <10	27%
	Blank	8/13/01	<10	
	DI Spike	8/13/01		56%
Propranolol	Marin Upstream	5/14/01	<10, <10	35%
	Marin Downstream	5/14/01	<10, <10	46%
	Marin Caisson	5/14/01	<10	28%
	Blank	5/14/01	<10	
	DI Spike	5/14/01		45%
	Witchita Fallas, TX Secondary	6/1/01	<10, 63	65%
	Witchita Fallas, TX Microfiltration	6/1/01	60, 55	66%
	Witchita Fallas, TX Blank	6/1/01	<10	
	Witchita Fallas, TX DI Spike	6/1/01		63%
	Marin Upstream	6/11/01	<10, <10	46%
	Marin Downstream	6/11/01	<10, <10	31%
	Marin Caisson	6/11/01	<10, <10	30%
	Blank	6/11/01	<10	
	Marin Upstream	7/11/01	<10, <10	56%
	Marin Downstream	7/11/01	<10, <10	46%
	Marin Caisson	7/11/01	<10, <10	25%
	Blank	7/11/01	<10	
	DI Spike	7/11/01		68%
	Delaware River near Drinking Water Intake	7/19/01	<10, <10	34%
	Delaware River near Wastewater Outfall	7/19/01	<10, <10	53%
	Blank	7/19/01	<10	
	DI Spike	7/19/01		34%
	Marin Upstream	8/13/01	<10, <10	29%
	Marin Downstream	8/13/01	<10, <10	34%

APPENDIX B:

Modified QA/QC Plan for Drugs Used in Human Therapy (Excluding Antibiotics)

Sample Collection: Grab samples will be collected in 1-L glass bottles with Teflon-lined screw caps. Each bottle will be kept in an individual polyethylene bag. Prior to sampling, bottles will be cleaned in our laboratory with Micro brand laboratory detergent, rinsed with water followed by methanol and deionized water between each analysis. Bottles will be shipped to participants in coolers with blue ice packs.

For samples collected from wastewater treatment plants or water treatment plants using chlorine for disinfection, $Na_2S_2O_3$ will be added to the samples bottle as a preservative. Each set of samples will be shipped with a field blank, which will be analyzed with the samples. Samples will be collected by field personnel who are familiar with trace organic sampling protocols. Field personnel will wear polyethylene gloves when handling bottles and will be instructed to minimize the amount of time that the bottle is kept uncapped outside of the cooler.

Sampling times, locations and personnel will be recorded on a log sheet that will accompany each set of samples. Each sample will be given a unique sequential sample identification number as indicated on the log sheet. To prevent bias, sample numbers will not provide any indication of sample locations. Samples will be shipped in the cooler via overnight mail. Upon arrival at UC Berkeley, samples and log sheets will be visually inspected and transferred to a 5 C storage area. Samples will extracted as soon as practical and within no more than 72 hours after arrival.

Sample Extraction and Analysis: Each set of ten samples will be analyzed in a batch that contains appropriate QA/QC standards. The following samples will be included with each set of samples.

(1) Field blank (1 L of deionized water that travels to and from the field site);

(2) Matrix recovery sample (1 sample from the site spiked with all analytes at 1,000 ng/L);

(3) Duplicate sample;

(4) Auxiliary standard consisting of a mixture of the derivatized analytes, as prepared by a third party in our laboratory.

All samples to be analyzed for acidic drugs will be amended with the equivalent of 100 ng/L of radiolabeled mecroprop prior to extraction. After elution from the SPE, all samples to be analyzed for beta-blockers wil be spiked with the equivalent of 100 ng/L of deoxyepinephrone. After derivitization, samples will be diluted to 1 mL prior to addition of the secondary internal standard, hexachlorobenzene.

The run sequence will consist of five standards followed by a randomized mixture of the samples and QA/QC samples. The calibration curve will be checked every ten samples by running a blank and a reslope standard from the middle of the calibration curve. If the calibration standard disagrees with the standard curve by more than 25% the samples in the following section will be rerun.

Our target for recoveries will be 60-120%. For any sample or batch of samples in which these values are not obtained, we will rerun all of the samples or repeat the analysis. If acceptable recoveries are not obtained, we will report the data with permanent qualifiers.

APPENDIX C:
LC/MS METHODS FOR ANTIBIOTICS

A HPLC/UV/MS system (Hewlett-Packard, Series 100 MSD G1946A, Palo Alto, CA) with electrospray ionization at positive ion mode was employed for analysis. Selected-ion monitoring (SIM) was used for compound detection and quantification. A 150 mm C18 column (2.1 mm, 5 μm particles, Agilent Technology) maintained at 30°C was used with an injection volume of 20 μL.

Method for Sulfonamides: The mobile phases included a solution containing 1 mM ammonia acetate (pH 6.47) and 10% acetonitrile (eluent A) and 100% acetonitrile (eluent B). At a flow rate of 0.25 ml/min, the gradient separation was as follows: 2 minutes isocratic 100% A followed by a gradient increase to 33.8% B in 16 minutes. The column was then flushed with 100% B for 6 minutes. A 8 minute post-time at isocratic 100% A was employed to allow the column to be equilibrated prior to the next injection. Sulfonamides were also monitored by UV absorption at 265 nm.

Mass spectra were acquired in positive-ion electrospray using selected-ion monitoring (SIM). Conditions of mass spectrometer were as follows: the drying gas at a flow rate of 10-L/min and 350 °C, the nebulizer pressure of 20 psi, the capillary voltage at 4000 V. For quantification, the fragmentor voltage was set to 70 V and only the molecular ion was monitored under SIM. For verification, the fragmentor voltage was set to 76 V and three ions (molecular ion and two characteristic ions) were monitored with SIM. The important ions for sulfonamides are: 254, 156 and 92 m/z for sulfamethoxazole, and 279, 186 and 156 m/z for sulfamethazine. The antibiotics were identified by their chromatographic retention time, molecular ion, fragment ions and the relative abundance between ions.

Method for Fluoroquinolones: The mobile phases included a solution containing 0.02 % acetic acid and 10% acetonitrile (eluent A) and 100% acetonitrile (eluent B). At a flow rate of 0.2 ml/min, the gradient separation was as follows: 2 minutes isocratic 100% A followed by a gradient increase to 16% B in 9 minutes and then 45 % in 20 minutes. The column was then flushed with 100% B for 5 minutes. A 9 minute post-time at isocratic 100% A was employed to

allow the column to be equilibrated *prior to the next injection. Fluoroquinolones were also* monitored by UV absorption at 278 nm.

The conditions of mass spectrometer were similar to those for sulfonamides except that the nebulizer pressure was 30 psi. For quantification, the fragmentor voltage was set to 70 V and the molecular ion was monitored under SIM. For verification, the fragmentor voltage was set to 80 V and two to three ions (molecular ion and 1-2 fragment ions) were monitored using SIM. The important ions for fluoroquinolones are: 332 and 274 m/z for ciprofloxacin; 360, 195 and 141 m/z for enrofloxacin; and 320 and 61 m/z for norfloxacin.

Appendix D: Revised Schedule

Note: We have extended the time required to complete method development activities for the antibiotics by approximately 3 months. All other tasks are proceeding according to schedule.

Year

1   2   3

1a. Draft literature review-drugs   1a. Update review

1b. Draft literature review-antibiotics   1b. Update review

2a. Method development-drugs excluding antibiotics

2b. Method development-antibiotics

3a. Site selection

3b. Sample collection and analysis

3c. Data analysis and synthesis
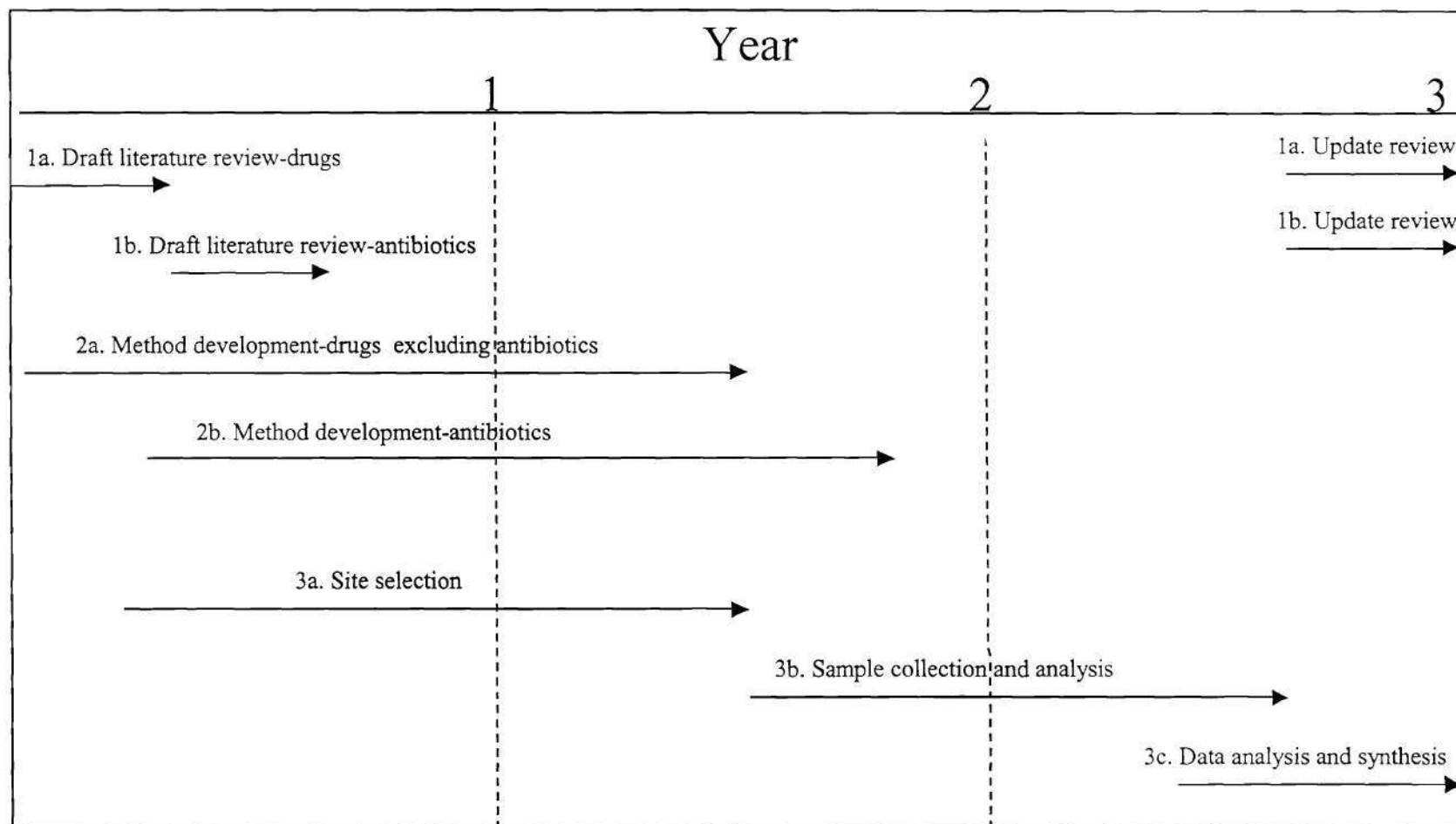
APPENDIX E: Responses to PAC Comments on the Third Periodic Report

Comment: 1: *PAC members were pleased with the third periodic report and found it "almost comprehensive and of good technical quality"*

Response: We aim to please!

General remarks:

Comment 2: *The analysis of PhACs in complex matrices such as municipal sewage is a difficult challenge for environmental analysts. This explains why some of the results reported for the development of the analytical methods are a little bit disappointing. Especially, the recoveries of the antibiotics are very poor and need to be improved before conducting the large monitoring investigations! The QA/QC procedures need further improvements to ensure higher accuracy of the analytical methods. There is, however, one aspect that is totally missing in this report and also in the time-schedule on page 50! What happened to the plan to elaborate and invent immunochemical methods for the detection of several pharmaceuticals in environmental samples? This aspect mentioned in the proposal and in the first report was one of the major advantages of this proposal and a major reason for its acceptance. Now, this aspect is totally missing and no efforts are mentioned following this direction???*

Response: As discussed in response to comment 18, we have been unable to pursue ELISA methods because we have been developing and testing GC/MS and LC/MS methods. As the PAC recalls from our initial meeting, we initially believed that we might be able to use ELISAs for analysis of antibiotics. Because the PAC insisted that we develop MS techniques for confirmatory analysis and the project budget is limited, we have been unable to pursue the development and testing of ELISA's.

Comment 3: *It seems the report is focusing on a lot of method development and it is very hard to summarize the many things that are being tried to make this a success. I think this report is much better in the detailing of what has been tried. However, effort seems to be going into make methods that work in actual samples instead of getting a method that may work in reagent water (eliminating all but matrix problems) and then working on matrix removal/problem strategies. Just a thought. The use of ion traps in any way for complex matrices should be avoided. The ion trap is very good looking for a single ion or multiple ions in clean matrices, but our experience is that in a complex matrix, do a very good separation, and then a very good analysis on quadrupoles.*

Response: During the first three progress reports we evaluated method performance in deionized water and in samples from candidate sites. We believe that our data illustrate the merits of conducting both types of analyses in parallel. For example, recoveries of acidic drugs tend to be better in environmental samples than in deionized water, presumably because dissolved ions and organic matter in the environmental samples improve the efficacy of the solid phase extraction resin or the derivitization process. If we had concentrated only on deionized water samples we would have never learned that our approach is acceptable for environmental samples. As for the

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use of quadrupoles, as we stated in our earlier discussions, we do not have access to a triple quadrupole GC/MS/MS system that would be needed for the type of analyses suggested by the reviewer. Since we are attempting to develop analytical methods that can be applied throughout the water industry and triple quadrupole GC/MS/MS systems are very expensive, we see merit in investigating the applicability of ion trap systems.

Specific comments:

Comment 4: p.7: Unfortunately, the attempts to include several other compounds into the analytical method failed. It was really surprising that it was also not possible to include caffeine because it can easily be detected without any derivatization. Could you report about the problems? It may still be beneficial to include this compound as it is often reported in publications and may be used to compare the results with those from other studies.

Response: As we stated in our progress report, we experienced poor sensitivity and poor chromatography during our attempts to analyze caffeine. The few previous reports of caffeine analysis by GC/MS used different columns than the one that we are using and we believe it would be very inconvenient to change columns for one analyte. Given concerns about sample contamination with caffeine reported elsewhere and the potential of sources other than sewage effluent, we do not believe that it is an appropriate compound for our study. If the reviewer can provide us with a reference that they believe to be useful, we will investigate this issue further.

Comment 5: p.23ff: Unfortunately, the recoveries reported for the antibiotics are still poor. You may avoid sentences such as "In the secondary effluent, recoveries ranged between 45 to 59% for ciprofloxacin, 45 to 46% for sulfamethoxazole, and 48 to 62% for sulfamethazine. In the final effluent, recoveries ranged between 120 to 174% for ciprofloxacin, and 20 to 31% for the sulfonamides" when only analyzing two samples from each kind of matrix. Analyzing only two recovery samples, containing each matrix, in parallel, the ranges of the recoveries may still be far from being representative. Thus, some additional experiments may be necessary to get reliable data. Method C seems to perform even better than method A but a recovery of 0%, reported for ciprofloxacin in spiked DI water (table 2), does not demonstrate the reliability of this method. Some further QA/QC procedures (e.g. radiolabeled ciprofloxacin) need to be included in this method to avoid non-precise or even false positive results? As long as the recoveries of the analytes are not improved significantly, it also seems to be highly questionable to correct the measured results by recovery. Especially, if the spiked amounts of the analytes significantly differ from the amounts of analytes detected in the samples (sulfonamides, p.44).

Response: The investigators agree with the PAC's comments. The recoveries reported in the previous report were intended to provide information regarding the progress of method development, and were used as guidance to improve analytical methods. The wide range of recoveries indicated the need to assess the steps where losses of analytes may occur and to modify the methods to minimize these losses. As described in this progress report, we conducted studies in this project period to address this problem. For instance, we reduced the losses of analytes during the blowdown step, improved the LC/MS methods and adopted internal standards in the analysis. These efforts have substantially improved the recoveries as well as sensitivity and precision of the analytical methods. Utilization of radiolabeled target antibiotics is

desirable for the QA/QC of analysis. We were unable to find vendors for these radiolabeled chemicals during the fourth project period. Instead, we evaluated structurally-related sulfonamides and fluoroquinolones as potential internal standards. The use of these internal standards facilitated assessment of LC/MS performance and the potential influence of matrix effect on analyte signals. In the next project period, we will select and evaluate some of these internal standard candidates as surrogate compounds to assess method recovery. If radiolabeled target antibiotics can be purchased, we will test the use of these compounds as surrogate standards in the analytical method as well.

During this project period, we have reduced the spiked concentrations of all antibiotics to 0.5-10 µg/L, and will use 1 µg/L spike concentration to assess analysis recovery in the future.

Comment 6: p.23: Cation exchange SPE may be very difficult, especially, if the analytes have to compete with a bulk of matrix. It is also mentioned that the flow rates are decisive to get higher recoveries (p.36). This is also consistent with observations in some other investigations. Slower flow rates may improve the recovery rates but they will also extent the analytical times. Could you give some details? What are your plans to improve recoveries at reasonable flow rates?

Response: We suspected that breakthrough was responsible for the poor recoveries of the cation exchange SPE. In addition, we discovered that some of the low recoveries seen previously may be the result of a buffer whose pH might not be adjusted properly. Furthermore, cation exchange resins from different manufactures yield considerably different extraction efficacy based upon our experience and other studies. During the fourth project period, we conducted tests for cation exchange SPE using the mixed-phase cation exchanger (3M) that has been used in a previous study to extract fluoroquinolones (Golet et al. 2001). This cation exchanger yielded greater than 90% of recoveries in reagent water and its use in extracting fluoroquinolones in wastewater matrices is currently being assessed and the results will be reported in the next progress report.

As discussed in this project report, the cation exchange SPE method can be used with HPLC/FLD analysis, thus providing an alternative method that can be used to confirm the analysis by LC/MS. For studies on the occurrence of antibiotics, the dual-cartridge SPE followed by LC/MS will be the primary analytical approach.

Comment 7: p.27, figure 10: HPLC/FL-detection seems to be heavily interfered (the analyte peak is one of the smallest peaks in a very complex chromatogram). How can you ensure the reliable identification and quantification of this compound in different kinds of matrices? Are the high recoveries reported in table 2 (final effluent) possibly only caused by matrix interferences (co-eluting peaks)? HPLC-MS or better MS/MS seems to be necessary to confirm these results? May at a later stage of the project only LC-MS be used for the analysis of ciprofloxacin? Is FL detection really necessary at all? figure 11 (p.28): 46µg/L: This seems to be the concentration of ciprofloxacin in the final concentrate. Probably, it may be less confusing if only the final concentrations of the analytes in the original sample (46 ng/L) are reported? (also figure 12)

Response: As discussed in the third progress report (pp. 26), the investigators agree with the PAC that quantification by HPLC/FLD is not accurate for fluoroquinolones in complicated wastewater extract (e.g., secondary effluent) due to the high amount of interfering compounds, unless further sample clean-up or a more selective extraction (e.g., cation exchange SPE) can be

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achieved. The HPLC/FLD analysis, however, works fairly well in effluent of advanced treatment systems where the problem of interfering compounds is much less significant. The higher recoveries obtained in the final effluent are reliable data based upon the clean chromatograms and high purity of the peak spectra. On the contrary, the co-eluting compounds in the secondary effluent samples probably resulted in underestimated recoveries since peak integration was conducted only at the area above the high baseline caused by interfering compounds.

Analysis by LC/MS methods is more appropriate, particularly for samples of complicated matrices such as secondary effluent. The LC/MS methods were developed and improved to analyze the target antibiotics and have yielded good results (see section 2B.2 in this report). The use of HPLC/FLD, when appropriate, will be only facilitative as a confirmatory method for results of fluoroquinolones by LC/MS.

Yes, the estimated concentration of ciprofloxacin in the original sample is 46 ng/L.

Comment 8: *p.29: Some problems (matrix interferences) using HPLC-MS for the analysis are described in this section. Matrix effects are a well-known problems using HPLC with MS detection. This effect is caused by co-eluting of matrix compounds and may result in both an overestimation and an underestimation of the analyte quantities. Thus, you may be aware that the use of internal standards which do not co-elute with the analytes do not solve problems with matrix effects. Due to the higher selectivity of MS detection, compared to UV detection, it may not be excluded that the lower recoveries are the right ones. The use of a standard addition method seems to be much more reasonable to assess and to minimize the impacts of matrix effects.*

Response: The matrix effect can probably be best evaluated using radiolabeled target analytes; however we were unable to find vendors for radiolabeled ciprofloxacin, sulfamethoxazole and sulfamethazine during the fourth project period. Instead, we conducted studies to improve the LC/MS methods to minimize the matrix effect by improving the chromatographic separation, and optimizing the mobile phase compositions and MS parameters. The matrix effect was considerably reduced by these efforts. We also spiked sulfonamide and fluoroquinolone compounds into wastewater extract and compared the analyte signals to those in reagent water at comparable concentrations. The results indicate that the matrix of secondary effluent reduced the analyte signal by 20 to 40%. The reduction in signals is comparable among sulfonamides and fluoroquinolones, respectively despite the differences in their retention time. The matrix effect is significantly less in less complicated water samples.

Comment 9: *p.30, figure 12: The lower figure is not readable in my copy.*

Response: This probably happened during transferring files between formats. The investigators apologize for this and will try to avoid such problems in the future.

Comment 10: *p.36. Are you going to try to improve recoveries of the 7 compounds listed in Appendix A? Since quite a few compounds were eliminated out of the original list (progress reports 1 and 2). Will you be able to expand the list to others beyond the 7 compounds?*

Response: We were able to analyze indometacine and plan to include carbamazepine in future analyses. We addressed the issue of recoveries in tis progress report. For the acidic compounds

recoveries should be acceptable. For the beta-blockers, we may not be able to obtain recoveries better than 40-60% with these methods.

Comment 11: *p.39-41, Appendix A: Have the calculated concentrations also been corrected by recovery? If yes, was this correction also used when the recoveries for the analytes were very low (metoprolol/propanolol)? Can the results really be regarded as being more than semi-quantitative?*

Response: We have not corrected any of the data for recoveries. We agree with the reviewer about results for beta-blockers in which recoveries are below 60%. However, we also believe that such "semi-quantitative" results will be useful in evaluating the scope of the problems posed by pharmaceuticals (i.e., we should be able to tell if they are removed during treatment, even if there is uncertainty associated with the exact results).

Comment 12: *p.39: How was the problem of the degradation of ibuprofen addressedwhen sewage samples were spiked for the recovery experiments?*

Response: The samples are spiked after filtration. The filtration of samples should have eliminated much of the bacteria. The acidification should also deactivate the bacteria.

Comment 13: *p.42. In the QC plan, I would recommend all glass bottles be amber glass bottles. Some of the target analytes do respond to light.*

Response: We realize that some of the analytes are sensitive to light and shield the samples from sunlight during sample collection using the coolers. We see no evidence of analyte loss when exposed to fluorescent light in the laboratory. We prefer to use clear glass because it is easier to evaluate the cleanliness of the bottles.

Comment 14: *p.43: "Our target for recoveries will be 60-120%. For any sample or batch of samples in which these values are not obtained, we will rerun all of the samples or repeat the analysis" What do you mean? The recoveries of the internal standards or those of the analytes obtained from spiked samples analyzed in parallel? The first approach would be more reasonable if the internal standards are able to compensate the analyte losses (or "gains") independent of the matrix.*

Response: We are referring to the recovery of the internal standards.

Comment 15: *p.44: 20µg/L of sulfonamides used for the spiking experiments are rather high and not very realistic compared to the concentrations that might be expected to occur in the environment. Of course, the use of such high concentrations is reasonable and necessary when using UV detection in method development as also pointed out clearly in the report. But it seem to be questionable that the recovery rates obtained are also applicable to samples containing much lower concentrations of the analytes?*

Response: In the studies conducted during the fourth project period, we have reduced the spiked concentrations of sulfonamides to 1-10 µg/L, and will use 1 µg/L spike concentration to assess

analysis recovery for all antibiotics in the future. We considered 1 µg/L to be appropriate since the estimated influent concentrations of the antibiotics range from 1.4 to 3.2 µg/L, and the preliminary results indicate that sulfamethoxazole is present at approximately 250 ng/L in the secondary effluent.

Conclusions:

Data analysis

Comment 16: *As also pointed out in the progress report and in the text above, several improvements of the analytical methods need to be done before conducting the monitoring analyses.*

Response: Improvements to analytical methods are addressed in responses to previous comments and in the progress report.

Progress to date

Comment 17: The efforts in method development need to be intensified.

Response: Almost all activities reported for this project period address method development.

Description and rationale for proposed changes to the scope of work.

Comment 18: What has happened to the development of ELISA methods?

Response: Immunoassays (e.g., ELISA) were described as one of the analytical approaches in the proposal. It is practical to use available ELISA kits from commercial manufacturers because custom-made ELISA kits can be highly costly and time-consuming thus not suitable for large number of samples. Our search indicated that commercial ELISA kits are not available for most of the drugs and antibiotics identified by our literature review as target analytes in the occurrence survey. Radio-immunoassay kits are available for selected antibiotics for screening purposes; however high cross-reactivity of these kits among structurally-related antibiotics renders their use for analyte quantitation difficult.

During the PAC meeting at the beginning of the project, the PAC raised the question regarding the uncertainties associated with using ELISA methods in complicated wastewater samples. When sample clean-up is not sufficient to eliminate interfering compounds, ELISA analysis may yield false-positive or false-negative results, and thus confirmation of the results by a second method such as GC/MS or LC/MS is necessary. Based upon the above considerations and the objective to obtain sufficient occurrence data for pharmaceuticals within the timeline and budget of the project, we think it is necessary to focus on GC/MS and LC/MS as the primary analytical methods because of their high selectivity and versatility for a wide range of compounds that have been identified by our literature review. As a result, our analytical method development has since been focused on GC/MS for drugs excluding antibiotics and LC/MS for antibiotics.

Projected work for the next period.

Comment 19: Work should be continued as proposed and planned in the report. The efforts in instrumental method development and the development of the ELISAs should be continued. Monitoring samples should only be analyzed when the analytical methods are completely validated.

Response: As described in response to the previous comments, we are not planning any activities related to ELISAs during the next project period. Analytical method development will be continued as described in the progress report. Occurrence survey samples will be analyzed for drugs other than antibiotics during the next project period because method development activities will be completed near the beginning of the next project period.

Appendix D

Comment 20: Based on the work you have done as part of your method development. The schedule does not indicate whether there will be an evaluation of "possible" indicator compounds for various groups (i.e. acidic, beta-blockers, etc).

Response: We will evaluate possible indicator compounds (e.g., EDTA and DOC) during the occurrence survey.

OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Fifth Progress Report

January 15, 2002

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SUMMARY

During the fifth project period, we continued to test and improve analytical methods for use in the occurrence survey. We also applied methods developed in the previous project periods to analyze samples for acidic drugs and beta-blockers from two advanced wastewater treatment plants and an engineered treatment wetland.

For the drugs other than antibiotics, we attempted to improve the analytical methods for beta-blockers and carbamazepine. After several attempts, we abandoned our search for an appropriate internal standard for the beta-blockers. Following the method provided by Dr. Heberer, we were able to recover and detect carbamazepine in several environmental samples. In addition, we collected and analyzed samples from the West Basin advanced wastewater treatment plant (AWWTP), the Orange County Water District's AWWTP and the Mt. View Sanitary District's engineered treatment wetland. Results indicated that microfiltration has no effect on the pharmaceuticals while reverse osmosis lowers pharmaceutical concentrations below detection limits. All of the compounds except gemfibrozil and naproxen were removed in the engineered treatment wetland.

For the antibotics, we improved the analytical methods for analysis of fluoroquinolones and sulfonamindes. The dual-cartridge SPE method followed by LC/MS analysis was further evaluated and extended to include several additional antibiotics. After making several modifications to the sample handling and analytical conditions, we were able to obtain satisfactory recoveries of the fluoroquinolones. However, recoveries of the sulfonamindes were still below our target values. Analysis of unspiked samples indicated that ciprofloxacin and sulfamethoxazole were present in secondary wastewater effluents but were removed by reverse osmosis. Additional analysis of the cation exchange SPE method followed by LC/fluorescence analysis indicated that the method is adversely affected by the organic matrices encountered in municipal wastewater effluent.

CONTENTS

FIGURES

TABLES

PROGRESS THIS PERIOD

TASK 1: LITERATURE REVIEW

The objective of this task is to identify compounds to be studied during the occurrence survey. The selection criteria for compounds in the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and availability of suitable analytical methods. Progress on the literature review was presented in the first and second progress reports. The literature review has been completed and will be updated prior to preparation of the final report. No additional progress related to task one was made during the current project period.

Sub-Task 1A: Drugs Used in Human Therapy

In the first progress report, we reviewed data on prescription drugs likely to be present in wastewater effluent in the United States. Using data on the number of prescriptions for the top 200 drugs and information on prescription formulations and metabolism, we estimated ranges of concentrations of 136 drugs expected to be present in wastewater effluent. In addition, we reviewed published occurrence data to identify other compounds of interest that did not appear among the common prescription drugs. A total of eleven drugs were identified for further consideration.

During the current project period no activity was conducted in association with this sub-task.

Sub-Task 1B: Drugs Used in Animal Husbandry

In the second progress report, we reviewed data on antibiotics used in livestock both therapeutically to treat diseases and sub-therapeutically as feed additives to promote growth. We focused our review on antibiotics used in animal feeding operations, because these sources could result in concentrated discharges of antibiotics. We also reviewed fate and transport properties and available analytical methods. On the basis of these results and our review of antibiotics used in human therapy (sub-task 1a), we identified five antibiotics that merit further consideration.

During the current project period no activity was conducted in association with this sub-task.

TASK 2: ANALYTICAL METHOD DEVELOPMENT AND TESTING

The objective of this task is to identify and test analytical methods that will be used during the occurrence survey. As part of the task, analytical methods for extraction, cleanup and derivitization will be tested by adding drugs to deionized water and to samples collected from sites being considered for inclusion in the occurrence survey. Analysis of antibiotics is included as a separate task from the other pharmaceutically active compounds (PhACs) because the analytical methods for antibiotics (e.g., HPLC/MS) are fundamentally different from the gas chromatography methods used for the other compounds.

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the first progress report, we described our preliminary efforts to develop suitable analytical methods for eleven compounds identified as part of the literature survey. We used solid phase extraction followed by derivitization and GC/MS/MS to quantify pharmaceuticals in deionized water and in samples collected from several of the candidate sampling sites. To quantify recoveries and matrix effects, all samples also were analyzed after addition of known quantities of analytes.

During the second project period, we focused our attention on improving the accuracy and precision of the analytical methods by analyzing samples from three sites being considered for inclusion in the occurrence survey. As a result of analytical difficulties, we did not analyze three of the compounds (i.e., carbamazepine, nabumetone and nadolol) during the second project period. For the remaining eight compounds, analysis of duplicate samples indicated that, with the exception of two beta-blockers, results were reproducible (i.e., we observed an average of 26% error between duplicate samples). Analysis of blank samples indicated that sample carryover and cross contamination were not significant problems. Analysis of spike recovery samples indicated variable recoveries, with values as low as 30% for some analytes. All of the drugs were detected in one or more of the unspiked wastewater effluent samples.

During the third project period, we continued to improve the analytical methods by identifying steps where analytes were lost during analysis. For the acidic drugs, we changed the solid phase extraction technique and added radiolabeled mecoprop as an internal standard. As a result of the new SPE method, spike recoveries improved significantly. For the beta-blockers, we increased the time of the drying step to improve the efficiency of derivitization, but this only

had a minor effect on spike recoveries. We also eliminated the use of PFE-lined containers, which resulted in losses of beta-blockers during storage. A QA/QC plan also was submitted to the PAC.

During the fourth project period, we attempted to resolve the remaining issues associated with the analytical methods. Attempts to use radiolabeled propranolol as an internal standard for the beta-blockers failed because the labeled compound could not be discriminated from the unlabeled compounds. Alternative surrogates for beta-blockers could be derivitized and analyzed, but were too polar to be retained during solid phase extraction. We also evaluated the variability in method performance for acidic drugs and beta-blockers by analyzing a total of 18 samples from two surface waters and an advanced wastewater treatment plant. Analysis of surface water samples from a site that was subjected to chlorine disinfection indicated that several of the analytes were lost in the presence of free chlorine. We also tested a GC/MS/MS technique for analysis of carbamazepine. The compound could be detected easily at high concentrations. However, sensitivity decreased significantly at low concentrations, possibly as the result of losses in the injection port of the GC.

During the fifth project period we evaluated the possible use of epinephrine and deoxyepinephrine as internal standards in the analysis of beta-blockers. We also attempted to improve the recovery of carbamazepine by modifying the SPE method and by conditioning the injection port liner and replacing it after each set of analyses. Results of these activities are summarized in the following paragraphs.

In the previous progress report we reported that we had derivitized and analyzed a high concentration standard containing epinephrine and deoxyepinephrine. During this project period we attempted to use these two compounds as internal standards to assess losses of the beta-blockers during sample blowdown, solvent transfer and derivitization. Unfortunately, we were unable to recover either of these compounds when they were added to extracts immediately after they were eluted from the SPE resins. Because the beta-blockers added as part of the spike recovery samples were recovered during this experiment, we concluded that epinephrine and deoxyepinephrine will not be useful as internal standards. Therefore, we have decided to abandon any further attempt to include internal standards for beta-blockers and will use spike recovery samples at a frequency of at least one spike recovery sample per three samples for quality control in our occurrence survey.

During the first part of the current project period, we were unable to obtain reproducible results for carbamazepine. However, after we received Dr. Heberer's advice as part of the PAC's comments on the fourth progress report we were able to recover the compound from environmental samples. During January, we analyzed three samples from the Sweetwater groundwater recharge system: two samples from the recharge pond (one with and one without added carbamazepine) and one from the deep, downgradient groundwater well. In our first attempt to analyze the extracts, we encountered some interference from co-eluting compounds that prevented accurate quantification. However, we detected relatively high concentrations of carbamazepine in the recharge pond. The compound was not observed in the samples from in the well. We are currently attempting to improve the chromatography to obtain accurate quantification. Results will be reported in the sixth progress report.

Sub-Task 2B: Antibiotics

Our analysis of antibiotics has focused on fluoroquinolone and sulfonamide antibiotics. Ciprofloxacin, sulfamethoxazole and sulfamethazine are the analytes selected for the occurrence survey. In the second and third progress reports, we reported our efforts to develop suitable analytical methods for these compounds. A dual-cartridge solid phase extraction (SPE) method was developed to extract antibiotics from water samples. Analysis of antibiotics was conducted by LC/MS and LC/FLD (fluorescence detection).

During the fourth progress report, several efforts were made to improve the analytical methods. We identified the steps where analytes were lost and made changes to minimize the losses. High-density polyethylene conical tubes were shown to yield the minimum losses of fluoroquinolones during the blow-down step and were used in all the later experiments. We determined that acidifying and storing samples in amber glass bottles best preserved analytes prior to solid-phase extraction and yielded minimum losses of analytes through adsorption to the container walls. Sample extracts were also found to be better preserved at 0°C than at 5°C when analysis by LC/MS could not be conducted immediately. To achieve better results, separate LC/MS methods were developed for sulfonamide and fluoroquinolone antibiotics respectively. LC/MS conditions were modified to reduce matrix effect and increase sensitivity. We investigated several structurally related sulfonamide and fluoroquinolone antibiotics as internal standards. Our studies indicate that sulfamerazine and enrofloxacin serve as appropriate internal standards. These research efforts have improved the method recovery to be above 60% and have also increased sensitivity in detection.

Near the end of the fourth project period, the cation-exchange extraction method for fluoroquinolones was found to work successfully in reagent water if we used a proper cation-exchanger (high-density, mixed-phase cation-exchange discs, 3M) and avoided errors in sample preparation. The cation-exchange extraction followed by HPLC/fluorescence detection is a simple and sensitive method that can be easily performed in most existing water utility labs and can also be used to independently confirm the analysis by the dual-cartridge SPE followed by LC/MS. Therefore, it was concluded that the cation-exchange SPE merited further investigation with wastewater matrices.

During this project period, we collected several more wastewater samples to examine the accuracy and precision of the dual-cartridge SPE and LC/MS methods, and conducted more tests

with the cation-exchange SPE for fluoroquinolones. Based upon the preliminary results, we concluded that it would be possible to include two addition antibiotics, trimethoprim and norfloxacin, as part of the occurrence study and began assessing the possibility of including these two compounds in the current analytical methods.

As a result of progress during the previous project periods, we have established a robust method (dual-cartridge SPE followed by LC/MS) for three fluoroquinolones ciprofloxacin, enrofloxacin and norfloxacin. The developed method for fluoroquinolones yield consistent recoveries that meet our QA/QC criteria and will be used in future occurrence studies. Trimethoprim was found to be easily accommodated by the developed method for sulfamethoxazole and sulfamethazine. However, the recoveries for sulfonamides were around 30-40%. Improvement of method recovery for sulfonamides will be further assessed in the next project period. Further studies on the cation-exchange SPE for fluoroquinolones indicated that consistent performance is difficult to obtain in more complicated wastewater matrices. Thus, the cation-exchange SPE will be not be utilized in the occurrence survey. The progress and results of this period are discussed in detail in the following sections.

Analysis by LC/MS

Wastewater samples were collected from three sites: Clayton WWTP in Atlanta, F. Wayne Hill (FWH) Advanced WWTP in Georgia and West Basin Advanced WWTP in *California. Secondary and final effluents were collected from* the Clayton WWTP which employs activated sludge biological treatment followed by UV disinfection; secondary and final effluents were collected there. Effluents of secondary treatment (activated sludge), GAC and final treatment (after GAC and ozonation) were collected at the FWH AWWTP. At the West Basin AWWTP, secondary effluent (i.e., influent to microfiltration), microfiltration effluent and reverse osmosis permeate were collected. For comparison, we also include the results of two analyses that were conducted near the end of the last project period into this report. Sampling and analysis were conducted three times at both Clayton WWTP and FWH AWWTP and one time at the West Basin AWWTP.

As part of our procedure, samples were collected in amber glass bottles, filtered by 0.5 µm glass fiber filters and acidified with phosphoric acid to approximately pH 3. Most samples were extracted by the combination of an anion exchanger and an Oasis HLB cartridge within

four days of sampling. After solid phase extraction, antibiotics were analyzed by LC/MS with electrospray ionization using positive ion mode and selected-ion monitoring (SIM).

During the fifth project period, we continued to optimize LC/MS conditions to improve our results. For analysis of sulfonamides, we increased the buffer concentration in the mobile phases from 1 mM ammonium acetate to 10 mM ammonia acetate (pH 5.74) with 0.007 % acetic acid in order to improve peak shape and stabilize compound retention time. For both sulfonamide and fluoroquinolone methods, the gradient separation was slowed down to increase the separation of the peaks. In addition, we increased the time to flush the column with 100 % acetonitrile at the end of each run to help clean the column between sample runs. We also increased the post-time to 15 minutes to allow the column to fully equilibrate prior to the next injection.

Previously we utilized a fragmentor voltage of 70 V to monitor molecular ions for quantification and utilized higher fragmentor voltages to monitor fragment ions in addition to molecular ion for compound verification. During this project period, we selected one fragmentor voltage for both verification and quantification to decrease the number of sample runs on LC/MS. After investigation, we determined that around 85 V and 83 V are optimal fragmentor voltages for sulfonamides and fluoroquinolones respectively to give good sensitivity of molecular ions and optimal fragmentation patterns. The retention time, molecular ion and confirming fragment ions for each antibiotic are summarized in Table 1. Among the antibiotics analyzed, sulfamerazine is used as an internal standard for quantification of sulfamethoxazole and sulfamethazine, and enrofloxacin is used as an internal standard for quantification of ciprofloxacin and norfloxacin. Neither sulfamerazine nor enrofloxacin are expected to be present at significant concentrations in municipal wastewater and this has been confirmed by our analyses. A fixed amount of internal standard was spiked into wastewater extracts after SPE and to the calibration standards.

Table 1. The retention time, molecular ion and fragment ions of antibiotics.

Antibiotic	Retention Time (min)	[MH]+ ion	Confirming ion 2	Confirming ion 3
Norfloxacin	13.6	320	302	276
Ciprofloxacin	15.4	332	314	288
Enrofloxacin	17.0	360	342	316
Sulfamerazine	9.7	265	156	
Sulfamethazine	12.9	279	156	
Trimethoprim	14.0	291	261	
Sulfamethoxazole	16.4	254	156	

The results of antibiotics obtained during the fifth project period are summarized in the Appendix B. In general, ciprofloxacin was detected in the secondary effluent collected at the West Basin AWWTP and the Clayton WWTP, but was near or below the detection limit in the secondary effluent of the FWH AWWTP. Sulfamethoxazole was detected in the secondary effluent of all three treatment plants. Microfiltration did not remove these two antibiotics. The preliminary results indicated that advanced treatment processes including GAC, ozonation and reverse osmosis removed these antibiotics. Sulfamethazine concentrations were below the detection limit (around 10-20 ng/L depending on sample matrix) in most wastewater samples. Method recoveries, however, varied considerably among the total 17 samples analyzed. Recoveries of ciprofloxacin, sulfamethoxazole and sulfamethazine are also shown in Figures 1-3. A detailed discussion of our results is included in the following sections:

(1) Results for Fluoroquinolones

For ciprofloxacin, the low recoveries of the Clayton 8/10/01 sample were caused by the use of an older LC/MS method. Modification on the LC/MS conditions later reduced matrix effect and improved method recoveries. Poor recoveries were obtained for the Clayton 11/1/01 samples and the FWH 11/21/01 samples. We suspect that eluting antibiotics from the cartridges by pure methanol rather than by methanol/acidified water mixture that had been used previously caused these low recoveries. The intent of using pure methanol rather than methanol/acidified water mixture for compound elution was to reduce the time consumed in the blow-down step. However, since this may cause lower recovery, we have resumed using methanol/acidified water mixture for compound elution in later experiments. Furthermore, the FWH 11/21/01 samples

were stored at 4°C (after filtration and acidification) for a week before SPE could be performed. Therefore, we discount results from the Clayton 11/1/01 and the FWH 11/21/01 samples due to the possible errors associated with these samples.

Recoveries of the West Basin samples were lower than those of the Clayton and FWH samples. The secondary effluent (i.e., microfiltration influent) and microfiltration effluent samples received from the West Basin AWWTP had considerably higher DOC levels than the secondary effluent samples collected from both Clayton and FWH treatment plants. Considerable amounts of insoluble precipitate formed after the blow-down step. Since we centrifuged the samples to remove insoluble precipitate prior to LC/MS injection to protect the electrospray tip, the lower recoveries in these samples may be related to the higher amounts of precipitate. The poor recoveries observed in the RO effluent samples probably do not reflect the actual results because we discovered that insufficient amount of extract may have been injected during sample injection. Excluding the Clayton 11/1/01, FWH 11/21/01 and West Basin RO samples, the rest wastewater samples yielded recoveries ranging from 49% to 121% and had an average recovery of 83±27% for ciprofloxacin. The average recovery of ciprofloxacin in DI water matrices was 100±29%.
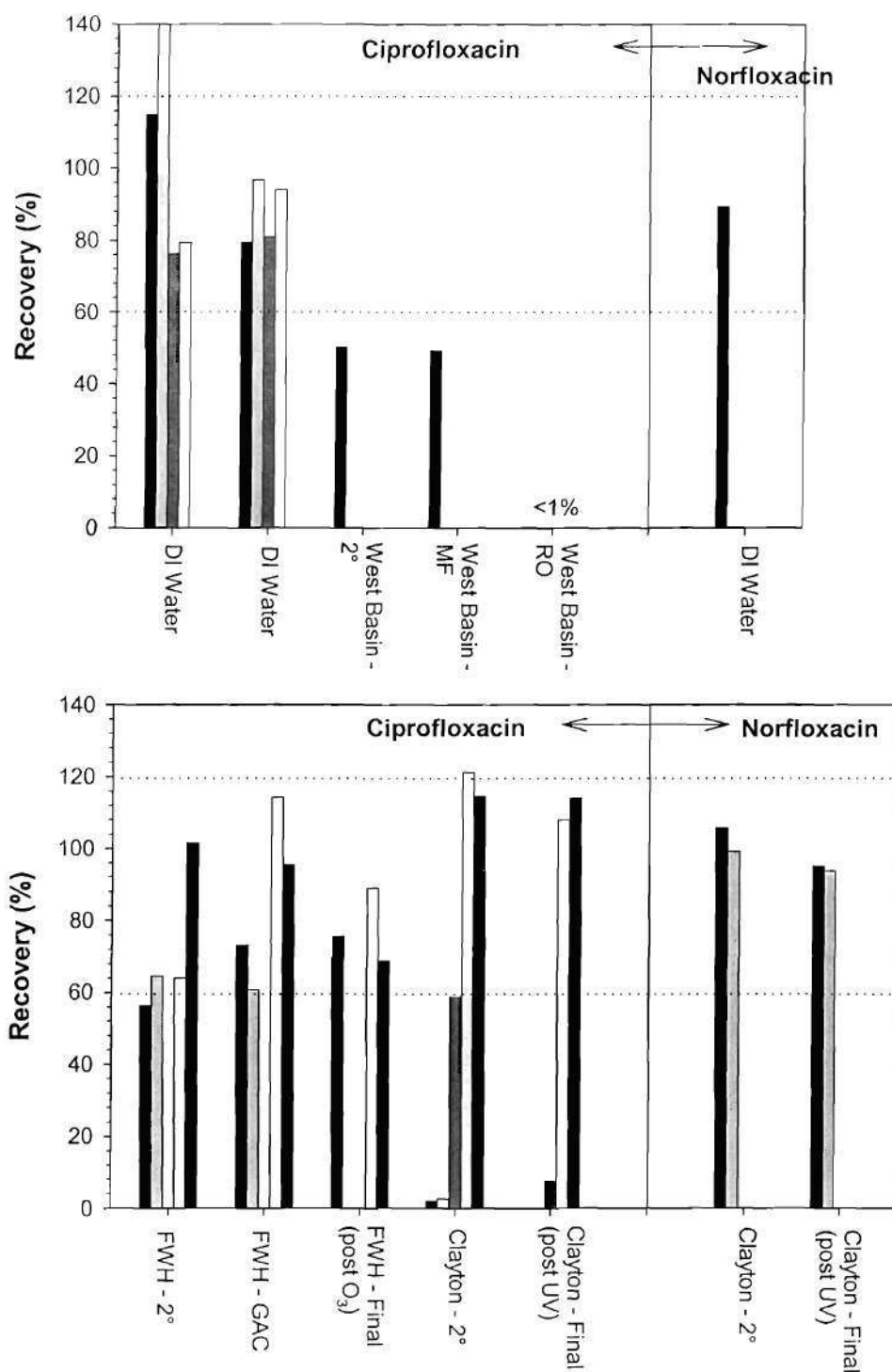
Figure 1. Recoveries of ciprofloxacin and norfloxacin in deionized water and wastewater effluent during the 5th project period. Ciprofloxacin and norfloxacin were spiked in all samples at 1.0 µg/L.

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As will be discussed in a later section, the preliminary results from the cation-exchange SPE followed by HPLC/FLD analysis suggested that norfloxacin might be present in wastewater samples. These results led us to consider including norfloxacin in the occurrence study in addition to ciprofloxacin and enrofloxacin. Norfloxacin was also considered as a potential internal standard in surface water samples in which enrofloxacin may be present due to releases from disposal of animal waste and agricultural runoff. The prescription data indicate that norfloxacin is used in the US at a considerably lower prescription rate than ciprofloxacin (not among the top 200 prescription drugs in 1999). Hence, the concentration of norfloxacin in surface water is likely to be low. We first assessed whether the existing fluoroquinolone LC/MS method can analyze norfloxacin in addition to ciprofloxacin and enrofloxacin in DI water matrices. The results indicated that the current method can readily accommodate norfloxacin without significant modifications. We then assessed the recovery of norfloxacin by spiking at 1.0 μg/L in DI water and in wastewater samples collected from the Clayton WWTP (1/8/02). Recovery of norfloxacin was 89% in the DI water spike and was above 94% in both secondary and final effluent samples. These results indicate that the developed analytical method is robust and reliable for all three fluoroquinolones and yields consistent recoveries that meet our QA/QC standards (between 60% and 120%). In the future occurrence analysis, all three fluoroquinolone antibiotics will be analyzed.

In addition, we recently discovered that lomefloxacin standard is available from a manufacturer. Lomefloxacin is a less frequently used fluoroquinolone antibiotic. We will assess the possibility of using lomefloxacin as an internal standard. If feasible, using lomefloxacin can facilitate analysis all three fluoroquinolones (cipro, norflo and enro) in the occurrence study

A unique phenomenon that occurred in the LC/MS analysis for fluoroquinolones is the changes of retention time in different sample matrices. The increase in retention time can be as much as 2 minutes in some sample matrices compared to DI water matrices. We attribute this phenomenon to the complicated protonation/deprotonation behavior of fluoroquinolones and their interactions with NOM in the matrices. This potential change in retention time further demonstrates the importance of confirming ions in compound verification and quantification. In all our analyses, we have verified each antibiotic by its molecular ion, at least one confirming ion and the relative abundance between ions.

(2) Results for Sulfonamides

For sulfamethoxazole and sulfamethazine, low recoveries were obtained for the Clayton 11/1/01 samples, the FWH 11/21/01 samples, and the West Basin secondary and microfiltration effluent samples. These low recoveries are likely caused by the same reasons as described above for the fluoroquinolones. In addition, we observed higher recoveries for both sulfonamides when a higher spiking concentration (10 µg/L) was employed. The average recovery was 77±19% and 72±38% for sulfamethoxazole and sulfamethazine respectively in both DI water and wastewater matrices. In later experiments, sulfonamides were spiked into samples at 1.0 µg/L and recoveries were generally lower. The average recovery of DI water spike is 57±20% and 33±9% for sulfamethoxazole and sulfamethazine respectively. The recoveries obtain in the recent FWH wastewater samples (1/4/02) yielded recoveries that were comparable to or lower than those in DI water.
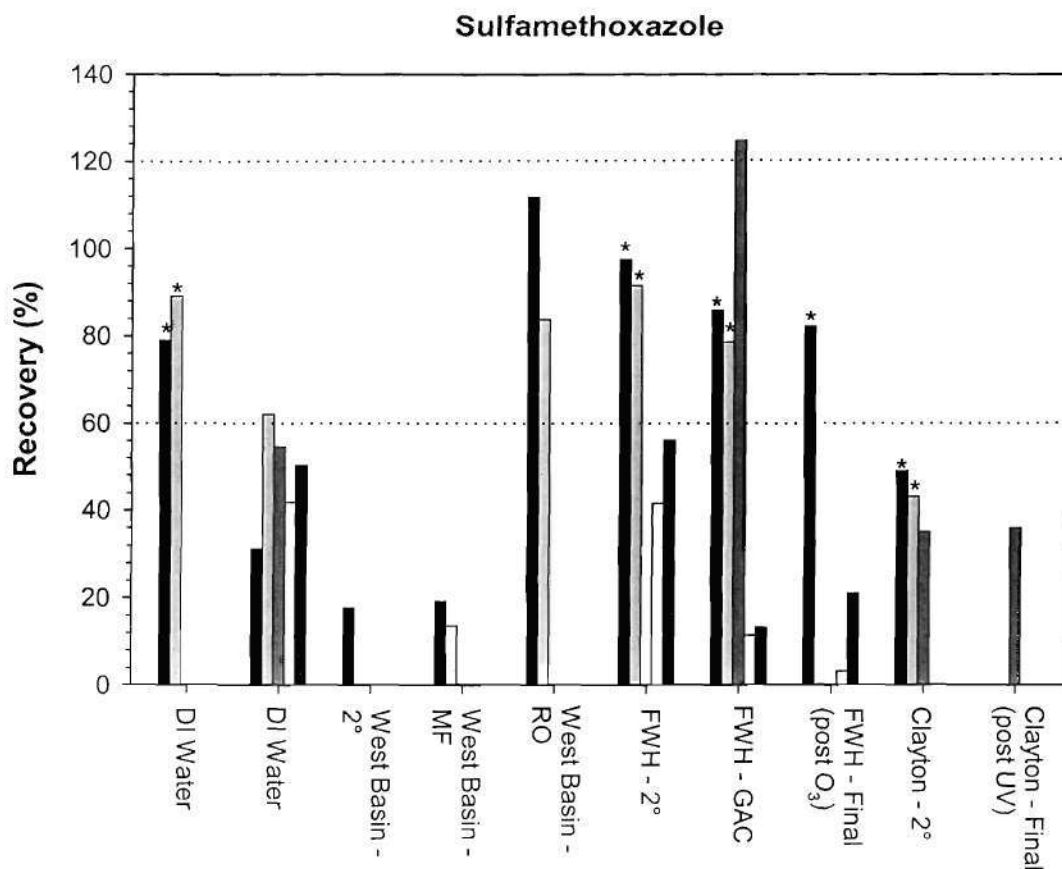
Sulfamethoxazole

Figure 2. Recoveries of sulfamethoxazole in deionized water and wastewater effluent during the 5th project period. Unless specified, sulfamethoxazole was spiked in all samples at 1.0 µg/L. *: Sulfamethoxazole was spiked at 10 µg/L.

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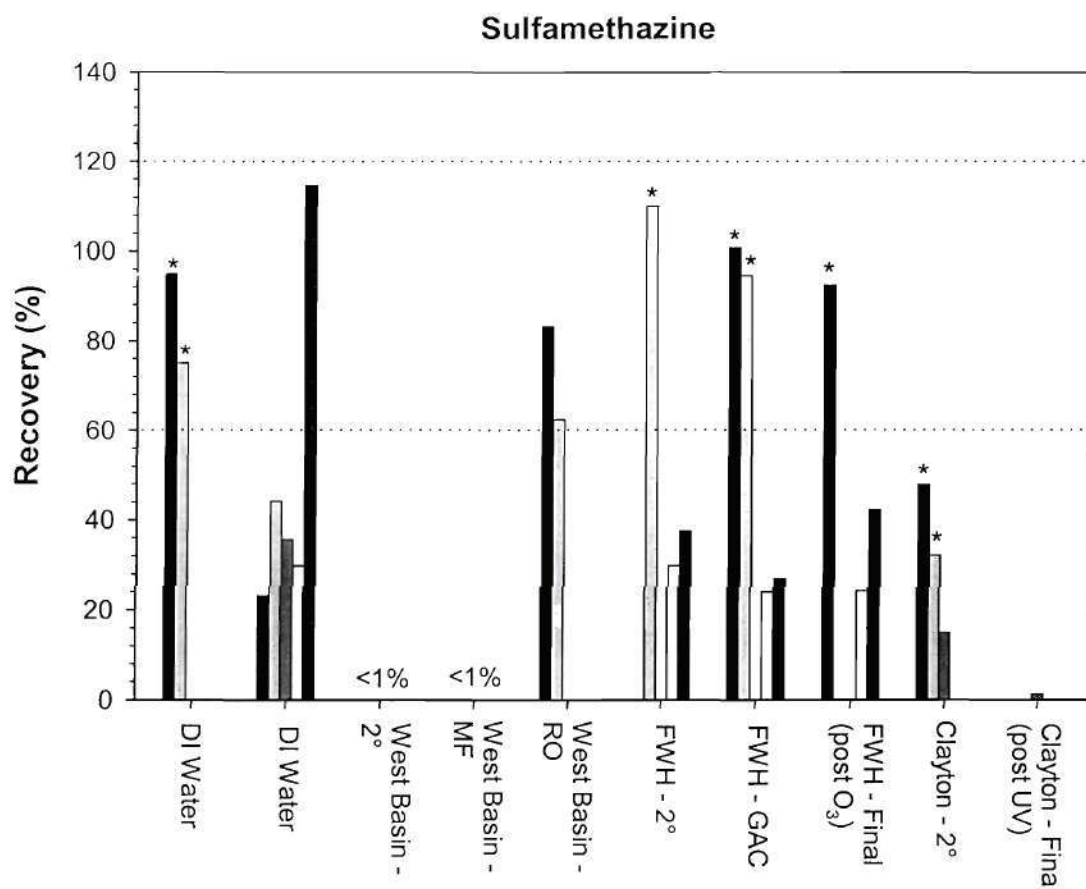
Figure 3. Recoveries of sulfamethazine in deionized water and wastewater effluent during the 5th project period. Unless specified, sulfamethazine was spiked in all samples at 1.0 µg/L. *: Sulfamethazine was spiked at 10 µg/L.

In future analyses, we plan to continue using the more representative spiking concentration of 1.0 µg/L for sulfonamides. Efforts will be made to improve the recovery. To improve method recovery, we considered two possibilities in addition to the reasons described earlier that might be contributing to lower recovery for sulfonamides. First, elution of antibiotics was not conducted immediately after sample extraction in a number of samples in the past. Instead, antibiotics were left in the cartridges overnight at 4°C and were eluted the following day. This delayed elution might cause the recovery to go down and will be avoided in future experiments. Second is the possibility of the anion exchanger to retain sulfonamides. Anion exchangers have been used to reduce the amount of NOM co-extracted with antibiotics thus reducing the matrix effect on LC/MS quantification. Although our previous investigation showed

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that negligible amounts of sulfonamide antibiotics were extracted by the anion cartridges, we will reconfirm this assertion and establish conditions that minimize this potential loss of analytes.

In the most recent wastewater samples collected from the Clayton WWTP, we extracted antibiotics only by HLB cartridges to assess whether recovery of sulfonamides could be improved this way. Excluding anion exchangers in the SPE resulted in visibly dirtier wastewater extracts than those extracted by the dual-cartridge SPE. The matrix effect in LC/MS was considerably greater and thus the method sensitivity was significantly reduced. As a result, neither ciprofloxacin nor norfloxacin could be detected in the unspiked wastewater samples. In the analysis of fluoroquinolones, the matrix effect for ciprofloxacin and norfloxacin was adequately corrected for by the internal standard enrofloxacin because of the close retention time and chemical properties of these three compounds.

The matrix effect was more pronounced in the analysis of sulfonamides, interfering with the analysis of the internal standard sulfamerazine as well as sulfamethoxazole and sulfamethazine. Quantification of compound concentrations and recoveries was not performed on these samples due to the significant matrix interferences that are likely to yield considerable errors. Therefore, utilization of anion exchangers with HLB cartridges in SPE is necessary for complicated wastewater matrices. In the future, the improvement of recovery will focus on establishing conditions that minimize sulfonamide loss through adsorption to the anion exchangers or via other potential ways. For instance, we will assess reducing the pH from 3 to 2.5 for sample acidification prior to SPE. At the lower pH, loss of proton from sulfonamides will be sufficiently inhibited, and protonation of sulfonamides may occur partially. In this manner, the loss of sulfonamides to anion exchangers will be minimized.

In most samples (when the dual-cartridge SPE was applied), quantification of antibiotics by methods with and without the internal standards yielded comparable results. Occasionally in dirtier samples we found that quantification by the internal standard method does not yield proper results due to co-eluting interfering compounds. For instance, in a couple of samples we found larger than expected peak area for sulfamerazine and suspected it was caused by co-eluting compounds in the matrices. Such interference can lead to low calculated concentrations of sulfamethoxazole and sulfamethazine as well as low calculated recovery. Although this does not appear to be a frequent problem thus far, we will utilize the standard addition method to obtain more accurate quantification if this problem occurs in the future.

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The detection of sulfamethoxazole in wastewater samples suggests that the synthetic antibiotic trimethoprim is also likely to be present in the wastewater samples since sulfamethoxazole is commonly prescribed in combination with trimethoprim, typically at the ratio of 5:1 by weight (PDR, 1999). Therefore, we conducted studies to assess whether trimethoprim can be included into the current method for sulfonamide antibiotics. The experiments with DI water spikes indicated that trimethoprim could be easily analyzed by the developed LC/MS method for sulfonamides without significant modifications on the method. Furthermore, we examined the recovery of trimethoprim in DI water and in wastewater samples from the Clayton WWTP (1/8/02). Recovery of trimethoprim was only 27% in the DI water spike. Since analysis of trimethoprim can be readily accommodated by the existing method with nominal extra efforts, we will include trimethoprim in the sulfonamide analysis and conduct further studies to assess its recovery and occurrence in water samples.

Cation Exchange SPE for Fluoroquinolones

Near the end of the fourth project period, we found that fluoroquinolone antibiotics can be successfully extracted from DI water by the 6 mL MPC-HD (mixed phase cation, high density) cation exchange discs (3M) using a method modified from Golet et al (2001). Fluoroquinolones were extracted from water samples by the cation-exchange SPE and analyzed by HPLC with fluorescence detection. During the fifth project period, we conducted further experiments to investigate the extraction efficiency of the MPC-HD discs for fluoroquinolones in effluent of primary, secondary and advanced wastewater treatment processes. To prevent breakthrough, we reduced the flow rate through the cation exchange discs in our tests from 1 mL/min that was reported in the previous study (Golet et al., 2001) to approximately one drop per second. Samples were eluted at < 1 mL/min with 2.5 mL of 15% methanol in an aqueous ammonia solution (5 mL 30% NH$_3$OH in 95 mL deionized water). High-density polyethylene conical tubes were used to collect the eluted samples and 0.5 mL of 85% phosphoric acid was added to each sample to acidify them.

Initially, higher than 100% of recoveries (140-225%) were obtained for ciprofloxacin, particularly in dirtier samples such as the primary effluent. The unusually high recoveries were likely caused by co-eluting interfering compounds in the matrices. Therefore, the HPLC gradient method was extended significantly to allow for better peak separation. With the extended

gradient method, it became clear that the primary effluent peak that had previously been thought to be only ciprofloxacin was likely ciprofloxacin and norfloxacin. The extended gradient program maintained good separation for three fluoroquinolones: ciprofloxacin, enrofloxacin and norfloxacin.

In the later experiments, the cation-exchange SPE method was tested on all three fluoroquinolones simultaneously. At 1.0 µg/L of spiking concentration, good recoveries (73-92%) were obtained for spiked deionized water samples. The co-eluting interfering compounds were no longer a problem with primary effluent samples with the new HPLC gradient, however recoveries in primary samples were significantly lower than those in the DI water spikes. Various methods were employed in an attempt to increase recoveries in dirtier matrices. Anion exchange cartridges were stacked upon the MPC-HD cartridges in an attempt to reduce the interference of wastewater organics in the cation exchange process. Little difference was seen in recoveries for fluoroquinolones with the stacked anion cartridges. In fact, primary effluent recoveries were slightly lower for the stacked anion samples than for the single cation cartridge samples.

Table 2 summarizes the average recoveries of the cation-exchange SPE method (single MPC cartridge) for wastewater samples collected from the Clayton WWTP and the FWH AWWTP:

Table 2: Average recoveries of the cation-exchange SPE for fluoroquinolones.

Sample Matrix	Ciprofloxacin %	Enrofloxacin %	Norfloxacin %
DI Water	92	90	98
Final Effluent	73	65	75
GAC Effluent	69	60	68
Secondary Effluent	62	57	66
Primary Effluent	22	18	34

Overall, the recoveries were within the reasonable range for the cleaner matrices (66±5%) but considerably lower in the primary effluent (25±8%). Curiously, the concentrations of ciprofloxacin and norfloxacin in the unspiked primary and some secondary effluent samples differ little from the concentrations in the spiked samples. These results have led to the conclusion that the volumes of wastewater extracted in these experiment (100 mL for primary

samples, 250 mL for secondary samples and 500 mL for advanced treatment samples) were too large and may have caused the capacity of the MPC-HD discs to be exceeded.

More experiments were then conducted with smaller extracting volumes of wastewater: 50 mL for primary samples, 100 mL for secondary samples and 250 mL for advanced treatment samples. However, recoveries were still not significantly improved in the primary and some secondary effluent. Further experiments were conducted with primary effluent and stacked cation discs that were eluted together, but recoveries could not be improved by this method. Stacking MPC-HD discs together rendered controlling the flow rate through the discs much more difficult and probably caused this approach to fail. Despite the low recoveries with the stacked MPC discs, in the same set of experiments recoveries were improved in the samples extracted by unstacked MPC discs. The increase in recoveries is encouraging and it is evident that the cation discs are extremely sensitive to experimental conditions and flow rate. Interestingly, norfloxacin was detected in the unspiked primary and secondary effluent samples collected from the FWH AWWTP. Therefore, norfloxacin has been added to the target analytes in the occurrence study by LC-MS as described in the earlier section.

In general, the MPC-HD SPE is a highly selective extraction method. Chromatograms are significantly cleaner than those produced using the Oasis HLB cartridges and fluoroquinolone compounds are readily identifiable by the highly sensitive HPLC/fluorescence detection (see chromatograms in Figure 4). Compared to the LC/MS analysis, the cation-exchange SPE followed by HPLC/FLD analysis is more sensitive and requires smaller concentration factors. In addition, this method is simple and can be easily performed in most existing water utility labs. However, the performance of MPC-HD discs is significantly affected by matrices, rendering their application in more complicated water samples questionable. Overall, we recommend the MPC-HD SPE coupled by HPLC/FLD as a simple and sensitive method suitable for screening cleaner wastewater (e.g., final effluent of tertiary treatment or other further treatment) and surface water samples for the presence of fluoroquinolones. For dirtier water matrices, the developed dual-cartridge and LC/MS analysis performs considerably better. Since the recoveries of the cation-exchange SPE cannot be significantly improved in more complicated water matrices after various modifications to the method, we do not see the merit of continuing pursuing the development of this method. In the future occurrence analysis, we will rely on the developed LC/MS method.
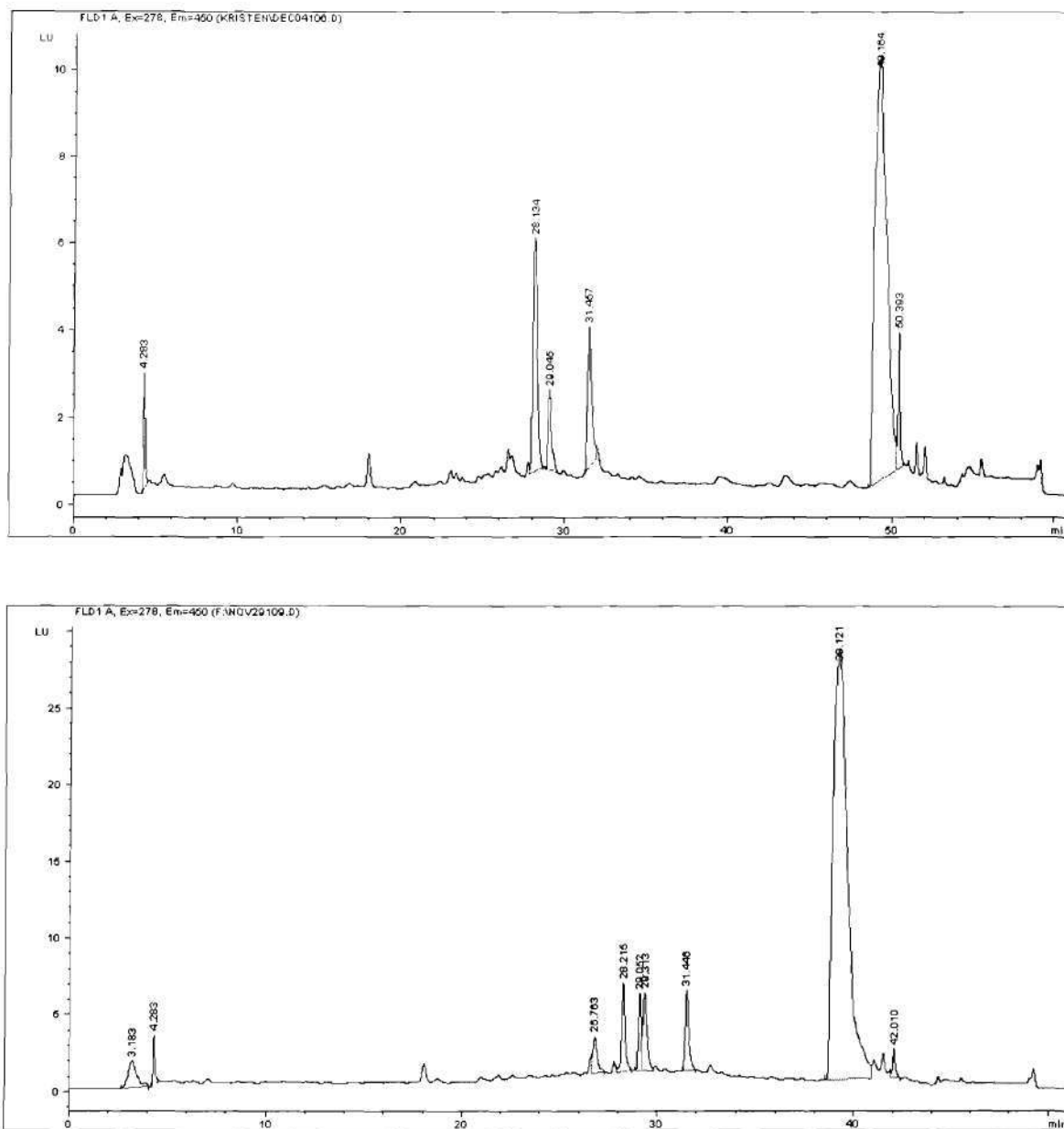
Figure 4. HPLC/FLD chromatograms for fluoroquinolones after MPC-HD disc extraction from spiked primary effluent (top) and secondary effluent (bottom). The spike concentration of fluoroquinolones is 1.0 µg/L. Compound Retention time: 28.2 min for norfloxacin, 29.0 min for ciprofloxacin, and 31.4 min for enrofloxacin.

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TASK 3: OCCURRENCE SURVEY

Sub-Task 3A: Site Selection

In the third progress report we provided a list of sites that we planned to include in the occurrence survey (see Table 3). As part of the site selection process, preliminary samples were collected during the first three project periods from sites that we considered for inclusion in the occurrence survey. The second column in Table 3 indicates the progress report in which preliminary samples were collected for each site on the list. While the quality of some of the preliminary data was acceptable, the samples were not analyzed using all of the steps ultimately incorporated into the analytical method described in the QA/QC plan. As a result, these previous results are only useful for screening purposes. During the fourth project period, we analyzed samples from two of the sites using the accepted analytical methods for acidic drugs and beta-blockers. Except for analysis of acidic drugs from the Witchita Falls AWWTP, these data met our QA/QC criteria and will be included in the occurrence survey. During the current project period, we analyzed a total of 11 samples for acid drugs and beta-blockers from four sites on the list. We also analyzed samples from three sites for antibiotics. Because method development for the antibiotics is not yet complete, the antibiotic data are only useful for screening purposes.

Table 3 also indicates dates for future sampling and analysis of samples from these sites. In the fourth column of the table, we have indicated our plans for collection and analysis of additional samples from each site. Three of the sites on our list may not be included in future sampling:

- Operation of the *Dublin/San Ramon AWWTP* has been discontinued because the project has been put on hold. The AWWTP has not been removed from the site and there is a possibility that may be re-started in the future. If the facility is re-started, we will collect and analyze samples.

- Initial conversations with the operator of the *Witchita Falls AWWTP* indicated that the site treated secondary wastewater effluent with microfiltration and reverse osmosis. After analysis of the samples during the fourth project period we learned that the facility used their reverse osmosis system to treat water from a lake which mainly received agricultural runoff. Because the pharmaceuticals that we have been measuring are unlikely to be present in the agricultural runoff, we do not believe that additional sampling of this site is appropriate.

Table 3. Summary of sample collection in the occurrence survey. The second column indicates preliminary samples collected as part of sample screening. The third column indicates data collected during the fourth and fifth progress periods. The fourth column indicates planned sample collection. Acid. = acidic drugs; β = beta-blockers; Anti. = antibiotics.

Location	Periods 1-3			Periods 4,5			Planned
	Acid.	β	Anti.	Acid.	β	Anti.	
Dublin/San Ramon WWTP		1					March 2002
Mt. View WWTP	2	2					Feb. 2002
Sweetwater WWTP[1]	2,3	2,3					Jan. 2002
San Jose/Santa Clara WWTP							March 2002
Hyperion WWTP[2]				5	5		Period 7
Clayton WWTP			3			4,5	Period 7
Dublin/San Ramon AWWTP		1					See text
West Basin AWWTP	3	3		5	5		Period 7
OCWD Pilot AWWTP				5	5		Period 7
FWH AWWTP			3			4,5	Period 7
Witchita Falls (TX) Pilot Plant				4*	4		See text
Mt. View Wetlands	2	2					Feb. 2002
Prado Wetlands							March 2002
Rio Hondo Spreading Basins							See text
Sweetwater Recharge Facility	2,3	2,3					Jan. 2002
Russian River	3	3		4, 5	4,5		Completed
Sacramento Delta							Feb. 2002
MWD Water	3	3					Period 7

Notes:

WWTP = conventional municipal wastewater treatment plant; AWWTP = advanced wastewater treatment plant; OCWD = Orange County (CA) Water District; FWH = F. Wayne Hill; MWD = Metropolitan (CA) Water District

[1] Sample collected from holding pond associated with recharge facility.

[2] Influent to the West Basin AWWTP

* Data unacceptable due to analytical problems.

- Despite several attempts, we have had considerable difficulty gaining access to the *Rio Hondo Spreading Basins* because the site has been under construction or has not been receiving wastewater effluent. We will remain in contact with the operators of the facility and collect and analyze samples if possible.

Sub-Task 3B: Sample Collection and Analysis

During the fifth project period samples collected from the Mt. View WWTP and associated treatment wetland, the West Basin AWWTP and the OCWD AWWTP were analyzed for acidic drugs and beta-blockers using finalized analytical methods. Samples from the OCWD AWWTP also were analyzed for antibiotics as part of method development activities, as described in the previous section. The data for acidic drugs and beta-blockers are presented in the following paragraphs along with descriptions of QA/QC results (where appropriate).

On September 4, 2001, samples were collected from the Mt. View WWTP and wetland (Figure 5). For the acidic drugs, acceptable recoveries were obtained for all sample locations except the plant effluent, where the recovery of mecoprop was 168%. Spike recoveries for the beta-blockers (i.e., metoprolol and propranolol) ranged from 7 to 68% (median value 46%). Relatively low recoveries for these two compounds have been discussed in the previous progress reports. Because higher recoveries cannot be obtained using the available methods, we will qualify our results for beta-blockers accordingly. Results of our analyses (Figure 5) are consistent with samples collected from this site during the second project period. Several observations are noteworthy:

- Concentrations of drugs are relatively high (e.g., 10,000 ng/L for ibuprofen) in the effluent from the trickling filter.

- Concentrations of acidic drugs decrease when the wastewater passes through the nitrification system. Concentrations of beta-blockers are unaffected by nitrification.

- With the exception of gemfibrozil and naproxen, concentrations of all of the compounds decrease to levels below the detection limit (i.e., 10 ng/L) in the engineered treatment wetland. Gemfibrozil is not removed in the treatment wetland while naproxen appears to be removed to a significant degree as the water passes through the first section of the wetland.
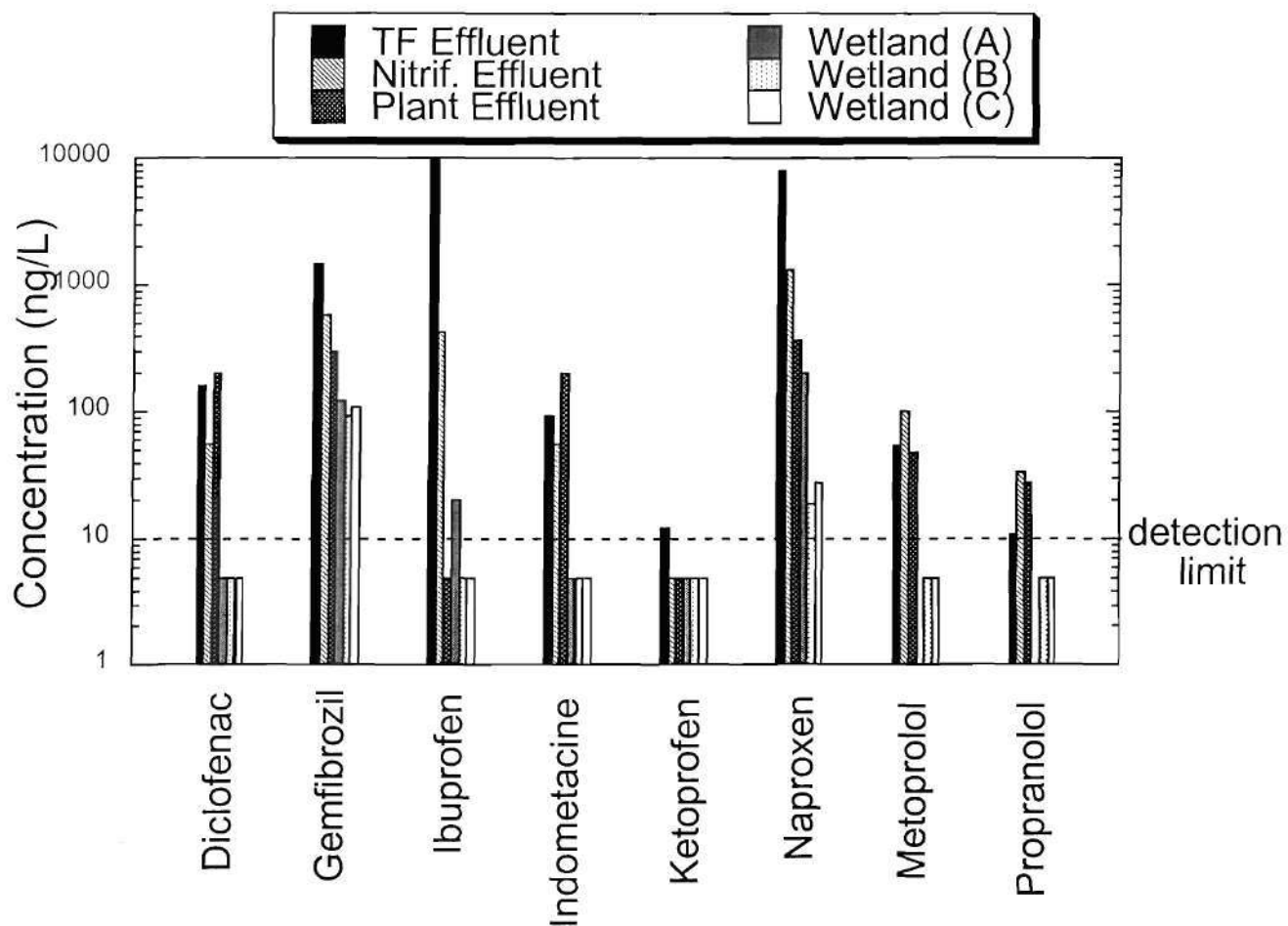
Figure 5: Concentrations of drugs detected at the Mt. View WWTP and associated treatment wetland. The detection limit for all compounds was 10 ng/L. Concentrations below the detection limit are plotted at half the detection limit.

On September 18, 2001, samples were collected from the West Basin AWWTP and analyzed for acidic drugs and beta-blockers. With the exception of the mecoprop recovery in the RO sample (161%) all data for acidic drugs met the QA/QC criteria. The spike recovery of beta-blockers in the MF sample was 47% for metoprolol and 48% for propranolol. Results of the analyses (Figure 6) are comparable to those obtained from this site during the third project period. Concentrations of drugs were comparable before and after microfiltration. More importantly, concentrations of all drugs decreased to levels below the method detection limit (i.e., 10 ng/L) after reverse osmosis treatment.

Figure 6: Concentrations of drugs detected at the West Basin AWWTP. The detection limit for all compounds was 10 ng/L. Concentrations below the detection limit are plotted at half the detection limit.

On September 12, 2001, samples were collected from the OCWD AWWTP. All QA/QC samples for the acidic drugs were acceptable except for the mecoprop recovery in the secondary effluent sample, which was above the QA/QC criteria. The recovery of beta-blockers ranged from 35-55%. Results of the analyses (Figure 7) are comparable to those obtained from the West Basin AWWTP. The only notable discrepancy in the data was the detection of beta-blockers in the reverse osmosis effluent. Although these data are suspect because the concentrations are higher than those measured in the microfiltration effluent, we did not note any obvious QA/QC
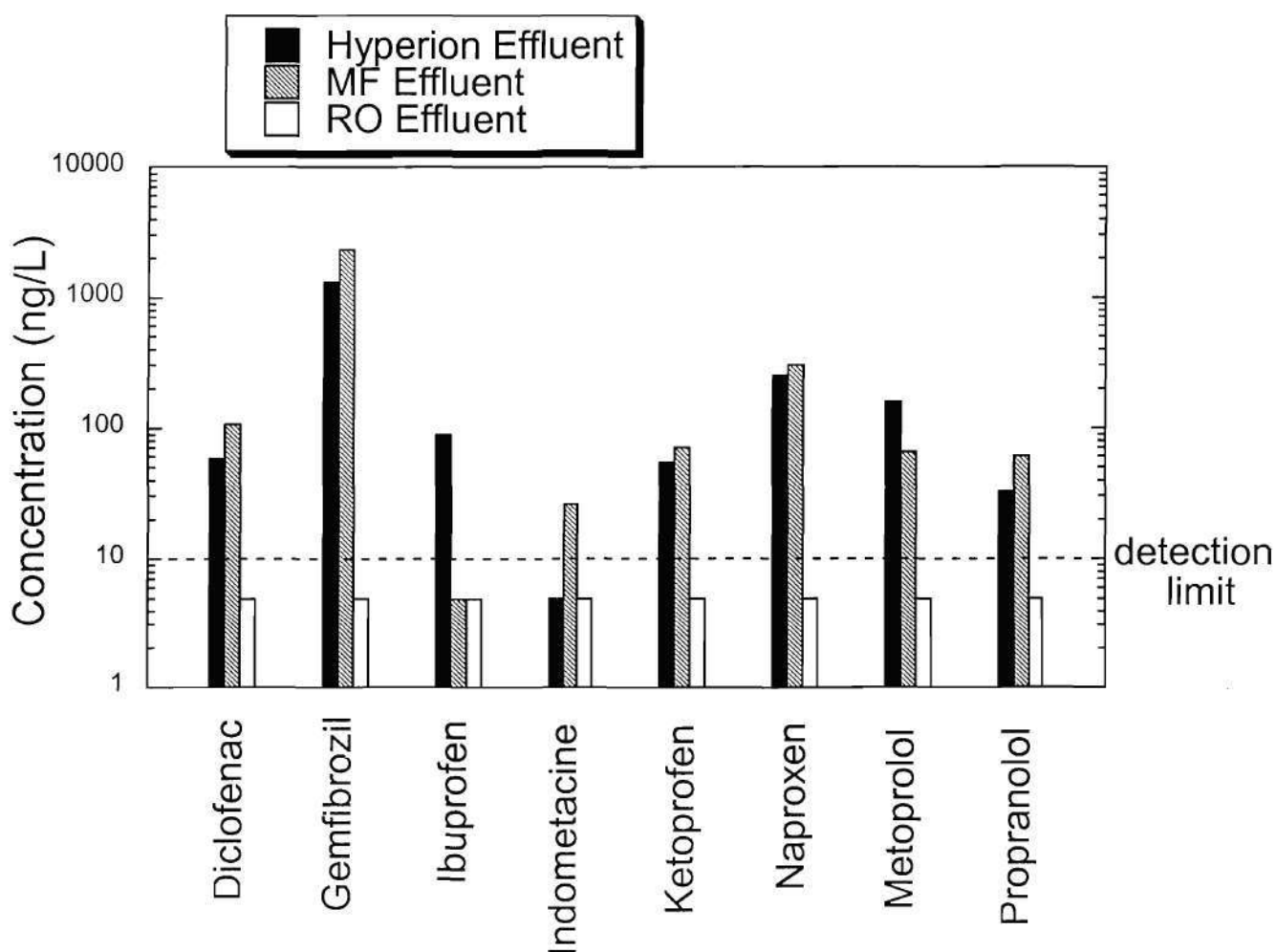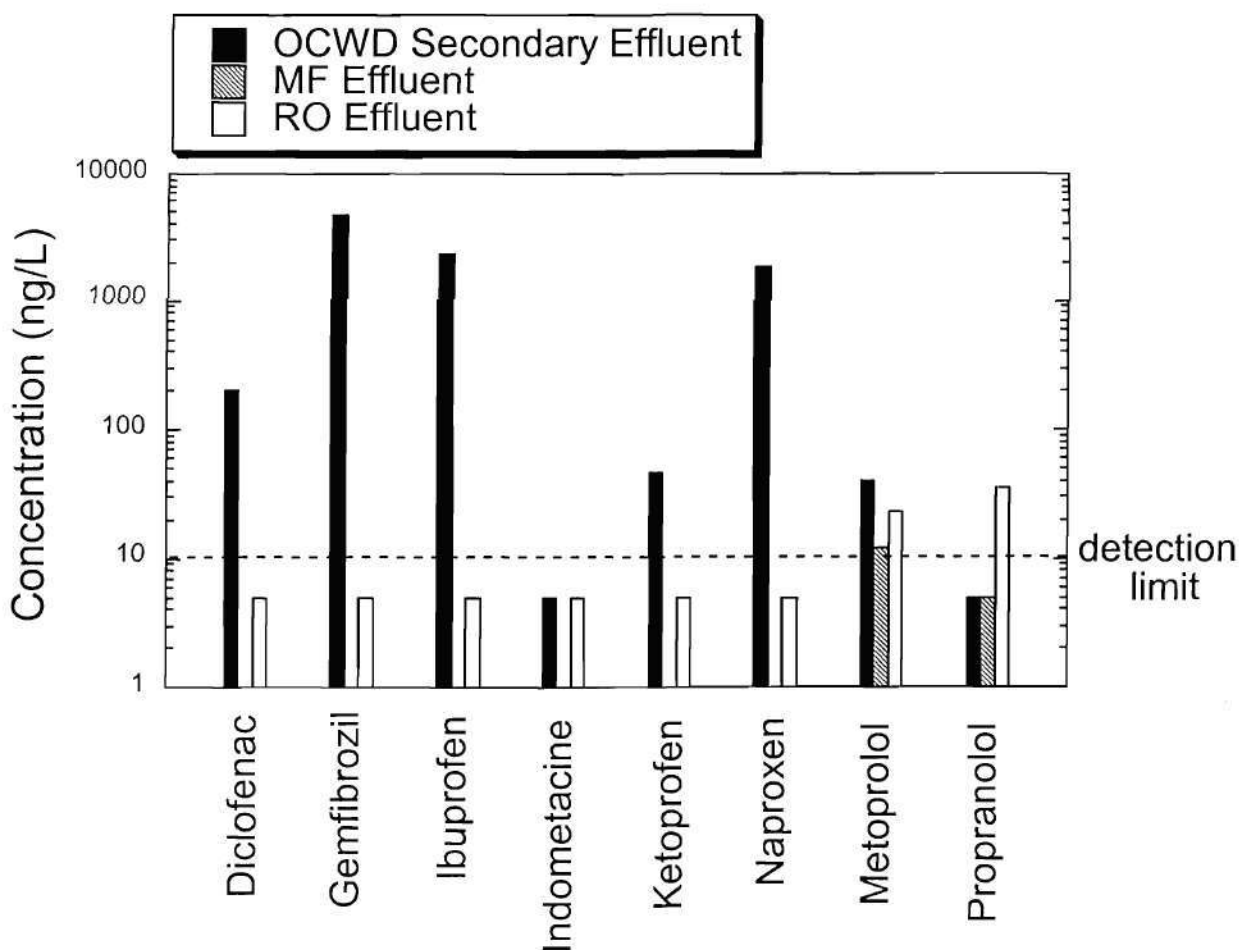

Figure 7: Concentrations of drugs detected at the OCWD AWWTP. The detection limit for all compounds was 10 ng/L. Concentrations below the detection limit are plotted at half the detection limit.

page 26:

Have really 250?g/L of sulfamethoxazole been found in secondary effluent from the Clayton STP? This is the highest concentration that has ever been reported for sewage effluents! Or is it rather ng instead of ?g/L?

Response: The concentrations were around 250 **ng**/L.

page 30:

The surface water results should be compared with those from the surface water monitoring of PhACs carried out by the USGS (100 streams survey 1999-2000, Kolpin et al., ES&T in press).

Response: We will compare our data to the USGS results when they are published.

Appendix A:

I really had some problems reading this table. Spike recovery: This is the recovery of the analytes in parallel analyses? Is this just an average of two analyses? Often two values are provided for one sample that differ significantly (e.g. gemfibrozil, TX microfiltration: 157 and <10 ng/L but 68% recovery). This is very confusing and not very convincing regarding the reliability of the method. In this case the sample with the <10 ng/L result seems to have a much lower recovery? Which one is the correct result? How has the recovery of the surrogate been and how has it been accounted for? Why wasn't this analysis repeated at third time to confirm any of these results? Mecoprop means most probably the radiolabeled compound?

Response: We apologize for any lack of clarity in the table. To clarify:
-Spike recovery refers to separate experiments performed by spiking a mixture of all of the pharmaceuticals into the water prior to extraction.
-Each value presented in the table represents a sample prepared and extracted in parallel with the other sample and not a duplicate measurement of one extract.
-When two values differ by a considerable amount and one sample is non-detect, we presume that the derivitization failed.

-The data have not been corrected for the internal standard or for recovery.

-Mecoprop does refer to the labeled form of the compound.

Appendix C:

The term m/z is most commonly placed in front of the numbers.

Response: This expression will be used in future reporting.

page 45/46 (response to comment 3):

The alternative to GC-iontrap MS analysis is not necessarily GC-quadrupole tandem MS analysis. Some investigations have shown that GC-EI-MS analysis is sufficient to obtain reliable and undoubtful results!

MS/MS often seems absolutely inevitable in environmental trace-analysis using LC. But LC-MS is totally different from GC-MS analysis because: 1. Modern API-interfaces are almost producing molecular ions but have little fragmentation (no fingerprint analysis as found with GC-EI-MS) and 2. GC has much better separation capabilities than HPLC! If the analytes are amenable to GC analysis, with or without derivatization, they can most certainly be analyzed using a cheap GC-quadrupole MS instrument (no triple-quad). There are only a few exceptions (e.g. analysis of dioxins). Sorry, that I react a little upset on this remark but regarding many other analyses carried out using ECD, NPD, FID, DAD, FLD or even UV detection it appears to be nonsense to discuss the reliability of GC-EI-MS analysis!

Response: None required.

page 46 (response to comment 4):

Kolpin et al. (ES&T, 2002) included caffeine and cotinine into their survey. We also analyze caffeine but we often have some quantitation problems.

Response: None required.

page 51 (response to comment 20):

DOC as a non-specific group parameter does not seem to be a good indicator? Isn't EDTA analysis rather complicated (adsorption problems) and time-consuming? How about boron? That's much easier to be analyzed!

Response: Boron is a good tracer for wastewater effluent when isotopic analysis is performed. Otherwise it is difficult to discriminate it from naturally occurring borate, which is common in California and other western states. We have considerable experience analyzing EDTA and plan to analyze it in the occurrence survey.

- It's a little unclear to me what the outcome has been on the additional compounds suggested by PAC members in 11/2000. Have any of those compounds been detected using the 3 identified analytical methods. Is what is explained on page 8 the results towards that effort? The beta-blockers' internal standards issue has not yet resolved itself. How much longer is the investigation going to take place before the lower recoveries are accepted? Finally, will the list of compounds expand from the original 11?

Response: As indicated in our previous progress report, we have not expanded our list of compounds beyond the original 11 and will include in the occurrence survey only those compounds discussed in this progress report.

problems during the analysis. Therefore, we will analyze this site again in a future round of sampling.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task were conducted during this project period.

PLANS FOR NEXT PERIOD

The following section describes research planned during the next project period. In addition, plans for the remainder of the project are described at the end of each section. A revised schedule for the project is presented in Appendix C.

Task 1: Literature Review

Sub-Task 1A: Drugs Used in Human Therapy

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Sub-Task 1B: Drugs Used in Animal Husbandry

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Task 2: Analytical Method Development and Testing

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

During the next project period wee will continue to evaluate and improve the analytical method for carbamazepine with the goal of including it in the occurrence survey.

Sub-Task 2B: Antibiotics

The developed method for fluoroquinolone antibiotics was shown to be robust and reliable. Except for the assessment of lomefloxacin as an internal standard, no further method

development is necessary. During the next project period we plan to continue applying this fluoroquinolone method in more sample matrices including wastewater and surface water samples to quantify the concentrations of ciprofloxacin, norfloxacin and enrofloxacin and to examine method recovery in various matrices.

We plan to continue addressing the low recovery issue associated with sulfonamide antibiotics and trimethoprim. To improve recovery, we will focus on establishing conditions that minimize antibiotics loss through adsorption to the anion exchangers or via other potential ways. For instance, acidification of samples to lower pH levels will be assessed for recovery improvement. Our primary objective is to obtain consistent recoveries for sulfonamides and trimethoprim. When possible, we plan to improve the recoveries to above the QA/QC standards. In parallel to method improvement, we will apply the sulfonamide method to various wastewater and surface water samples to assess the occurrence of sulfamethoxazole, sulfamethazine and trimethoprim and method recoveries in different sample matrices.

Task 3: Occurrence Survey

Sub-Task 3A: Site Selection

We have chosen all of the sites to be included in the occurrence survey (Table 3). Additional sites will be included if time permits. No further activity is planned in association with this task.

Sub-Task 3B: Sample Collection and Analysis

As indicated in Table 3, we plan to collect and analyze samples from eight sites during the next project period. Samples will be analyzed for acidic drugs and beta-blockers using previously described procedures. Samples also will be analyzed for antibiotics using methods described in the previous section of this progress report. After completion of these activities, we will have completed our first round of sampling. A second round of sampling at each site and possibly additional locations will be performed in the following project periods.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task are planned during this project period. After completion of the occurrence survey, data will be evaluated to identify trends meriting further study. Data will be compared with expectations based on physical/chemical properties of the compounds as well as results reported by other researchers.

In light of our available data and results of studies conducted elsewhere (e.g., the NGWA International Conference on Pharmaceuticals and Endocrine Disrupters in Minneapolis) we believe that it would be appropriate to publish the results from our project shortly after it is completed. Our present plan is to prepare two manuscripts during Fall 2002. The first manuscript will describe results of the occurrence survey and will identify areas for future research related to pharmaceuticals in source waters. The second manuscript will describe new analytical methods developed during this project for measurement of antibiotics. We are considering publishing the first manuscript in *Water Research* and the second in *the Journal of Chromatography*. We would appreciate any opinions about these plans from the PAC.

REFERENCES

Golet, E. M.; Alder, A. C.; Hartmann, A.; Ternes, T. A.; Giger, W. (2001)."Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection". *Anal. Chem.*, 73, 3632-3638.

PDR (1999). *Physicians Desk Reference*; Medical Economics Company: Montvale, NJ.

APPENDIX A: Summary of Data for Acidic Drugs and Beta-Blockers
during the Third Project Period

Compound	Location	Date	Concentration (ppt)
Diclofenac	Blank	9/4/01	<10
	Mt. View Trickling Filter Effluent	9/4/01	160, <10
	Mt. View Nitrification Effluent	9/4/01	30, <10
	Mt. View Plant Effluent	9/4/01	<10
	Mt. View Wetland Beginning	9/4/01	<10
	Mt. View Wetland Middle	9/4/01	<10
	Mt. View Wetland End	9/4/01	<10
	Water Factory 21 Secondary Effluent	9/12/01	200
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	<10
	Blank	9/18/01	<10
	West Central Basin Secondary Effluent	9/18/01	59
	West Central Basin Microfiltration Effluent	9/18/01	110
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10
Gemfibrozil	Blank	9/4/01	<10
	Mt. View Trickling Filter Effluent	9/4/01	1500, 1200
	Mt. View Nitrification Effluent	9/4/01	590, 420
	Mt. View Plant Effluent	9/4/01	300
	Mt. View Wetland Beginning	9/4/01	120
	Mt. View Wetland Middle	9/4/01	92
	Mt. View Wetland End	9/4/01	110
	Water Factory 21 Secondary Effluent	9/12/01	4600
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	<10
	Blank	9/18/01	<10
	West Central Basin Secondary Effluent	9/18/01	1300
	West Central Basin Microfiltration Effluent	9/18/01	2300
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10
Ibuprofen	Blank	9/4/01	<10
	Mt. View Trickling Filter Effluent	9/4/01	10000, 9600
	Mt. View Nitrification Effluent	9/4/01	430, 340
	Mt. View Plant Effluent	9/4/01	<10
	Mt. View Wetland Beginning	9/4/01	20
	Mt. View Wetland Middle	9/4/01	<10
	Mt. View Wetland End	9/4/01	<10
	Water Factory 21 Secondary Effluent	9/12/01	2300
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	<10
	Blank	9/18/01	<10
	West Central Basin Secondary Effluent	9/18/01	91
	West Central Basin Microfiltration Effluent	9/18/01	<10
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10
Indometacine	Blank	9/4/01	<10
	Mt. View Trickling Filter Effluent	9/4/01	94, 100
	Mt. View Nitrification Effluent	9/4/01	56, 43
	Mt. View Plant Effluent	9/4/01	200
	Mt. View Wetland Beginning	9/4/01	<10
	Mt. View Wetland Middle	9/4/01	<10
	Mt. View Wetland End	9/4/01	<10
	Water Factory 21 Secondary Effluent	9/12/01	<10
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	<10
	Blank	9/18/01	<10
	West Central Basin Secondary Effluent	9/18/01	<10
	West Central Basin Microfiltration Effluent	9/18/01	26
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10

Compound	Location	Date	Concentration (ppt)
Ketoprofen	Blank	9/4/01	<10
	Mt. View Trickling Filter Effluent	9/4/01	12, <10
	Mt. View Nitrification Effluent	9/4/01	<10, <10
	Mt. View Plant Effluent	9/4/01	<10
	Mt. View Wetland Beginning	9/4/01	<10
	Mt. View Wetland Middle	9/4/01	<10
	Mt. View Wetland End	9/4/01	<10
	Water Factory 21 Secondary Effluent	9/12/01	47
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	<10
	Blank	9/18/01	<10
	West Central Basin Secondary Effluent	9/18/01	55
	West Central Basin Microfiltration Effluent	9/18/01	72
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10
Naproxen	Blank	9/4/01	<10
	Mt. View Trickling Filter Effluent	9/4/01	7900, 7800
	Mt. View Nitrification Effluent	9/4/01	1300, 1100
	Mt. View Plant Effluent	9/4/01	370
	Mt. View Wetland Beginning	9/4/01	200
	Mt. View Wetland Middle	9/4/01	19
	Mt. View Wetland End	9/4/01	28
	Water Factory 21 Secondary Effluent	9/12/01	1800
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	<10
	Blank	9/18/01	<10
	West Central Basin Secondary Effluent	9/18/01	250
	West Central Basin Microfiltration Effluent	9/18/01	300
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10
Mecoprop*	Mt. View Trickling Filter Effluent	9/4/01	120%, 138%
	Mt. View Nitrification Effluent	9/4/01	120%, 152%
	Mt. View Plant Effluent	9/4/01	168%
	Mt. View Wetland Beginning	9/4/01	122%
	Mt. View Wetland Middle	9/4/01	113%
	Mt. View Wetland End	9/4/01	109%
	Water Factory 21 Secondary Effluent	9/12/01	180%
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	98%
	Blank	9/18/01	119%
	West Central Basin Secondary Effluent	9/18/01	64%
	West Central Basin Microfiltration Effluent	9/18/01	161%
	West Central Basin Reverse Osmosis Effluent	9/18/01	126%

* Recovery of labeled mecoprop (internal standard) added to samples at 1,000 ng/L.

Compound	Location	Date	Concentration (ppt)	Recovery (%)
Metoprolol	Blank	9/4/01	<10	
	Mt. View Trickling Filter Effluent	9/4/01	37, 72	
	Mt. View Nitrification Effluent	9/4/01	110, 92	
	Mt. View Plant Effluent	9/4/01	48	53%
	Mt. View Wetland Middle	9/4/01	<10	39%
	Mt. View Wetland End	9/4/01	<10	7%
	Water Factory 21 Secondary Effluent	9/12/01	40, 20	
	Water Factory 21 Microfiltration Effluent	9/12/01	12	55%
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	23	46%
	Blank	9/18/01	<10	
	West Central Basin Secondary Effluent	9/18/01	160	
	West Central Basin Microfiltration Effluent	9/18/01	67	48%
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10	
Propranolol	Blank	9/4/01	<10	
	Mt. View Trickling Filter Effluent	9/4/01	16, <10	
	Mt. View Nitrification Effluent	9/4/01	46, 21	
	Mt. View Plant Effluent	9/4/01	28	62%
	Mt. View Wetland Middle	9/4/01	<10	34%
	Mt. View Wetland End	9/4/01	<10	23%
	Water Factory 21 Secondary Effluent	9/12/01	<10, <10	
	Water Factory 21 Microfiltration Effluent	9/12/01	<10	45%
	Water Factory 21 Reverse Osmosis Effluent	9/12/01	36	35%
	Blank	9/18/01	<10	
	West Central Basin Secondary Effluent	9/18/01	33	
	West Central Basin Microfiltration Effluent	9/18/01	61	47%
	West Central Basin Reverse Osmosis Effluent	9/18/01	<10	

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APPENDIX B: Summary of Data for Antibiotics during the Fifth Project Period

Compound	Location	Date	Concentration (μg/L)[*]	Spike Recovery
Ciprofloxacin	Clayton WWTP, GA - Secondary	8/10/01	<LOD, <LOD	2%, 3%[(a)]
	DI Water Spike	8/10/01		115%, 158%[(a)]
	FWH AWWTP, GA - Secondary	9/1/01	<LOD, <LOD	56%, 65%[(b)]
	FWH AWWTP, GA - GAC	9/1/01	<LOD, <LOD	73%, 61%[(b)]
	FWH AWWTP, GA - Final (after O$_3$)	9/1/01	<LOD, <LOD	76%[(b)]
	West Basin, CA - Secondary	9/19/01	0.19, 0.25	50%
	West Basin, CA - Microfiltration	9/19/01	0.35, 0.18	49%, 0%
	West Basin, CA - Reverse Osmosis	9/19/01	<LOD, <LOD	0%, 0%[(c)]
	Clayton WWTP, GA - Secondary	11/1/01	0.15, 0.21	59%
	Clayton WWTP, GA - Final (after UV)	11/1/01	<LOD, <LOD	7%
	FWH AWWTP, GA - Secondary	11/21/01	<LOD, <LOD	0%
	FWH AWWTP, GA - GAC	11/21/01	<LOD, <LOD	0%
	FWH AWWTP, GA - Final (after O$_3$)	11/21/01	<LOD, <LOD	0%
	DI Water Spike	12/20/01		76%, 79%
	DI Water Spike	12/20/01		97%, 81%
	FWH AWWTP, GA - Secondary	1/4/02	0.02, <LOD	64%, 101%
	FWH AWWTP, GA - GAC	1/4/02	<LOD, <LOD	114%, 95%
	FWH AWWTP, GA - Final (after O$_3$)	1/4/02	<LOD, <LOD	89%, 69%
	Clayton WWTP, GA - Secondary	1/8/02	<LOD, <LOD	121%, 115%
	Clayton WWTP, GA - Final (after UV)	1/8/02	<LOD, <LOD	108%, 114%
	DI Water Spike	1/8/02		94%
Sulfamethoxazole	Clayton WWTP, GA - Secondary	8/10/01	0.24, 0.25	49%, 43%[(d)]
	DI Water Spike	8/10/01		79%, 89%[(d)]
	FWH AWWTP, GA - Secondary	9/1/01	<LOD, <LOD	98%, 91%[(d)]
	FWH AWWTP, GA - GAC	9/1/01	<LOD, <LOD	86%, 79%[(d)]
	FWH AWWTP, GA - Final (after O$_3$)	9/1/01	<LOD, <LOD	82%[(d)]
	West Basin, CA - Secondary	9/19/01	0.16, 0.20	18%
	West Basin, CA - Microfiltration	9/19/01	0.19, 0.2	19%, 14%
	West Basin, CA - Reverse Osmosis	9/19/01	<LOD, <LOD	112%, 84%
	Clayton WWTP, GA - Secondary	11/1/01	0.53, 0.10	35%
	Clayton WWTP, GA - Final (after UV)	11/1/01	0.57, 075	36%
	FWH AWWTP, GA - Secondary	11/21/01	0.30, 0.39[(b)]	NA
	FWH AWWTP, GA - GAC	11/21/01	0.52, 0.57[(b)]	128%[(b)]
	FWH AWWTP, GA - Final (after O$_3$)	11/21/01	<LOD, <LOD	0%[(b)]
	DI Water Spike	12/20/01		31%, 62%
	DI Water Spike	12/20/01		54%, 80%
	FWH AWWTP, GA - Secondary	1/4/02	0.55, 0.46	42%, 56%
	FWH AWWTP, GA - GAC	1/4/02	0.04, 0.04	11%, 13%
	FWH AWWTP, GA - Final (after O$_3$)	1/4/02	0.05, 0.03	3%, 21%
	Clayton WWTP, GA - Secondary	1/8/02	<LOD, <LOD	NA[(e)]
	Clayton WWTP, GA - Final (after UV)	1/8/02	<LOD, <LOD	NA[(e)]
	DI Water Spike	1/8/02		50%

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Compound	Location	Date	Concentration (ug/L)[*]	Spike Recovery
Sulfamethazine	Clayton WWTP, GA - Secondary	8/10/01	<LOD, <LOD	48%, 32% [d]
	DI Water Spike	8/10/01		99%, 75% [d]
	FWH AWWTP, GA - Secondary	9/1/01	<LOD, <LOD	0%, 110% [d]
	FWH AWWTP, GA - GAC	9/1/01	<LOD, <LOD	101%, 95% [d]
	FWH AWWTP, GA - Final (after O₃)	9/1/01	<LOD, <LOD	92% [d]
	West Basin, CA - Secondary	9/19/01	<LOD, <LOD	0%
	West Basin, CA - Microfiltration	9/19/01	<LOD, <LOD	0%, 0%
	West Basin, CA - Reverse Osmosis	9/19/01	<LOD, <LOD	83%, 62%
	Clayton WWTP, GA - Secondary	11/1/01	<LOD, <LOD	15%
	Clayton WWTP, GA - Final (after UV)	11/1/01	<LOD, <LOD	1%
	FWH AWWTP, GA - Secondary	11/21/01	<LOD, <LOD	NA
	FWH AWWTP, GA - GAC	11/21/01	<LOD, <LOD	0%
	FWH AWWTP, GA - Final (after O₃)	11/21/01	<LOD, <LOD	0%
	DI Water Spike	12/20/01		23%, 44%
	DI Water Spike	12/20/01		36%, 30%
	FWH AWWTP, GA - Secondary	1/4/02	0.2, <LOD	30%, 37%
	FWH AWWTP, GA - GAC	1/4/02	<LOD, <LOD	24%, 27%
	FWH AWWTP, GA - Final (after O₃)	1/4/02	<LOD, <LOD	24%, 42%
	Clayton WWTP, GA - Secondary	1/8/02	<LOD, <LOD	NA [e]
	Clayton WWTP, GA - Final (after UV)	1/8/02	<LOD, <LOD	NA [e]
	DI Water Spike	1/8/02		114%
Norfloxacin	Clayton WWTP, GA - Secondary	1/8/02	<LOD, <LOD	106%, 99%
	Clayton WWTP, GA - Final (after UV)	1/8/02	<LOD, <LOD	95%, 94%
	DI Water Spike	1/8/02		89%
Trimethoprim	Clayton WWTP, GA - Secondary	1/8/02	<LOD, <LOD	NA [e]
	Clayton WWTP, GA - Final (after UV)	1/8/02	<LOD, <LOD	NA [e]
	DI Water Spike	1/8/02		27%

Note: The reported concentrations were not corrected by recoveries.

Unless specified, spike concentration is 1.0 µg/L.

*: The LOD (limit of detection) is around 10 ng/L except for the Clayton 1/8/02 samples.
The Clayton 1/8/02 samples have higher LOD due to a different SPE method.

(a) Internal standard was not used for quantitation; method was still being optimized to minimize matrix effect
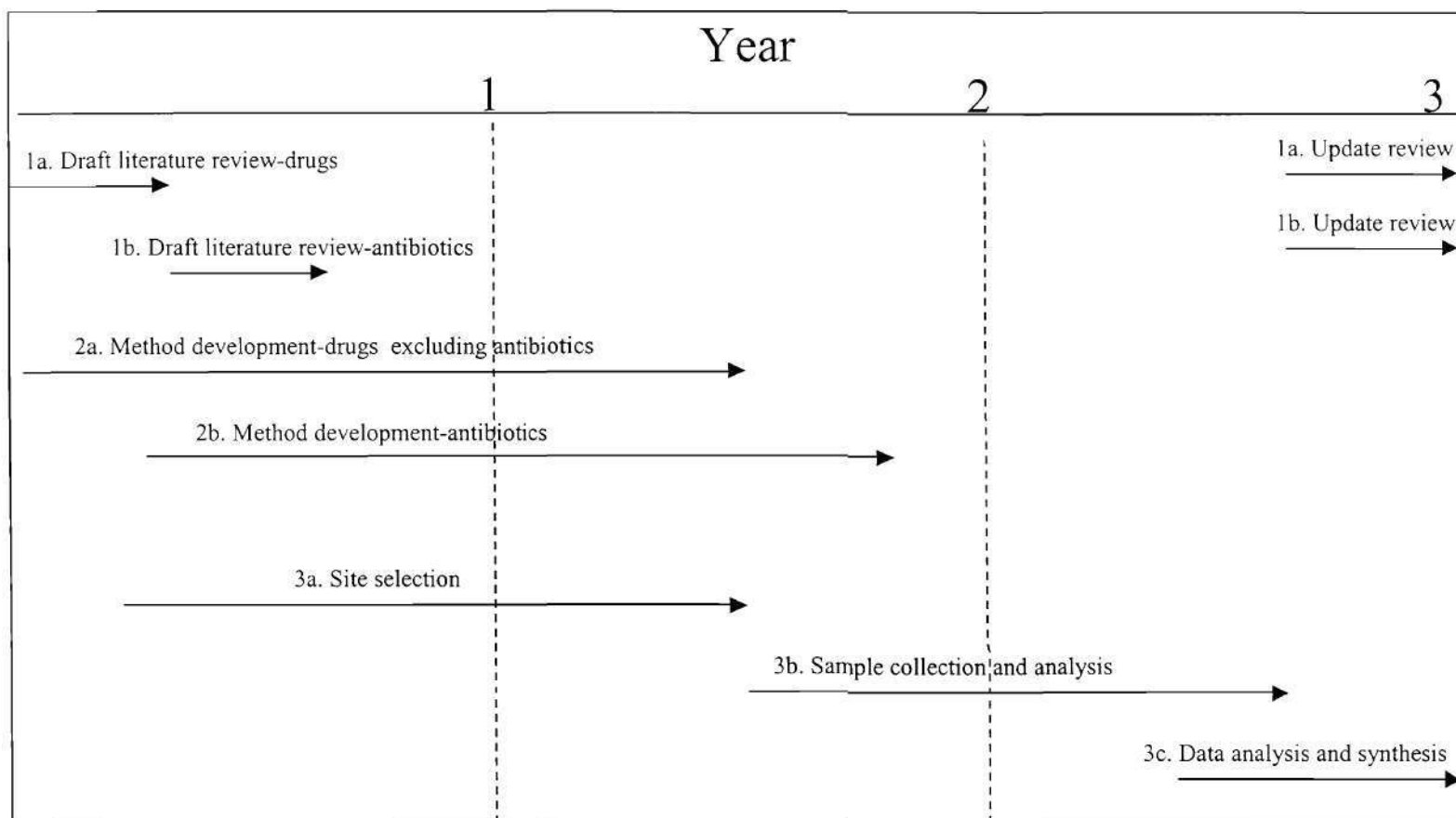
(b) Internal standard was not used for quantitation

(c) low recoveries may be caused by insufficient injection volume that occurred during sample injection

(d) Internal standard was not used for quantitation; spike concentration was 10 µg/L

(e) Compound quantification was hampered by matrix interferences

Appendix C: Revised Schedule

Year

1   2   3

1a. Draft literature review-drugs

1a. Update review

1b. Update review

1b. Draft literature review-antibiotics

2a. Method development-drugs excluding antibiotics

2b. Method development-antibiotics

3a. Site selection

3b. Sample collection and analysis

3c. Data analysis and synthesis

APPENDIX D: Responses to PAC Comments on the Third Periodic Report

PAC Comments on 4th periodic report for Project 2617
Occurrence Survey of Pharmaceutically Active Compounds

At first, please allow me to emphasize that I really like this report. I appreciated, especially, the almost objective descriptions of efforts and progress but also the mentioning of several problems encountered during method development. Unfortunately, the researchers do not longer follow one of the main objectives (development of ELISA methods) of their proposal. Nevertheless, the explanations why you had to skip this issue are very reasonable and consistent with concerns expressed earlier (proposal review)

PAC comments

page 7, 2nd paragraph: "Attempts to use radiolabeled propanolol ... failed because the labeled compound could not be discriminated from the unlabeled compounds.". Please give some more details! Is that because they have the same non-labeled fragments used for identification? Please identify these ions. Normally, MS is able to differentiate coeluting compounds with different molecular weights.

Response: The problem arose because we attempted to use MS/MS for analysis of the compound. Because the fragment of interest did not contain the label, it could not be discriminated from the unlabeled compound. We could differentiate the two compounds using GC/MS, but it decreased the overall sensitivity and prevented us from detecting propranolol in any environmental samples.

pages 7 and 8: Instead of the radio-labeled propanolol two other much more polar compounds are used as internal standards. Both compounds can not be added before sample extraction. Thus, they are not used as surrogate standards but only as internal standards to check sample injection. I really doubt that this approach is useful because only little information is provided by the

recovery rates measured for both compounds. This approach doesn't accurately reflect matrix influences. No information will be provided about the success of the solid-phase extraction and it is also not sure that a successful derivatization of the target analytes can be guaranteed even if the derivatization of the internal standards was successful.

Response: We agree with the reviewer that the effort to use these imperfect internal standards *probably is not worthwhile. Therefore, we have decided to rely upon spike recovery experiments* in our evaluation of the beta-blockers. After the expenditure of considerable effort, we are unable to improve the recoveries of beta-blockers beyond approximately 40-60%. Therefore, we will qualify all of the data when it is reported.

Page 8. The addition of deoxyepinphrine after the solid-phase extraction is an acceptable practice if and only if this step is explained in detail to the data users. I have come across data with similar practices that use this kind of addition to change (recovery correct the data) without disclosing this practice to the data users. It can give a false sense of recovery, accuracy, and/or precision. The explanation is good but needs to be used in any journal articles or other publication of the data.

Response: See response to previous comment.

page 8 and 9 (carbamazepine):
In Minneapolis, I already talked with David and we discussed the problems of carbamazepine analysis. So, here are a few additional hints that will hopefully help in overcoming the difficulties:
- The insert liners need to be deactivated and prepared before analysis. Before samples or standards are analyzed we always inject (3 times) the reagent used for derivatization (MTBSTFA).
- The use of a suitable surrogate standard is highly recommended, especially, when matrix-containing samples (sewage) shall be quantified!
- The use of ion-trap instruments without further deactivation of the metal surfaces (e.g., provided by Varian) may be problematic.

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Page 9. The poor recovery of the carbamazepine may be enhanced by putting sodium chloride (1 to 10 g/L) into the sample before extraction. We have used this technique to enhance the extraction of polar compounds from surface water samples. In addition, the use of teflon sample bottles with salt has improved recovery. Just a suggestion.

Response: We appreciate the suggestions and will try to use them in the future.

page 13:

The fact that the recovery experiments with samples from Caisson failed for diclofenac, gemfibrozil, naproxen and indomethacine sounds really problematic for the evaluation of results obtained with chlorinated samples? Is the reasonable explanation that this might be due to transformation by chlorine just a suggestion made to explain this result or are there any other indications? May the residual chlorine also affect your method and the recoveries?

Response: We have performed preliminary experiments evaluating the reactions between the acidic drugs and free and combined chlorine. The data confirm our hypothesis that the compounds are transformed by chlorine and that quenching the chlorine can eliminate the artifact. These experiments will be summarized in our next progress report.

page 13:

The low recoveries for propanolol and metaprolol seem not suitable for an accurate evaluation of the results (calculation of total loads etc.)? But I also have no final clue to this problem.

Response: As indicated previously, we believe that the data for metoprolol and propranolol will be adequate for qualitative purposes and will report them with suitable qualifications.

-Page 13 The differences between the Russian River samples and other sites back east. Could there be interference because waters have different origin composition? (i.e. industrial vs. wastewater vs. storm water, etc) I'm wondering whether the Russian River has a high component of a certain water origin that could cause a lot of the interference.

Response: The samples from the Russian River are pristine relative to the wastewater effluent and reclaimed water sampled at other locations. Therefore, any interference is due to natural organic matter or, in the Caisson sample, chlorine.

… Page 13. The variable recovery for the beta-blockers may be from two sources. The ionic strength may be playing a part of the variable recovery. The addition of salt may help this. The other may be to use the cationic cartridge (or some other cartridge) to remove some of the matrix and then do the derivitization. This would be similar to the antibiotic extraction. Again, the USGS has had success at adding salt and or removing matrices before analysis to improve recovery. Again, just suggestions.

Response: We appreciate the suggestion and have used salt to improve the extraction efficiency. We do not plan to investigate alternative SPE methods because we are uncertain that organic matter is responsible for the low and variable recoveries.

page 21:

The use of related antibacterial compounds as surrogates that are still used appears to be highly problematic even if these compounds are only used at very low quantities or in veterinary medicine. In several investigations we observed positive results for such compounds that we didn't expect to occur in those samples. In trace analysis a single application of one compound may cause significant contaminations (compared to other trace analytes) in a single sample. We also observed veterinary compounds in municipal sewage most probably originating from pet application. Might it be possible to identify and obtain out-of-use compounds as surrogates or just prepare an easy to synthesize compound?

Response: The related antibiotics (enrofloxacin and sulfamerazine) have been added after the SPE step, and used as internal standards for quantification, rather than as surrogates to assess method recovery. Matrix addition of target analytes has been used to assess method recovery. Thus far, all the wastewater samples analyzed have shown that these two antibiotics are absent in those samples. However, we agreed with the PAC that the absence of these compounds in samples needs to be confirmed to avoid any potential analytical errors. In the future, we plan to

replace enrofloxacin with lomefloxacin as the internal standard for fluoroquinolones. Due to its lower frequency in use, we expect the concentration of lomefloxacin to be very low whether in municipal wastewater or in surface water. During our search for proper internal standards, we found it difficult to obtain out-of-use antibiotics from commercial sources. Recently, we have identified a commercial supplier that provides ^{14}C-labeled ciprofloxacin and ^{3}H-labeld sulfamethazine, however, the prices for these radiochemicals are fairly high. If the PAC have better suppliers or compound candidates to recommend, we very welcome such suggestions and can investigate further on this issue.

page 24-26:

The recoveries for sulfamethoxazole and sulfamethazine have significantly been improved. Are there any plans to carry out recovery experiments at lower concentration levels (<=100 ng/L) reported for these compounds in surface waters by several authors? Please describe how the original loads of the secondary effluents with the investigated analytes have been taken into consideration when calculating the recoveries (any figures?).

Response: During the 5th project period, all the recovery experiments with sulfonamide antibiotics were conducted using the spiking concentration of 1 μg/L (instead of 10 μg/L). Unfortunately, we observed lower recoveries for both sulfamethoxazole and sulfamethazine with the lower spiking concentration (30-40%) compared to those with the higher spiking concentration (>75%). We think 1 μg/L is an appropriate spiking concentration and plan to continue using it in the future. In the next project period, we will conduct study to improve the recovery. For instance, we will assess whether salt addition to the matrices will improve the recovery.

In our method development, we routinely performed a total of four SPE for each sample: two for matrix additions and two for samples without fortification. The percent recovery was calculated from the measured total matrix addition concentration divided by the sum of background level and added amount (i.e., average background level + added amount). During the 4th project period, the only positive detection of sulfonamide antibiotics in samples was the presence of sulfamethoxazole in the secondary effluent of Clayton WWTP at 240 and 250 ng/L.

OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Sixth Progress Report
May 15, 2002

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SUMMARY

During the sixth project period, we continued method development activities and analyzed samples from several sites as part of the occurrence survey.

For the drugs other than antibiotics, most of our efforts involved analysis of samples as part of the occurrence survey. Analysis of samples from before and after chlorine disinfection at two municipal wastewater treatment plants did not show evidence of transformation of either acidic drugs or beta blockers. Data collected at a groundwater recharge facility corroborated previous measurements indicating removal of PhACs during groundwater infiltration. However, unlike the previous results, low concentrations of beta-blockers were detected in the sample from the deep well. Analysis of samples from the Santa Ana River indicated the presence of several compounds at concentrations slightly above the limit of quantification.

For the antibiotics, further method development activities improved the accuracy and precision of the analytical methods. In particular, addition of concentrated NaCl and use of standard addition yielded better recoveries and more accurate data. The analysis also was streamlined by combining the analysis of all of the antibiotics in one method. Although the analyses were not conducted using the finalized analytical methods, some interesting observations were made. Antibiotics were present at concentrations ranging from approximately 100 to 3,000 ng/L in effluent from conventional wastewater treatment plants. Antibiotics were detected in effluent from an advanced wastewater treatment plant, an engineered treatment wetland and a groundwater recharge facility.

CONTENTS

FIGURES

TABLES

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PROGRESS THIS PERIOD

TASK 1: LITERATURE REVIEW

The objective of this task is to identify compounds to be studied during the occurrence survey. The selection criteria for compounds in the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and availability of suitable analytical methods. Progress on the literature review was presented in the first and second progress reports. The literature review has been completed and will be updated prior to preparation of the final report. No additional progress related to task one was made during the current project period.

Sub-Task 1A: Drugs Used in Human Therapy

In the first progress report, we reviewed data on prescription drugs likely to be present in wastewater effluent in the United States. Using data on the number of prescriptions for the top 200 drugs and information on prescription formulations and metabolism, we estimated ranges of concentrations of 136 drugs expected to be present in wastewater effluent. In addition, we reviewed published occurrence data to identify other compounds of interest that did not appear among the common prescription drugs. A total of eleven drugs were identified for further consideration.

During the current project period no activity was conducted in association with this sub-task.

Sub-Task 1B: Drugs Used in Animal Husbandry

In the second progress report, we reviewed data on antibiotics used in livestock both therapeutically to treat diseases and sub-therapeutically as feed additives to promote growth. We focused our review on antibiotics used in animal feeding operations, because these sources could result in concentrated discharges of antibiotics. We also reviewed fate and transport properties and available analytical methods. On the basis of these results and our review of antibiotics used in human therapy, we identified that sulfonamide and fluoroquinolone antibiotics as the most probable water contaminants, followed by macrolide antibiotics. Among these antibiotic classes, we identified five antibiotics that merit further consideration.

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During the current project period no activity was conducted in association with this sub-task

TASK 2: ANALYTICAL METHOD DEVELOPMENT AND TESTING

The objective of this task is to identify and test analytical methods that will be used during the occurrence survey. As part of the task, analytical methods for extraction, cleanup and derivitization will be tested by adding drugs to deionized water and to samples collected from sites being considered for inclusion in the occurrence survey. Analysis of antibiotics is included as a separate task from the other pharmaceutically active compounds (PhACs) because the analytical methods for antibiotics (e.g., HPLC/MS) are fundamentally different from the gas chromatography methods used for the other compounds.

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the first progress report, we described our preliminary efforts to develop suitable analytical methods for eleven compounds identified as part of the literature survey. We used solid phase extraction followed by derivitization and GC/MS/MS to quantify pharmaceuticals in deionized water and in samples collected from several of the candidate sampling sites. To quantify recoveries and matrix effects, all samples also were analyzed after addition of known quantities of analytes.

During the second project period, we focused our attention on improving the accuracy and precision of the analytical methods by analyzing samples from three sites being considered for inclusion in the occurrence survey. As a result of analytical difficulties, we did not analyze three of the compounds (i.e., carbamazepine, nabumetone and nadolol) during the second project period. For the remaining eight compounds, analysis of duplicate samples indicated that, with the exception of two beta-blockers, results were reproducible (i.e., we observed an average of 26% error between duplicate samples). Analysis of blank samples indicated that sample carryover and cross contamination were not significant problems. Analysis of spike recovery samples indicated variable recoveries, with values as low as 30% for some analytes. All of the drugs were detected in one or more of the unspiked wastewater effluent samples.

During the third project period, we continued to improve the analytical methods by identifying steps where analytes were lost during analysis. For the acidic drugs, we changed the

solid phase extraction technique and added labeled mecoprop as an internal standard. As a result of the new SPE method, spike recoveries improved significantly. For the beta-blockers, we increased the time of the drying step to improve the efficiency of derivitization, but this only had a minor effect on spike recoveries. We also eliminated the use of PFE-lined containers, which resulted in losses of beta-blockers during storage. A QA/QC plan also was submitted to the PAC.

During the fourth project period, we attempted to resolve the remaining issues associated with the analytical methods. Attempts to use labeled propranolol as an internal standard for the beta-blockers failed because the labeled compound could not be discriminated from the unlabeled compounds. Alternative surrogates for beta-blockers could be derivitized and analyzed, but were too polar to be retained during solid phase extraction. We also evaluated the variability in method performance for acidic drugs and beta-blockers by analyzing a total of 18 samples from two surface waters and an advanced wastewater treatment plant. Analysis of surface water samples from a site that was subjected to chlorine disinfection indicated that several of the analytes were lost in the presence of free chlorine. We also tested a GC/MS/MS technique for analysis of carbamazepine. The compound could be detected easily at high concentrations. However, sensitivity decreased significantly at low concentrations, possibly as the result of losses in the injection port of the GC.

During the fifth project period we evaluated the possible use of epinephrine and deoxyepinephrine as internal standards in the analysis of beta-blockers. While it was possible to analyze the derivatives, they were lost during the extraction, solvent transfer and blow-down steps to a much greater degree than the other compounds. Therefore, we decided to limit our assessment of recovery to matrix spike recovery measurements with both beta-blockers for each set of samples.

During the fifth project period we also attempted to improve the recovery of carbamazepine by modifying the SPE method and by conditioning the injection port liner and replacing it after each set of analyses. Because these activities were not completed at the time of the report, we did not include any of our carbamazepine results. During the sixth project period we completed the carbamazepine studies begun during the fifth project period. Results of our analyses indicated that carbamazepine was present at a concentration around 1,000 ng/L in the effluent of the Mt. View WWTP and in the pond at the Sweetwater recharge facility. However,

we were unable to obtain reproducible, linear standard curves because the injection port liners could only be used for a few samples before they had to be replaced. Given the numerous analytical challenges associated with the analysis of carbamazepine and the need to complete the occurrence survey, we decided to forgo any further attempt to analyze this compound.

Sub-Task 2B: Antibiotics

Our analysis of antibiotics has focused on fluoroquinolone and sulfonamide antibiotics. Ciprofloxacin, sulfamethoxazole and sulfamethazine are the selected target analytes of occurrence analysis. In the second and third progress reports, we reported our efforts to develop suitable analytical methods for these compounds. A dual-cartridge solid phase extraction (SPE) method was developed to extract antibiotics from water samples. Antibiotics were analyzed using LC/MS and LC/FLD (fluorescence detection).

During the fourth progress report, we attempted to improve the analytical methods. We identified the steps where analytes were lost and made changes to minimize the losses. High-density polyethylene conical tubes were shown to yield the smallest losses of fluoroquinolones during the blow-down step and were used in all the later experiments. We determined that acidifying and storing samples in amber glass bottles best preserves analytes prior to solid-phase extraction and yields the smallest losses of analytes through adsorption to the container walls. Sample extracts were better preserved at 0°C than at 5°C when analysis by LC/MS could not be conducted immediately. To achieve better results, separate LC/MS methods were developed for sulfonamide and fluoroquinolone antibiotics respectively. LC/MS conditions were modified to reduce matrix effects and increase sensitivity. We investigated several structurally related sulfonamide and fluoroquinolone antibiotics as internal standards. Our studies indicate that sulfamerazine and enrofloxacin serve as appropriate internal standards. These research efforts have improved the method recovery to above 60% and have also increased sensitivity in detection.

Near the end of the fourth project period, the cation-exchange extraction method for fluoroquinolones was found to work successfully in reagent water after finding a proper cation-exchanger (high-density, mixed-phase cation-exchange discs, 3M) and avoided errors in sample preparation. The cation-exchange extraction followed by HPLC/fluorescence detection is a simple and sensitive method that can be easily performed in most existing water utility labs and

can also be used to independently confirm the analysis by the dual-cartridge SPE followed by LC/MS. Therefore, it was concluded that the cation-exchange SPE merited further investigation with wastewater matrices.

During the fifth project period, we examined the accuracy and precision of the dual-cartridge SPE and LC/MS methods in several more wastewater samples and included two additional antibiotics, trimethoprim and norfloxacin, in the analysis. The results indicated consistent recoveries meeting the QA/QC criteria for the fluoroquinolones (norfloxacin and ciprofloxacin). However, the recoveries for sulfonamides and trimethoprim were still below our target values. Further studies on the cation-exchange SPE followed by LC/fluorescence detection method indicated that the method is adversely affected by the organic matter encountered in more complicated wastewater matrices, rendering consistent performance difficult. It was concluded that the method is only suitable for qualitative screening purposes for fluoroquinolones and may be suitable for compound quantitation in relatively clean water samples.

During this project period, significant improvements were made to the analytical method. The fluoroquinolones ofloxacin and enrofloxacin were added to the analysis, yielding a total of four fluoroquinolones (i.e., ofloxacin, enrofloxacin, ciprofloxacin and norfloxacin) in the occurrence survey. Lomefloxacin was used as an internal standard for the fluoroquinolones. The recoveries for sulfonamides and trimethoprim were greatly improved after using salt addition prior to the SPE step. For more accurate quantification, the standard addition method was used for quantification. Results were compared to data obtained using the internal standard method. Analytical efficiency was enhanced after combining the two LC/MS methods for fluoroquinolones and sulfonamides. Finally, the LC/MS sensitivity was improved by lowering the eluent buffer concentrations while still maintaining sufficient buffering capacity. The aforementioned improvements resulted in a robust and sensitive method and thus we consider the method development for antibiotics near completion. Samples collected from several wastewater treatment systems were analyzed with the improved analytical methods. Results (Appendix B) provided insights to the occurrence of the seven target antibiotics and the efficacy of a range of treatment processes.

Sample Collection and Method Optimization

Wastewater samples were collected from five sites: F. Wayne Hill (FWH) Advanced Wastewater Treatment Plant (AWWTP) in Georgia, Clayton WWTP in Atlanta, South Cobb WWTP in Georgia, Sweetwater Recharge Facility in Arizona and Mt. View WWTP/engineered wetland in California. Samples were collected from the Sweetwater Recharge Facility twice.

Secondary and final effluents were collected from South Cobb WWTP, which employs activated sludge biological treatment followed by chlorination. Effluents of primary treatment, secondary treatment (activated sludge), GAC and final treatment (after GAC and ozonation) were collected at the FWH AWWTP. Secondary and final effluents were collected from the Clayton WWTP that employs biological treatment followed by UV disinfection. Three effluents were collected from the Sweetwater Recharge Facility. The Sweetwater Recharge Facility infiltration basins receive secondary influent from Roger Road WWTP and Tucson Water Reclamation Plant. The infiltration basin pond, a shallow and deep well below the pond were all sampled at this site. The samples collected from Mt. View WWTP included trickling filter effluent, nitrification effluent and wetland entrance and exit samples. The wastewater at this site is treated with UV disinfection prior to the wetland entrance.

Samples were collected in amber glass bottles, filtered by 0.5-µm glass-fiber filters and acidified with phosphoric acid to approximately pH 2.5. In addition, 0.1 M of NaCl was added to the samples. The addition of NaCl greatly improved the recoveries for the sulfonamides and trimethoprim by enhancing the salting out of these antibiotics. Addition of NaCl also helped stabilize the recoveries for the fluoroquinolones. Samples were extracted by a combination of an anion exchanger and an Oasis HLB cartridge stacked vertically. In the study of matrix spike recoveries, approximately 1.0 µg/L of each target antibiotic was added to the samples prior to extraction. Sulfamerazine was used as an internal standard for the quantification of the sulfonamides and trimethoprim while lomefloxacin was used as an internal standard for the fluoroquinolones. A fixed amount of internal standard was spiked into wastewater extracts after SPE and to the calibration standards.

After solid phase extraction, antibiotics were analyzed by LC/MS with electrospray ionization using positive ion mode and selected-ion monitoring (SIM). Additional optimization of the LC/MS method was accomplished during this project period. For effluent that received conventional wastewater treatment, a significant amount of signal suppression was caused by the

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matrix effect. To detect the antibiotics in these samples, the eluent buffer concentration was reduced. This adjustment minimizes the signal suppression due to the eluent buffer and allows antibiotics in the dirtier matrices to be detected. The eluent buffer was changed to 0.002% acetic acid and the eluent gradient was slowed in order to increase chromatographic separation, which also minimized signal suppression. The LC/MS methods for fluoroquinolones, sulfonamides and trimethoprim were also combined and thus decreased the number of LC/MS runs. The fragmentor voltage for the optimized method was set to 85 V. To confirm the presence of the antibiotics, the samples were also run with a higher fragmentor voltage of 120 V to identify the confirming fragment ion. A description of the modified method used for analysis of antibiotics is provided in Appendix C.

For compound quantification, both the internal standard method and the standard addition method were used. The accuracy of the internal standard method depends on the matrix enhancing or suppressing the internal standard by the same proportion as the analyte being quantified. Because the effect is not always uniform in environmental samples, overestimation and underestimation has been observed in our analysis using the internal standard method. Variation in the effect of organic matter on instrument response is not as important in the standard addition method. In the standard addition method, one unspiked sample from each matrix was divided into two parts after extraction. For half of the sample, a known amount of each antibiotic analyzed was added. No antibiotics were added to the other half. After analysis with the LC/MS, equation 1 was used to calculate the amount of antibiotics in sample while taking into account volume changes.

$$X = SI_X/(I_S-I_X) \qquad \text{(Eq. 1)}$$

where X is the amount of antibiotic in extract, S is the amount of antibiotic spiked into extract, I_S is the signal intensity of antibiotic in spiked solution, and I_X is the signal intensity of antibiotic in unspiked solution. Use of standard addition method is appropriate since all the antibiotics exhibit linear calibration curves within the investigated concentration range ($r^2 > 0.98$).

Matrix Spike Recoveries

The recoveries for all fluoroquinolones during the sixth project period are shown in Figures 1-4. The average recovery for all matrices for ciprofloxacin, norfloxacin, enrofloxacin, and ofloxacin were 103±23%, 101±23%, 96±21% and 125±27%, respectively. Hence, the

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recoveries are generally within the QA/QC target range. In addition, the standard deviation of ciprofloxacin recoveries has decreased as the method has been improved. Overall, the method is robust and reliable for the analysis of fluoroquinolones in different wastewater matrices.

The recoveries for the sulfonamides and trimethoprim have been greatly improved for this reporting period (Figures 5-7). The average recovery for all matrices for sulfamethoxazole, sulfamethazine and trimethoprim were 69±25%, 57±31% and 159±114% respectively.

Figure 1. Recoveries of ciprofloxacin (spiked at 1.0 µg/L) in deionized water and wastewater effluent during the 6th project period. All recoveries were calculated based on the internal standard quantification method in which lomefloxacin was the internal standard.
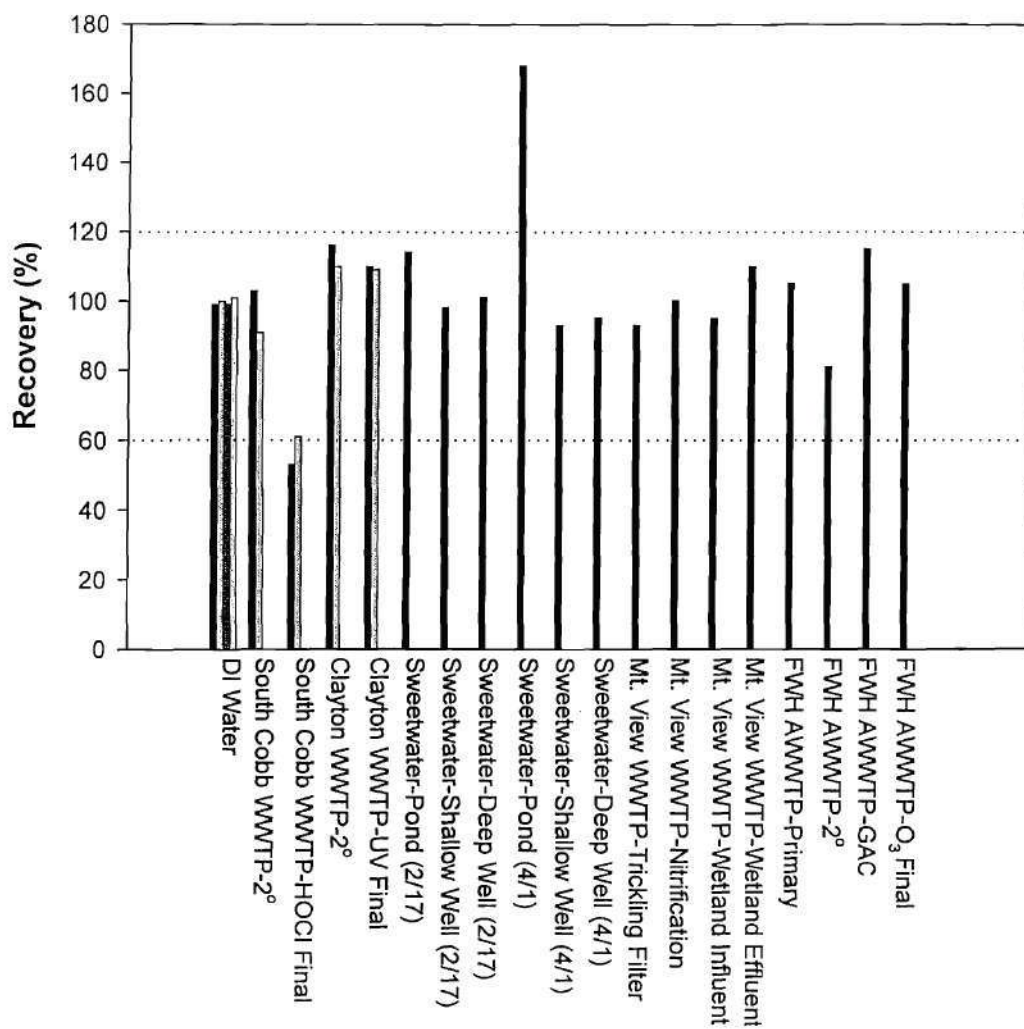
Figure 2. Recoveries of norfloxacin (spiked at 1.0 µg/L) in deionized water and wastewater effluent during the 6th project period. All recoveries were calculated based on the internal standard quantification method in which lomefloxacin was the internal standard.

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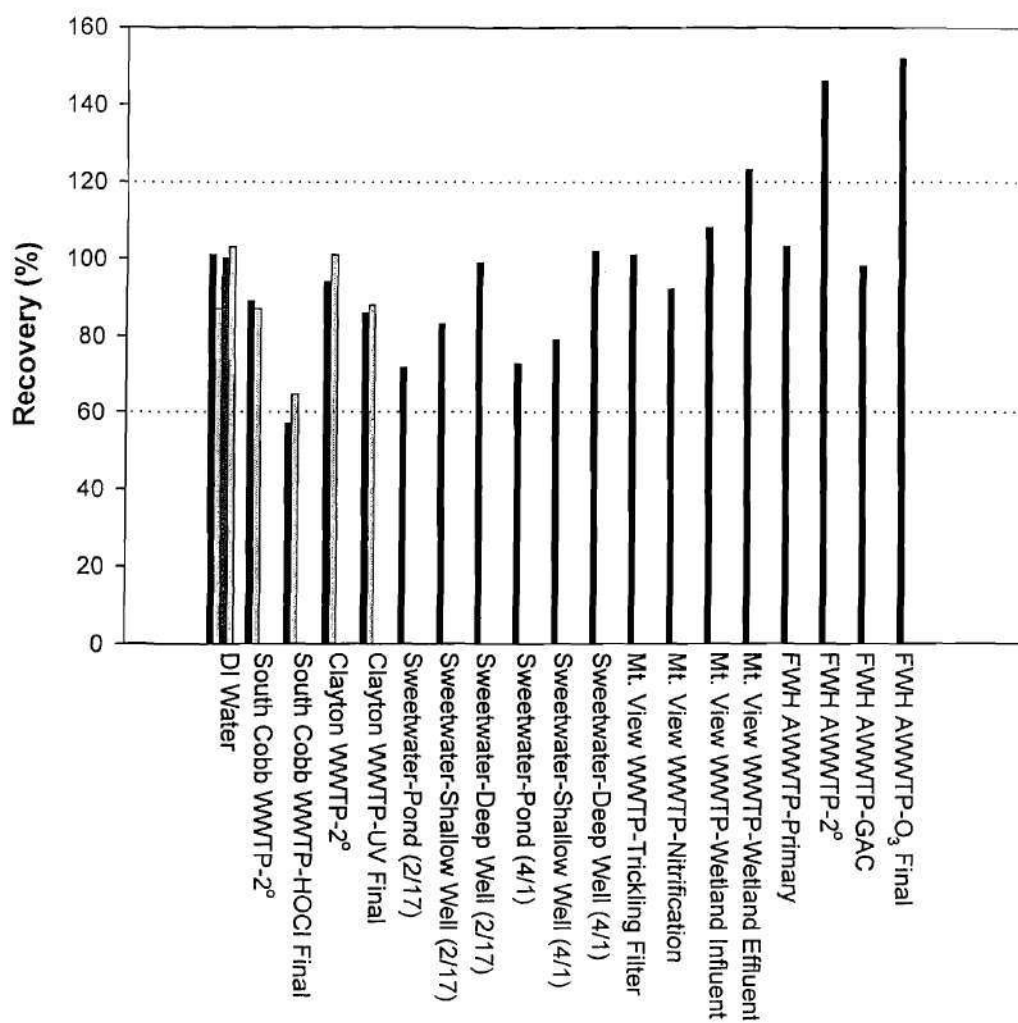
Figure 3. Recoveries of enrofloxacin (spiked at 1.0 µg/L) in deionized water and wastewater effluent during the 6th project period. All recoveries were calculated based on the internal addition method in which lomefloxacin was the internal standard.
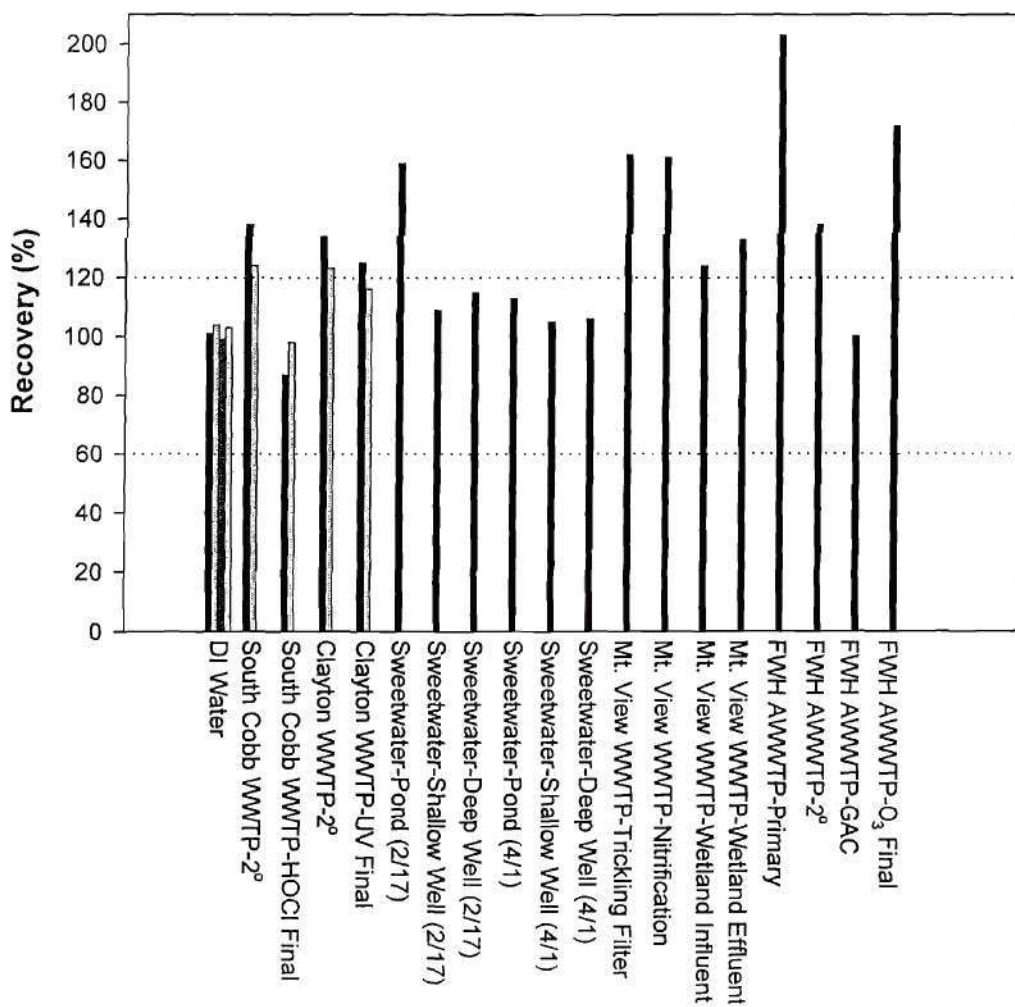
Figure 4. Recoveries of ofloxacin (spiked at 1.0 µg/L) in deionized water and wastewater effluent during the 6th project period. All recoveries were calculated based on the internal standard quantification method in which lomefloxacin was the internal standard.
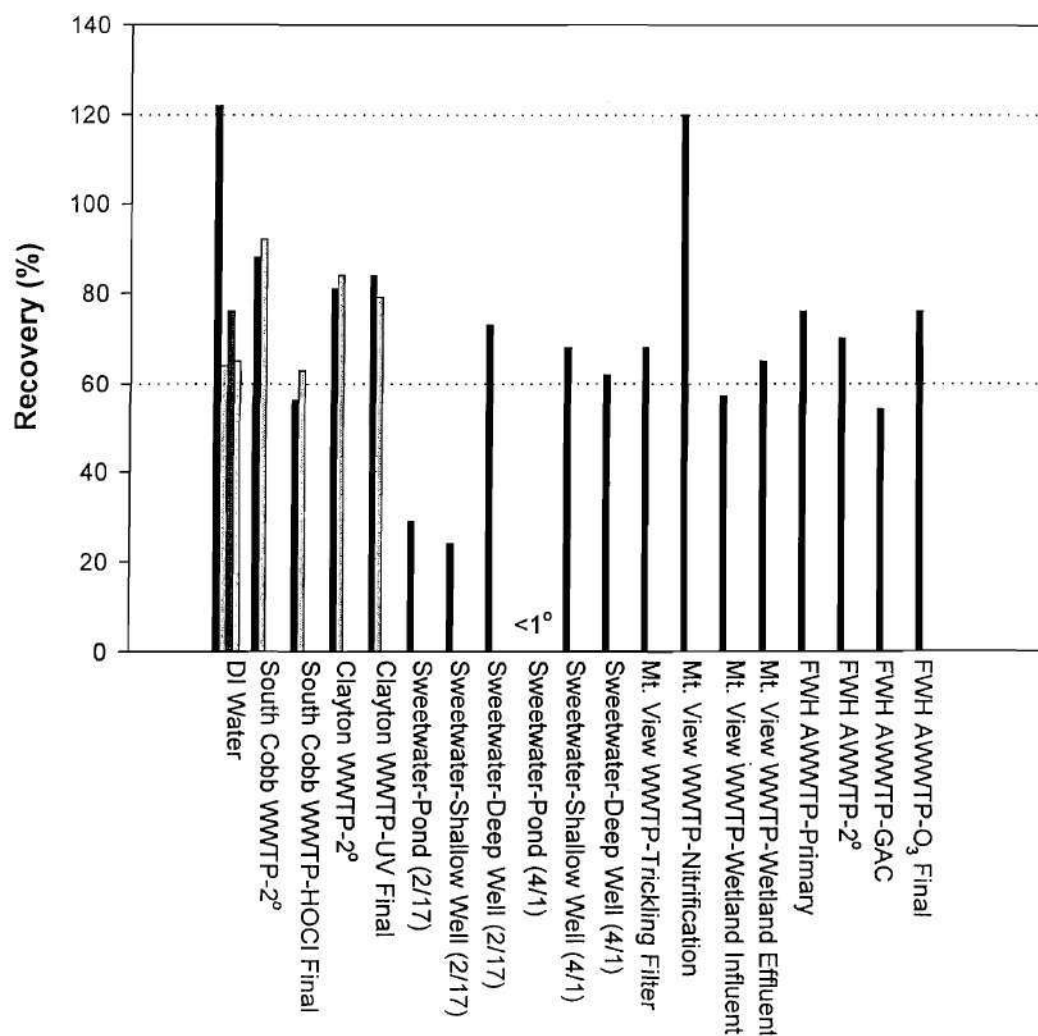
Figure 5. Recoveries of sulfamethoxazole (spiked at 1.0 µg/L) in deionized water and wastewater effluent during the 6th project period. All recoveries were calculated based on the internal standard quantification method in which sulfamerazine was the internal standard.

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Figure 6. Recoveries of sulfamethazine (spiked at 1.0 μg/L) in deionized water and wastewater effluent during the 6th project period. All recoveries were calculated based on the internal standard quantification method in which sulfamerazine was the internal standard.
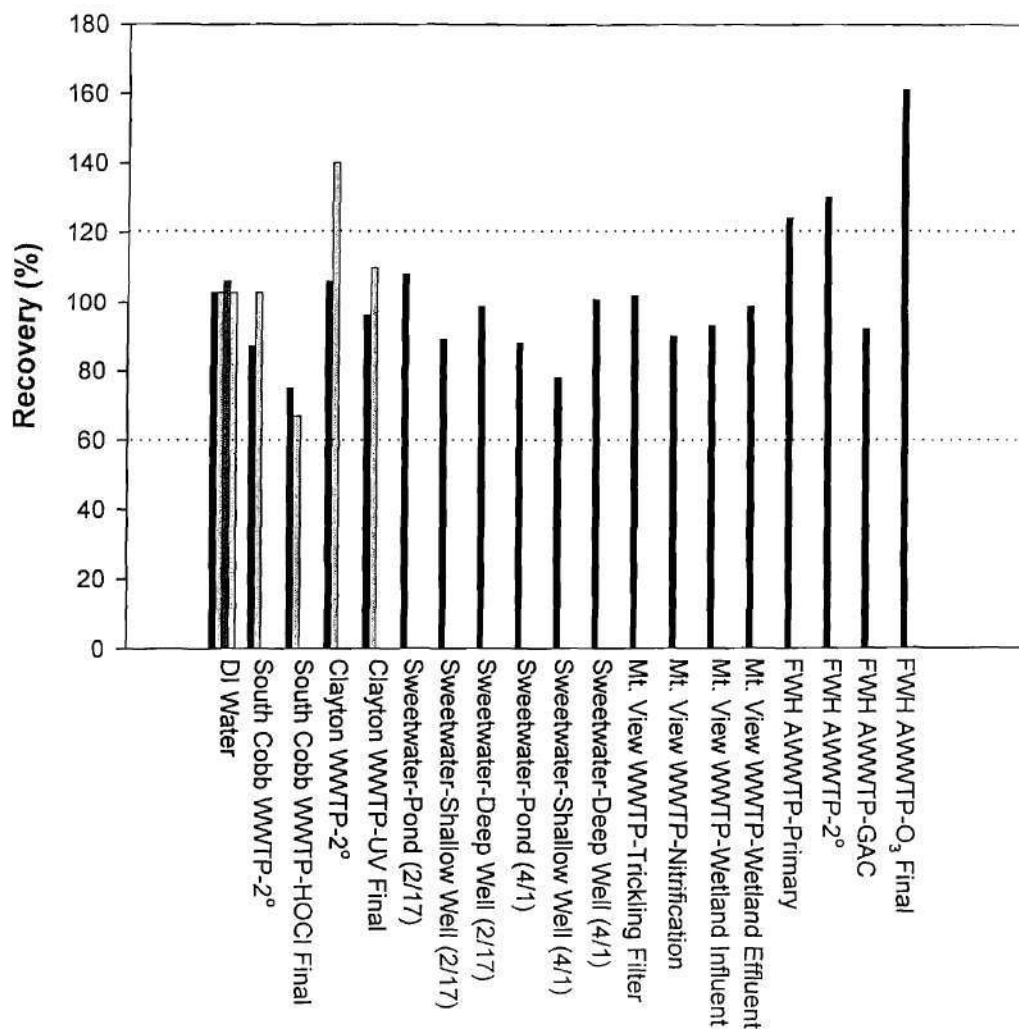
Figure 7. Recoveries of trimethoprim (spiked at 1.0 µg/L) in deionized water and wastewater effluent during the 6th project period. All recoveries were calculated based on the internal standard quantification method in which sulfamerazine was the internal standard.
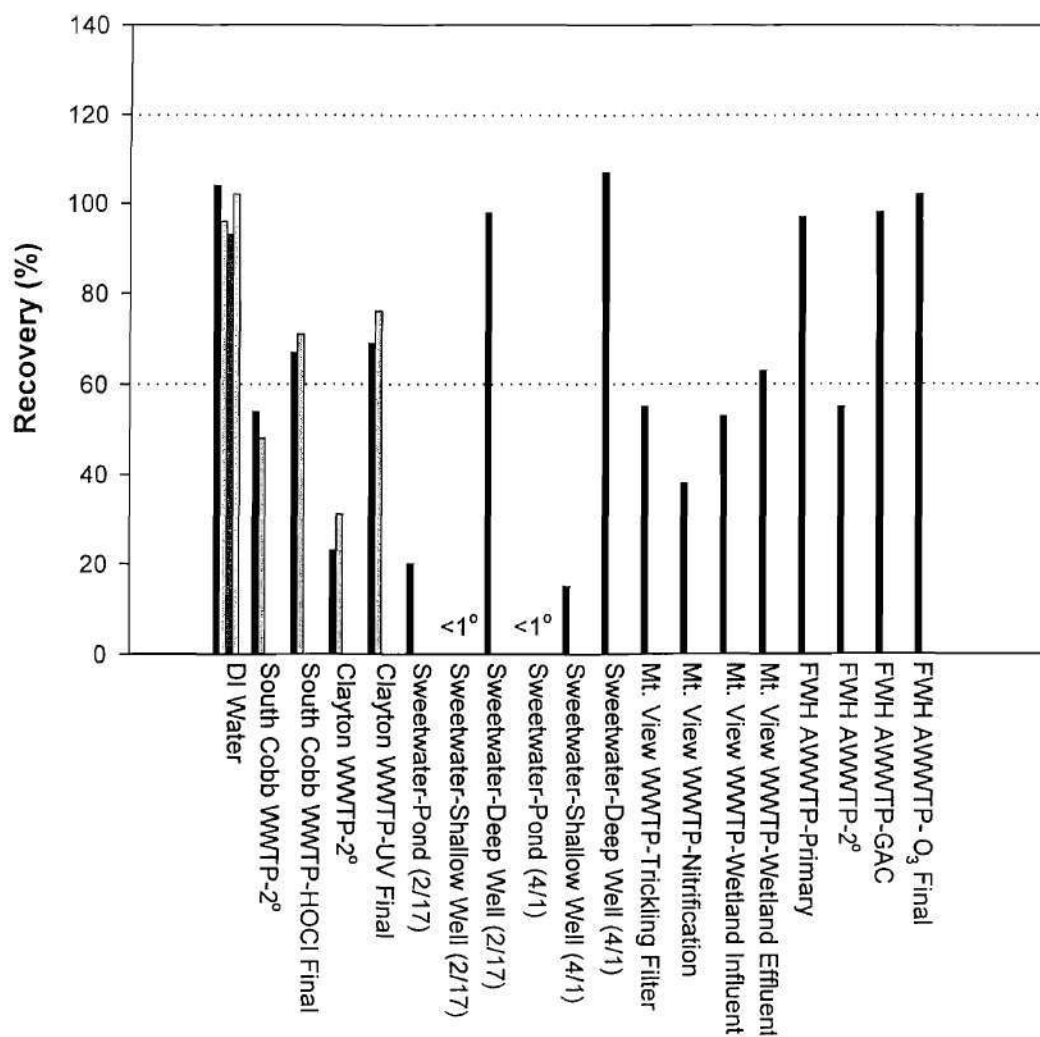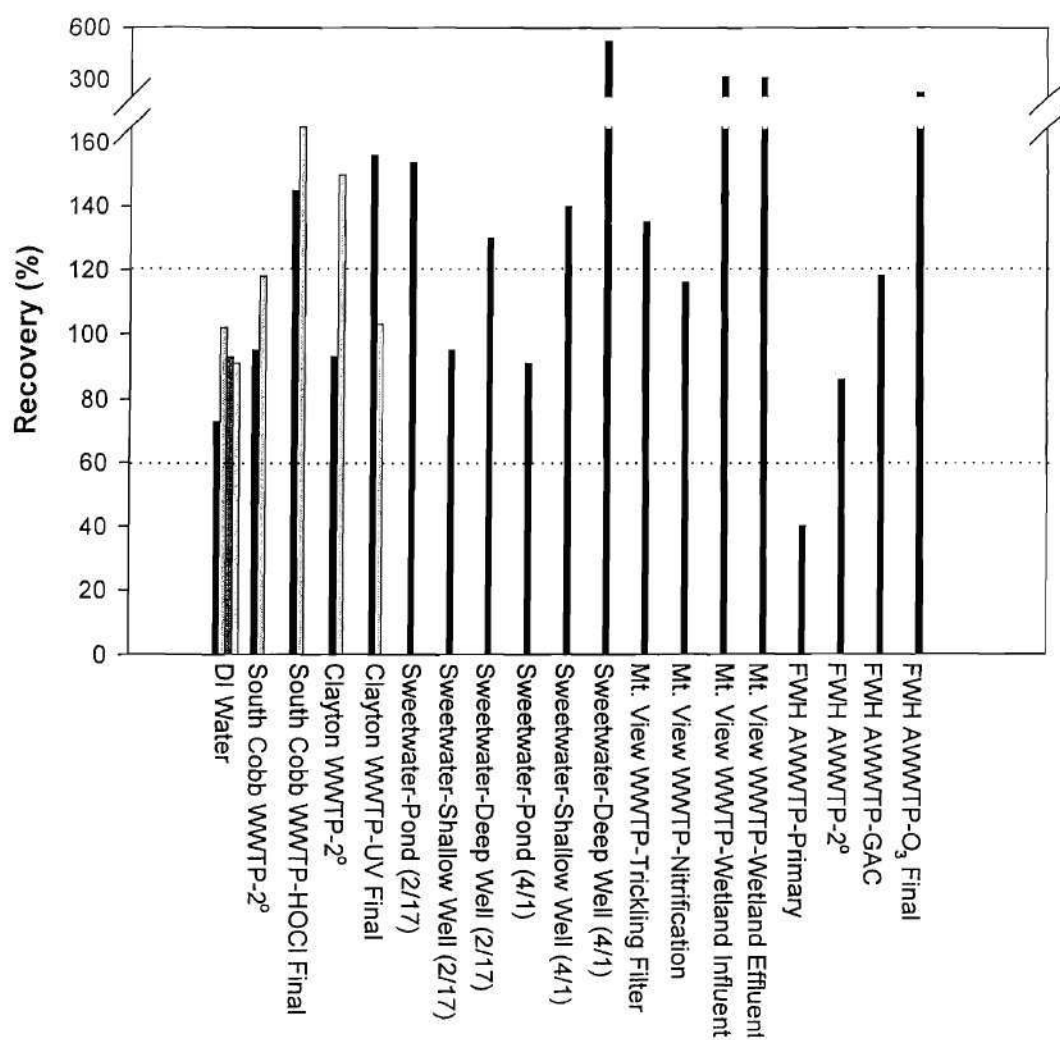
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The recoveries for trimethoprim are overstated due to the use of sulfamerazine as an internal standard and can be explained by two factors. Firstly, the internal standard should be as similar as possible to the analyte in order to ensure accurate quantification. Since the structure of trimethoprim is not as close to sulfamerazine as the other sulfonamides, the internal standard quantification is not as accurate for trimethoprim. Secondly, the retention times for the sulfonamides and trimethoprim ranged from 9.7 min to 18.4 min. Thus constituents of the matrix that coelute with the internal standard (sulfamerazine) could be considerably different from the constituents that coelute with the analytes (sulfamethazine, sulfamethoxazole and trimethoprim). As a result, the magnitude of signal suppression for the internal standard could be substantially different from that of the analytes. As seen in this study, such discrepancy in signal suppression can also vary among matrices.

Figures 8 and 9 illustrate the signal suppression effect for the antibiotics in samples from the Sweetwater Recharge Facility. The signal suppression is defined as the percent decrease in signal intensity for an antibiotic in a sample extract versus in a deionized water matrix and was calculated using equation 2. In those samples, the internal standards were assumed to be absent in the unspiked samples.

$$\text{Signal Suppression (\%)} = (1 - \left[\frac{I_s - I_x}{I_{DI}}\right]) * 100 \qquad \text{(Eq. 2)}$$

where I_s is the signal intensity of antibiotic in the spiked solution of the matrix of interest, I_X is the signal intensity of antibiotic in unspiked solution of the matrix of interest, I_{DI} is the signal intensity in deionized water spiked with the same amount of antibiotic as the matrix of interest. The volume changes were also taken into account.

The UV_{254} absorbance (a surrogate measure of the concentration of dissolved organic carbon DOC) for the deep well, shallow well and pond samples prior to extraction were 0.028, 0.267 and 1.117, respectively. Clearly, signal suppression increases with increasing DOC in the sample and the difference in signal suppression between the analytes and the internal standards exists in all matrices. Figure 8 indicates that trimethoprim typically exhibits a lower susceptibility toward signal suppression than the sulfonamides. As a result, the concentration of trimethoprim is overestimated based on the sulfamerazine internal standard quantification. If the

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approximately 35% overestimation due to signal suppression is taken into account for trimethoprim, the average recovery of trimethoprim (159%) would be closer to 103% (i.e., 159%×65%).

The difference in signal suppression between lomefloxacin and the other fluoroquinolones is smaller (Figure 9). This is likely due to the fact that the structures and retention times of fluoroquinolones are more similar to each other. However, ofloxacin and norfloxacin exhibit lower signal suppression than lomefloxacin and thus their recoveries may also be overestimated.
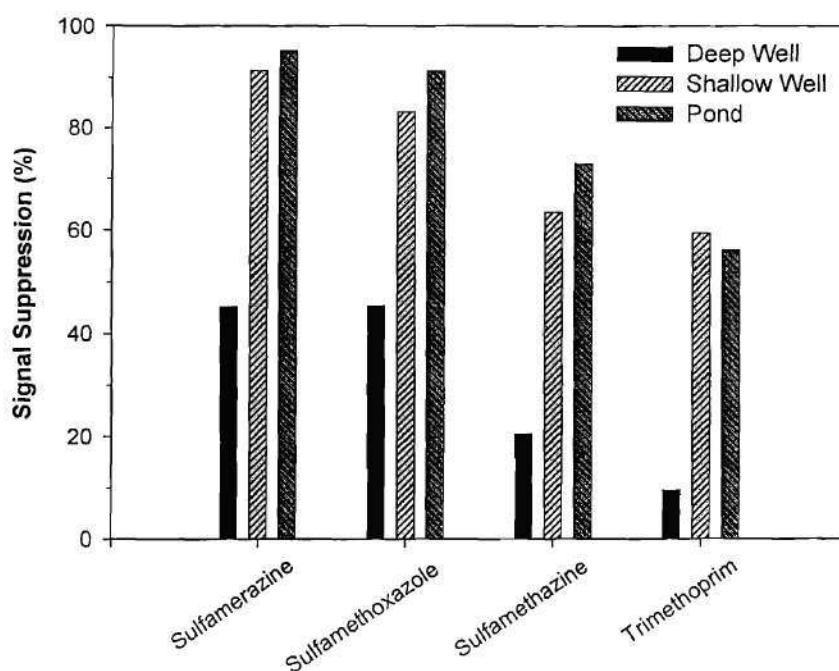
Figure 8. Signal suppression for sulfinamides and trimethpoprim caused by the matrix effect from the Sweetwater Recharge Facility 4/1/2002 samples.
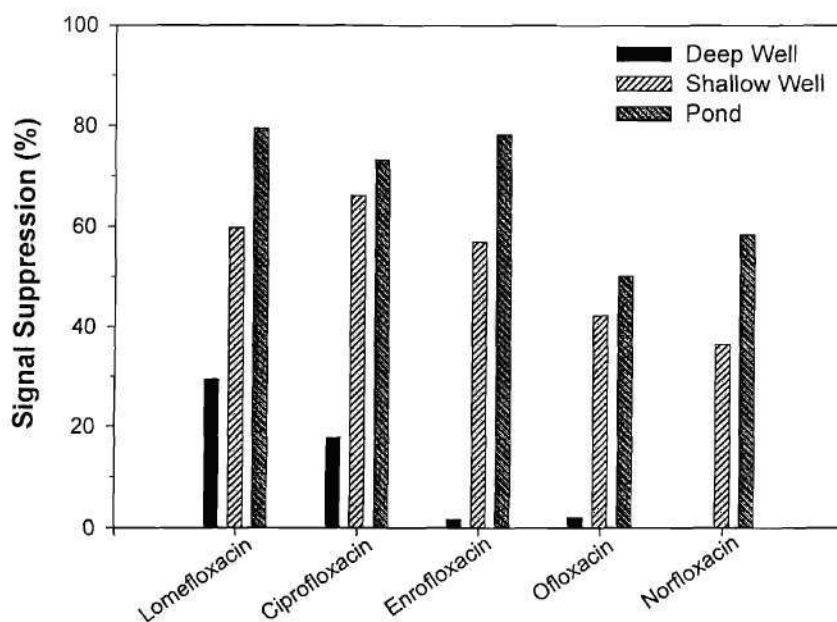
Figure 9. Signal suppression for fluoroquinolones caused by the matrix effect from the Sweetwater Recharge Facility 4/1/2002 samples.

As shown in Figure 5 and 6, a few occasions of high amounts of DOC caused low recoveries (<30%) for sulfamethoxazole and sulfamethazine (i.e., the pond and shallow well samples from the Sweetwater Recharge Facility). A 40% recovery for trimethoprim was obtained in the primary effluent from the FWH AWWTP (Figure 7). However, sampling of primary effluent objective is not an important objective of our study. Despite these limitations, the initial attempt to assess the feasibility of quantifying antibiotic concentrations in the primary effluent yielded encouraging results. Unusually high recoveries for trimethoprim were obtained

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on a few occasions. These very high recoveries could be caused by overestimation by the internal standard method and by other errors in sample preparation.

Overall, the current analytical method recovers antibiotics from the complicated wastewater samples very effectively and is robust and reliable for assessing the occurrence of antibiotics in many water samples. It is evident that the usefulness of internal standard is limited due to the inherent matrix effect in electrospray ionization unless the labeled versions of each target analyte are used (which is not feasible and very costly). As a result, the standard addition method is a more accurate quantification method for all antibiotics. Unless stated otherwise, the concentrations of antibiotics presented in the later sections were calculated based upon the standard addition method. In the future, we also plan to use the standard addition method to determine the matrix spike recovery.

A QA/QC plan is also included in the Appendix D for the occurrence survey of antibiotics. In our method, recoveries will be assessed by spiking the matrices with the target antibiotics. Antibiotic quantification will be conducted by the standard addition method. Lomefloxacin and sulfamerazine will be used primarily for evaluating the LC/MS performance in each run. We will also investigate samples that are not spiked with lomefloxacin and sulfamerazine to evaluate their potential presence in the samples.

TASK 3: OCCURRENCE SURVEY

Sub-Task 3A: Site Selection

In the third progress report, we provided a list of sites that we planned to include in the occurrence survey (see Table 1). As part of the site selection process, preliminary samples were collected during the first three project periods from sites that we considered for inclusion in the occurrence survey. The second column in Table 3 indicates the progress report in which preliminary samples were collected for each site on the list during the first three project periods. While the quality of some of the preliminary data was acceptable, the samples were not analyzed using all of the steps ultimately incorporated into the analytical method described in the QA/QC plan. As a result, these previous results are only useful for screening purposes. During the fourth project period, we analyzed samples from two of the sites using the accepted analytical methods for acidic drugs and beta-blockers. Except for analysis of acidic drugs from the Witchita Falls AWWTP, these data met our QA/QC criteria and will be included in the occurrence survey. During the fifth project period, we analyzed a total of 11 samples for acid drugs and beta-blockers from four sites on the list. We also analyzed samples from three sites for antibiotics. Because method development for the antibiotics was not completed at that time, the antibiotic data are only useful for screening purposes.

During the sixth project period, we analyzed samples from a total of seven sites (Appendices A and B). During the next project period, we plan to sample at nine of the sites listed in Table 1. Sites will be sampled for a second time during the eighth project period.

A total of ten samples were analyzed for acidic drugs and beta-blockers from three wastewater treatment plants, a groundwater recharge facility and an effluent-dominated surface water. Samples from two of the wastewater treatment plants were collected before and after chlorination to assess for transformation reactions related to reactions with chlorine. Samples from the groundwater recharge facility and Santa Ana River were collected to assess the importance of natural attenuation. For the acidic drugs, the samples from the Sweetwater groundwater recharge pond and the sample from the Prado Wetland did not meet our QA/QC target. At least one replicate of each of the other samples met our QA/QC target.

Table 1. Summary of sample collection in the occurrence survey. The second column indicates preliminary samples collected as part of sample screening. The third column indicates data collected during the fourth and fifth progress periods. The fourth column indicates planned sample collection. Acid. = acidic drugs; β = beta-blockers; Anti. = antibiotics.

Location	Periods 1-3			Periods 4-6			Planned
	Acid.	β	Anti.	Acid.	β	Anti.	
Dublin/San Ramon WWTP		1		6	6		August 2002
Mt. View WWTP	2	2				6	June 2002
Sweetwater WWTP[1]	2,3	2,3		6	6	6	Period 8
San Jose/Santa Clara WWTP				6	6		August 2002
Hyperion WWTP[2]				5	5		July 2002
Clayton WWTP			3			4,5,6	August 2002
Dublin/San Ramon AWWTP		1					See text
West Basin AWWTP	3	3		5	5		July 2002
OCWD Pilot AWWTP				5	5		July 2002
FWH AWWTP			3			4,5,6	August 2002
South Cobb WWTP						6	May 2002
Witchita Falls (TX) Pilot Plant				4*	4		See text
Mt. View Wetlands	2	2				6	June 2002
Prado Wetlands				6	6		Period 8
Rio Hondo Spreading Basins							See text
Sweetwater Recharge Facility	2,3	2,3		6	6	6	Period 8
Russian River	3	3		4, 5	4,5		Completed
Sacramento Delta							July 2002
MWD Water	3	3					July 2002

Notes:

WWTP = conventional municipal wastewater treatment plant; AWWTP = advanced wastewater treatment plant; OCWD = Orange County (CA) Water District; FWH = F. Wayne Hill; MWD = Metropolitan (CA) Water District

[1] Sample collected from holding pond associated with recharge facility. [2] Influent to the West Basin AWWTP ; * Data unacceptable due to analytical problems.

Sub-Task 3B: Sample Collection and Analysis

During the sixth project period, samples collected from the San Jose/Santa Clara WWTP, Dublin/San Ramon WWTP, Sweetwater groundwater recharge facility and the Santa Ana River were analyzed for acidic drugs and beta-blockers using finalized analytical methods. Samples from several sites also were analyzed for antibiotics as part of method development activities, as described in the previous section. The data for acidic drugs and beta-blockers are presented in the following paragraphs.

In the fourth progress report, we described the loss of diclofenac, gemfibrozil, indometacine and naproxen from samples that were not dechlorinated after collection (i.e., the Caisson samples from the Russian River). These results combined with subsequent laboratory studies indicated that the compounds were transformed by chlorine and that addition of a quenching agent, such as sodium thiosulfate is needed to avoid artifacts due to transformation after sample collection.

During this project period, we attempted to evaluate the potential for transformation of the compounds in chlorine disinfection systems employed in municipal wastewater treatment plants. To accomplish this goal, we collected samples from the San Jose/Santa Clara (SJSC) and the Dublin/San Ramon (DSR) municipal wastewater treatment plants. The SJSC WWTP employs biological nutrient removal to remove dissolved inorganic nitrogen while the DSR WWTP does not remove ammonia from the effluent. Therefore, the effluent from the SJSC WWTP is subjected to free chlorine (i.e., HOCl/OCl$^-$) while the effluent from the DSR WWTP is subjected to combined chlorine (i.e., NH_2Cl and organic chloramines). Grab samples were collected from each treatment plant in containers that contained sodium thiosulfate ($Na_2S_2O_3$) to quench the chlorine. Due to logistical considerations, it was impossible to follow the same parcel of water through the disinfection process. However, the chlorine contact time at these facilities was relatively short (~ 1 hour), and it is unlikely that the composition of the water changed greatly during the period in question. Results of the analyses suggest that none of the compounds were transformed to an appreciable degree during disinfection (Figure 10).
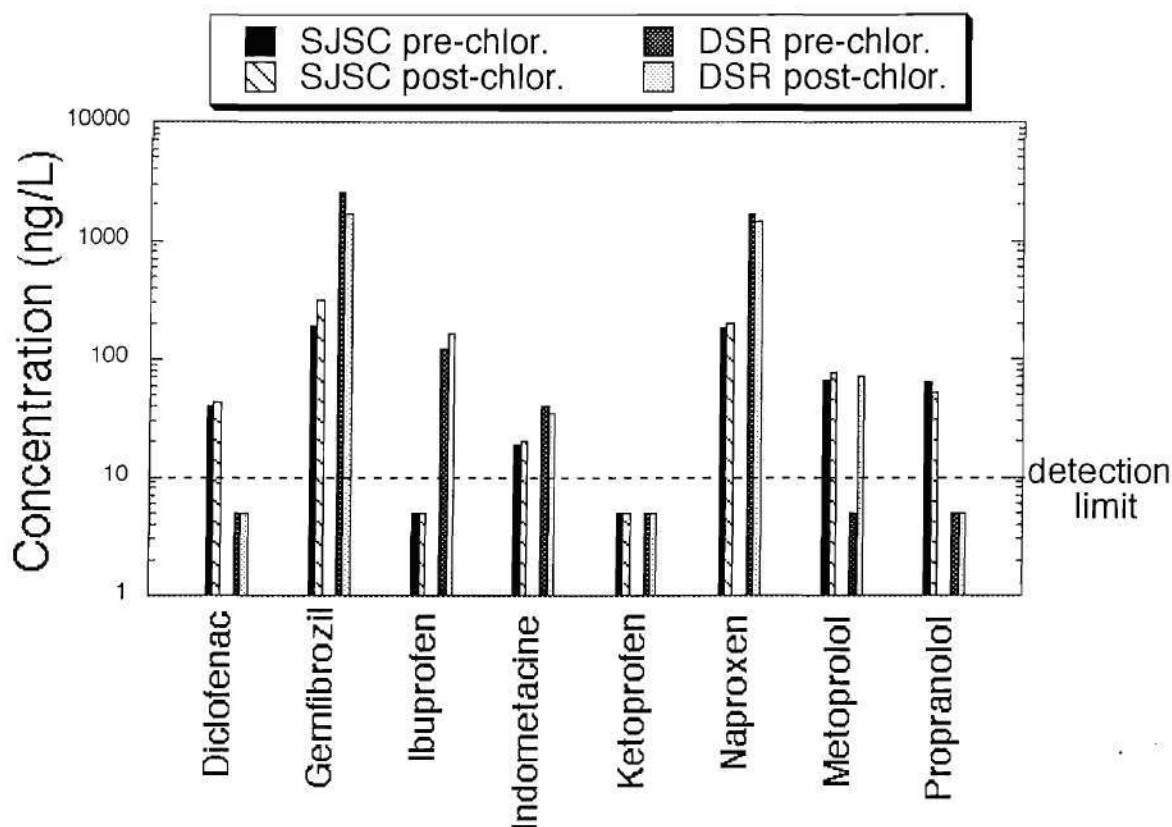
Figure 10. Concentrations of drugs detected at the San Jose/Santa Clara (SJSC) and Dublin/San Ramon (DSR) Wastewater Treatment Plants. The detection limit for all compounds was 10 ng/L. Concentrations below the detection limit are plotted at half the detection limit.

At the Sweetwater recharge facility, we collected samples from the pond as well as the shallow and deep groundwater wells. Results were consistent with our previous findings that pharmaceuticals were attenuated during infiltration (Figure 11). As previously observed, little removal occurred between the pond and the shallow well. No acidic drugs were detected in the deep well. Beta-blockers were detected at concentrations slightly higher than the limit of quantification in the deep well.

Samples also were analyzed for acidic drugs and beta-blockers in three samples from the Santa Ana River, located in Orange County, CA. The river consists mainly of wastewater effluent during the dry season. One sample was collected from above the Prado engineered
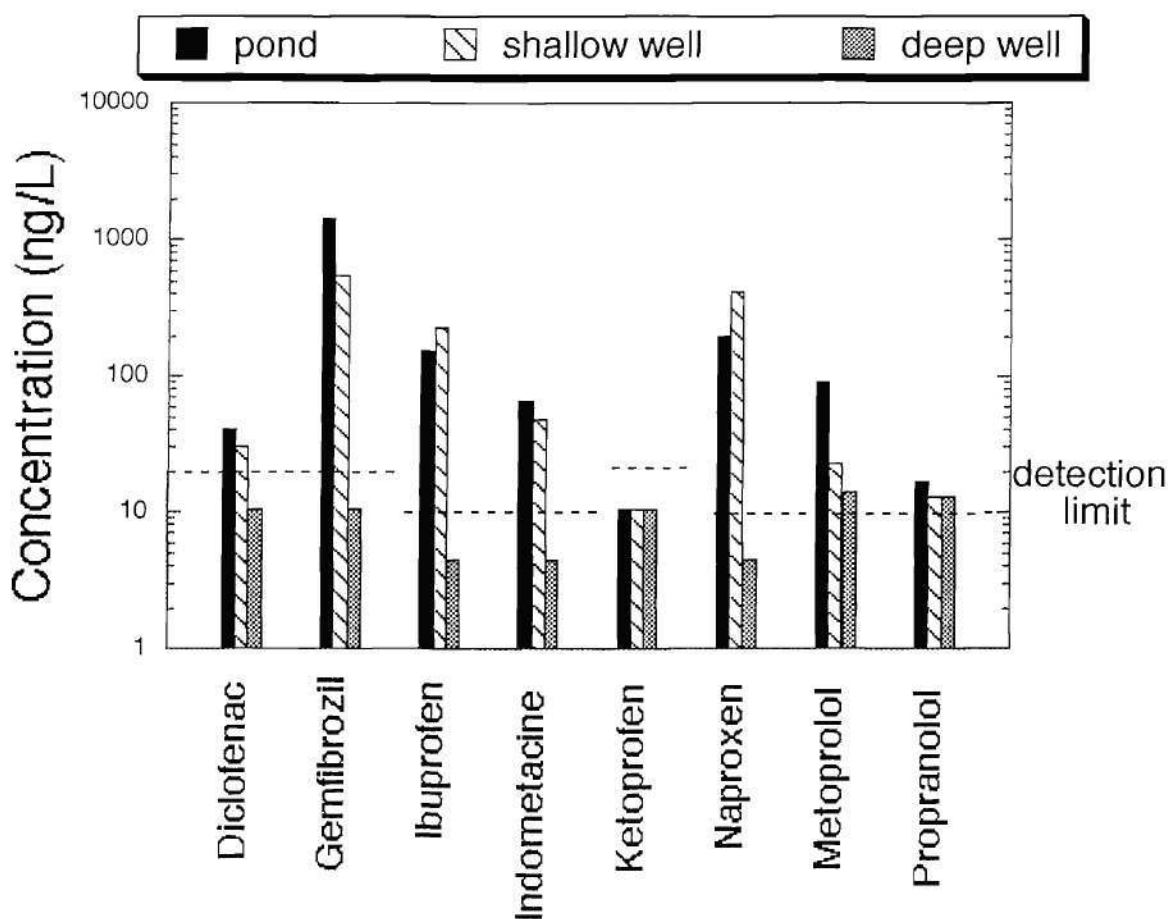
Figure 11: Concentrations of acidic drugs and beta-blockers detected at the Sweetwater Groundwater recharge facility. Concentrations below the detection limit are plotted at half the detection limit.

wetland, one sample was collected within the wetland and one sample was collected below the wetland. Gemfibrozil, metoprolol and propranolol were the only compounds detected (Appendix A) and concentrations were only slightly higher than the limit of quantification. The relatively low concentrations detected in these samples may be attributable to the dilution of wastewater effluent with groundwater and surface runoff.

The results of antibiotics obtained during the sixth project period are shown in Figures 12-18 and are summarized in the Appendix B. Unless stated otherwise, antibiotic concentrations were determined by standard addition method and were not corrected by recovery. Although

these data were collected using the accepted analytical method, a complete QA/QC plan was not followed (e.g., lab blanks were analyzed instead of field blanks). Therefore, these data are considered preliminary and useful for screening purposes. In general, sulfamethoxazole, trimethoprim, ciprofloxacin and ofloxacin were detected in most samples. Sulfamethazine and enrofloxacin was detected in samples from Mt. View WWTP/engineered wetland, FWH AWWTP and Sweetwater Recharge Facility. Norfloxacin was detected in samples from Mt. View WWTP/engineered wetland and FWH AWWTP.

Figure 12. Occurrence of sulfamethoxazole in wastewater effluent during the 6th project period. Unless otherwise stated concentration is based on standard addition quantification. *: Concentration based on internal standard quantification.
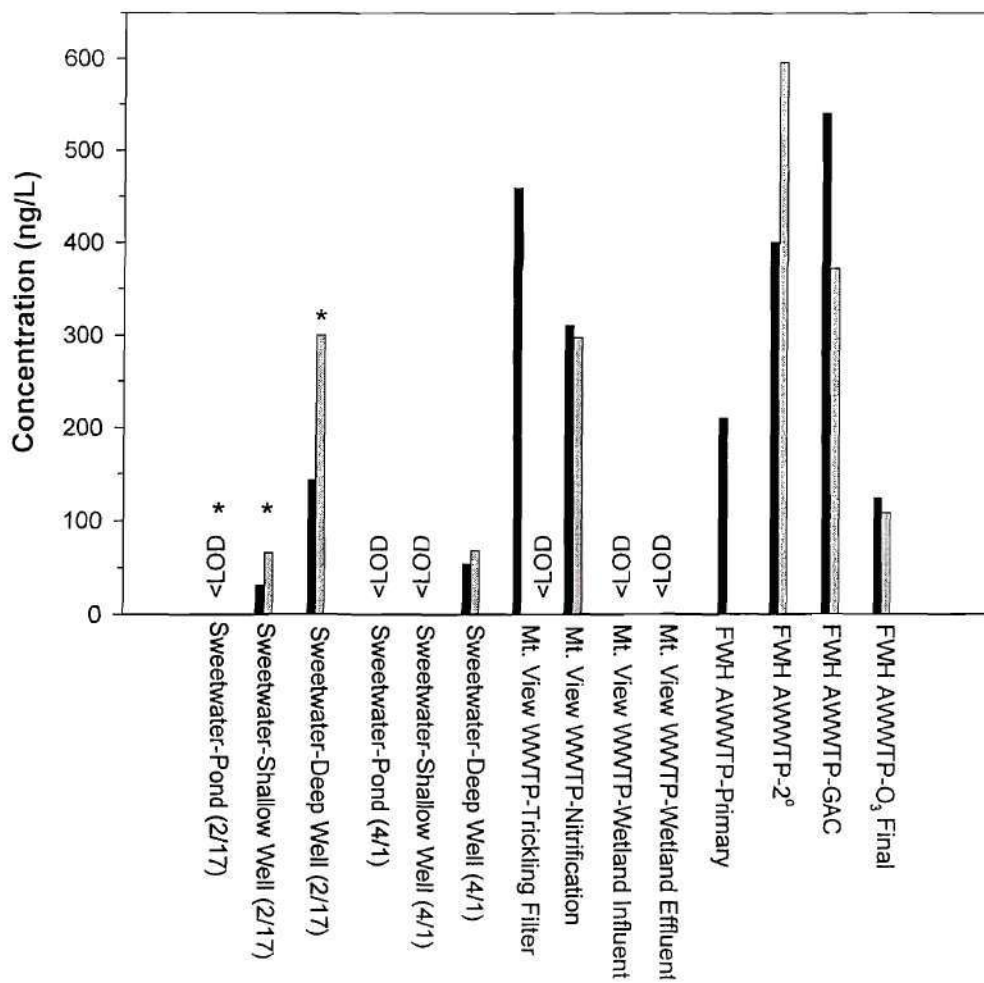
Figure 13. Occurrence of sulfamethazine in wastewater effluent during the 6th project period. Unless otherwise stated concentration is based on standard addition quantification. *: Concentration based on internal standard quantification.
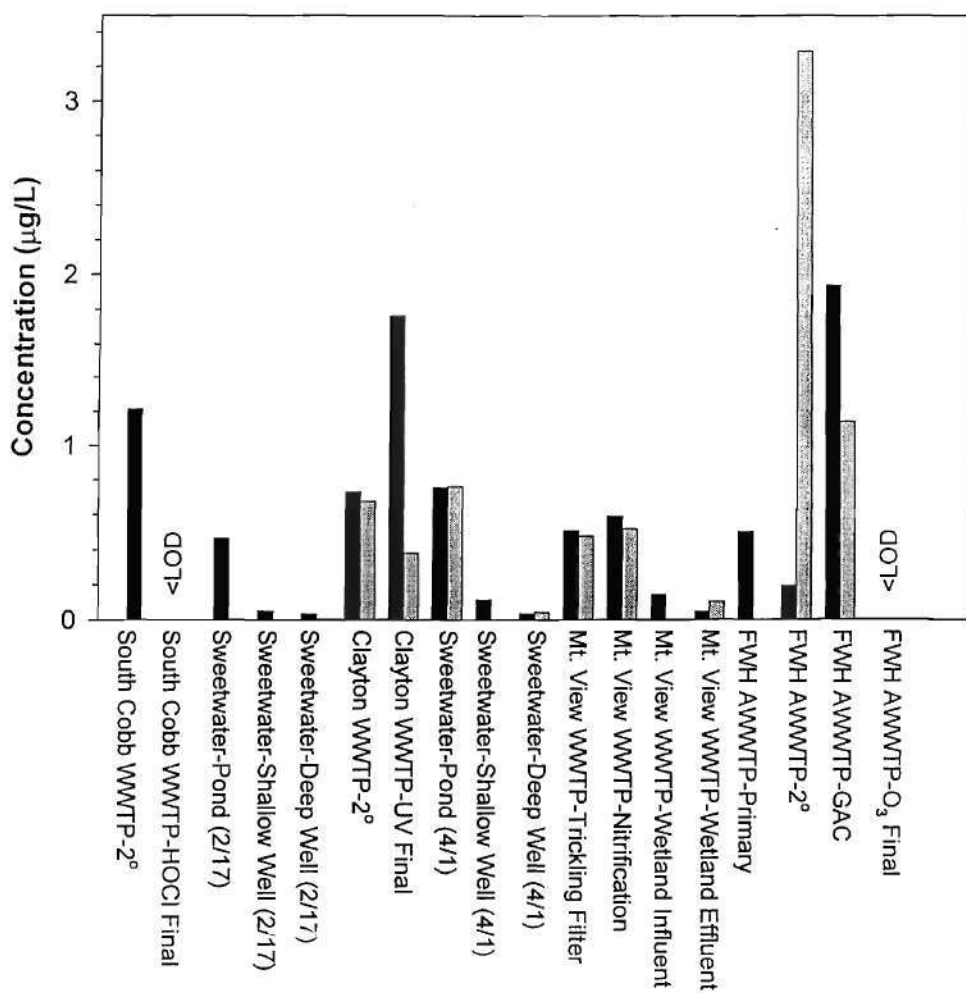
Figure 14. Occurrence of trimethoprim in wastewater effluent during the 6th project period. Unless otherwise stated concentration is based on standard addition quantification. *: Concentration based on internal standard quantification.
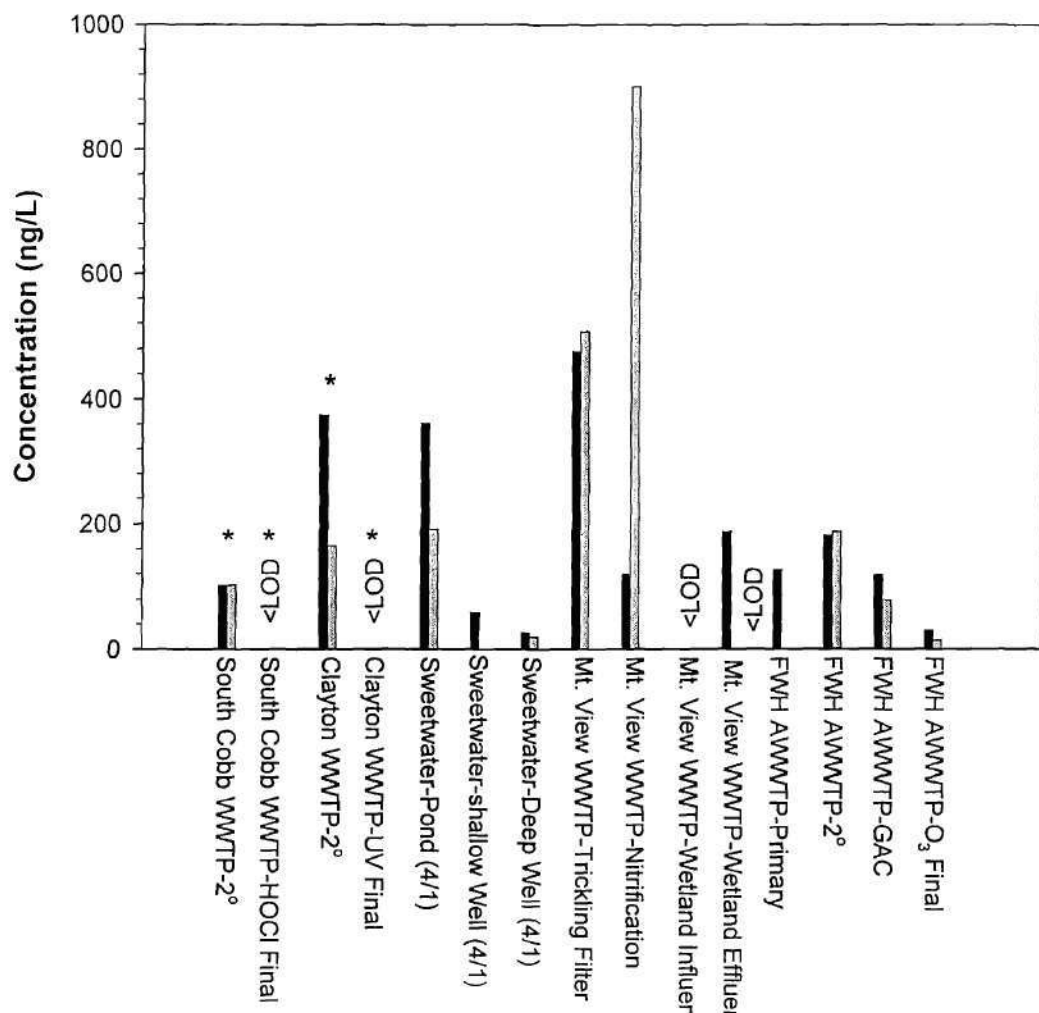
Figure 15. Occurrence of ciprofloxacin in wastewater effluent during the 6th project period. Unless otherwise stated concentration is based on standard addition quantification. *: Concentration based on internal standard quantification.
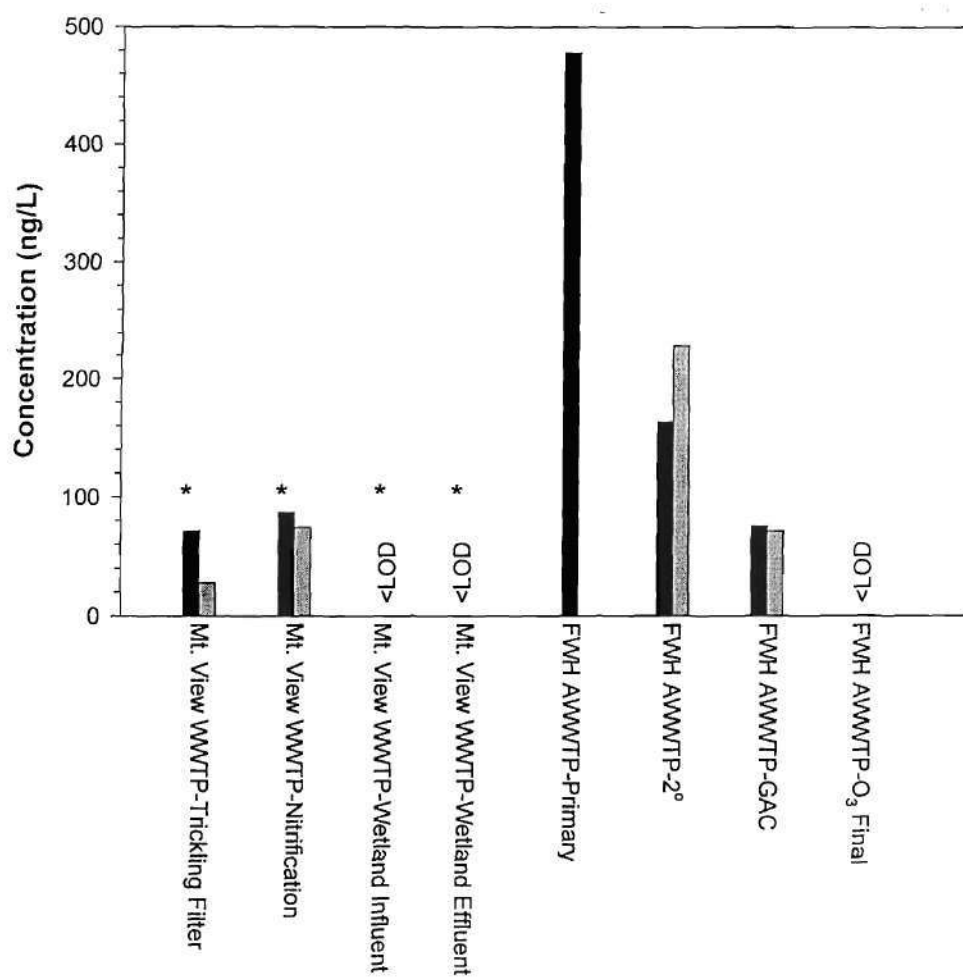
Figure 16. Occurrence of norfloxacin in wastewater effluent during the 6th project period. Unless otherwise stated concentration is based on standard addition quantification. *: Concentration based on internal standard quantification.
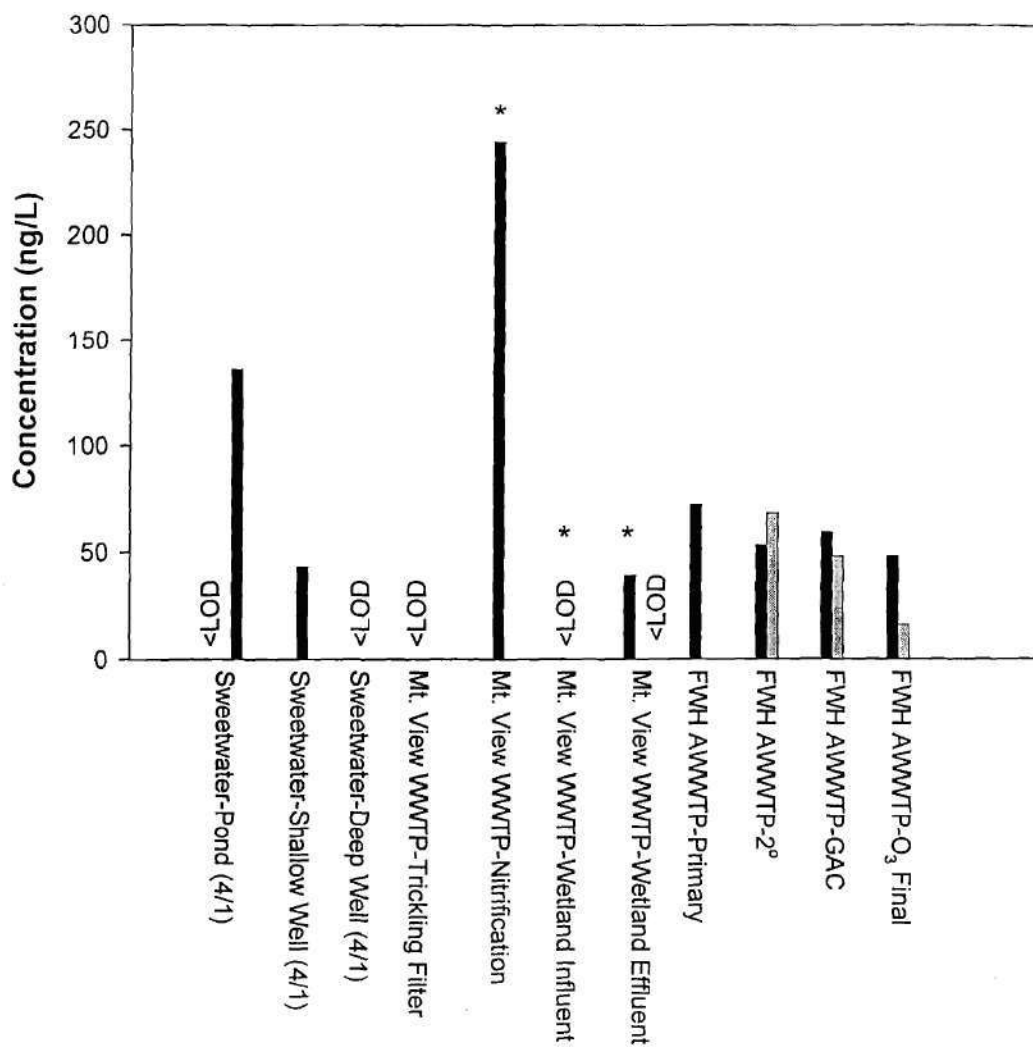
Figure 17. Occurrence of enrofloxacin in wastewater effluent during the 6th project period. Unless otherwise stated concentration is based on standard addition quantification. *: Concentration based on internal standard quantification.
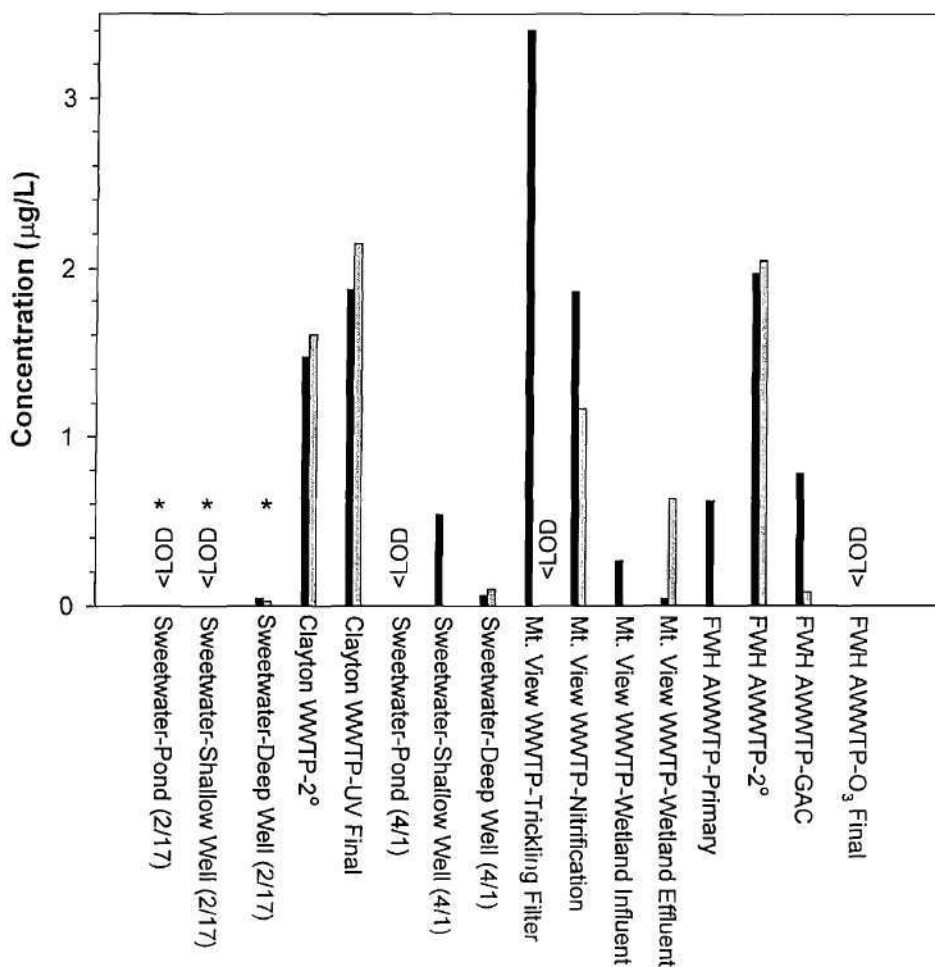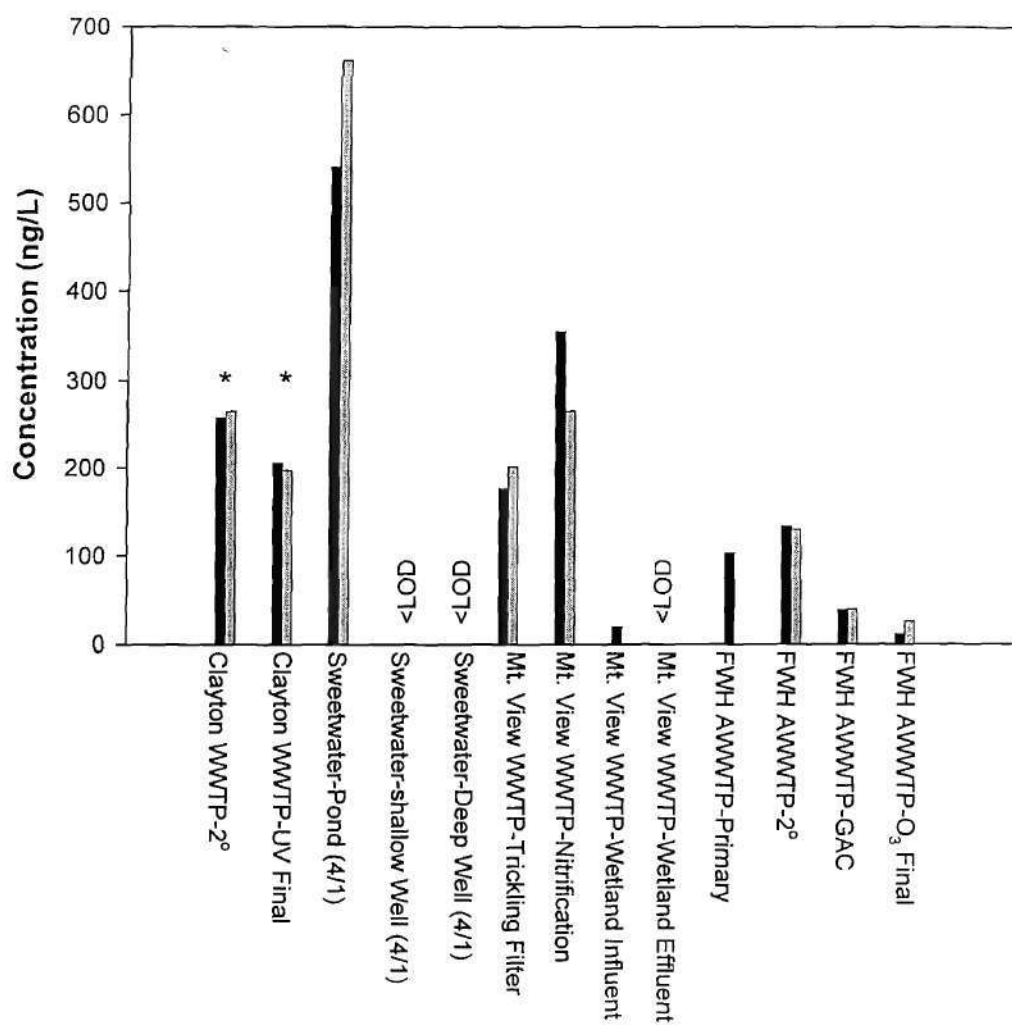
Figure 18. Occurrence of ofloxacin in wastewater effluent during the 6th project period. Unless otherwise stated concentration is based on standard addition quantification. *: Concentration based on internal standard quantification.

Based upon the preliminary occurrence results, several important observations can be summarized:

- Concentrations of antibiotics in the effluent of tricking filters or activated sludge are relatively high and the concentrations of fluoroquinolones are generally lower than those of sulfonamides (i.e., 1470-3400 ng/L for sulfamethoxazole, 470-3290 ng/L for trimethoprim, 180-510 ng/L for ciprofloxacin, 130-660 ng/L for ofloxacin, and 90-230 ng/L for norfloxacin).

- Veterinary antibiotics (sulfamathazine and enrofloxacin) are detected in the municipal wastewater samples, but at considerably lower concentrations than those of human health antibiotics (i.e., 400-600 ng/L for sulfamethazine and 50-230 ng/L for enrofloxacin in the effluent of trickling filters or activated sludge).

- Concentrations of antibiotics were high in the infiltration basin of the Sweetwater Recharge Facility. Sulfamethoxazole and sulfamethazine concentrations were not determined due to matrix interference. Concentrations of antibiotics were lower in the shallow well and were further decreased in the deep well, indicating that antibiotics were removed by soil infiltration. Despite the significant removal of antibiotics by soil infiltration, antibiotics can still be detected at low concentrations in the deep well samples.

- At the Mt. View WWTP/engineered wetland, concentrations of antibiotics did not appear to be lowered by nitrification following the trickling filters. Sulfamethoxazole and trimethoprim were detected in the wetland influent (260 ng/L and 140 ng/L, respectively) and their concentrations were approximately unchanged in the wetland effluent.

- The results from the three WWTPs (South Cobb, Clayton and FWH) suggest that chlorination may remove trimethoprim and ciprofloxacin. However, the samples were not quenched upon sample collection and then analyses must be repeated. UV disinfection appears to be ineffective in removing sulfonamides but may eliminate fluoroquinolones, GAC appears to remove significant amounts of antibiotics but its success may still be limited, and ozonation following GAC may effectively reduce the antibiotic concentrations.

These observations are provided to guide the PAC in their evaluation of the data and require confirmatory sampling prior to drawing any conclusions about antibiotic fate.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task were conducted during this project period.

PLANS FOR NEXT PERIOD

The following section describes research planned during the next project period. A revised schedule for the project is presented in Appendix E.

Task 1: Literature Review

Sub-Task 1A: Drugs Used in Human Therapy

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Sub-Task 1B: Drugs Used in Animal Husbandry

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Task 2: Analytical Method Development and Testing

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

During the next project period no new research is planned is association with this task.

Sub-Task 2B: Antibiotics

The developed method for fluoroquinolone antibiotics was shown to be robust and reliable. Except for the assessment of lomefloxacin as an internal standard, no further method development is necessary. During the next project period we plan to continue applying this fluoroquinolone method in more sample matrices including wastewater and surface water samples to quantify the concentrations of ciprofloxacin, norfloxacin and enrofloxacin and to examine method recovery in various matrices. Unless we receive comments from the PAC

instructing us to substantially change the method, we will use the method described in this progress report as part of the occurrence survey.

Task 3: Occurrence Survey

Sub-Task 3A: Site Selection

We have chosen all of the sites to be included in the occurrence survey (Table 1). Additional sites will be included if time permits. No further activity is planned in association with this task.

Sub-Task 3B: Sample Collection and Analysis

As indicated in Table 1, we plan to collect and analyze samples from ten sites during the next project period. Samples will be analyzed for acidic drugs and beta-blockers using previously described procedures. Samples also will be analyzed for antibiotics using methods described in the previous section of this progress report. We will complete our second round of sampling at each site during the eighth project period and prepare the final report during the ninth project period.

Due to budget constraints, the project had planned for a 2-year participation in the study for Dr. Huang at Georgia Tech. With respect to antibiotics, the original scope of the study included literature review, method development and selected occurrence survey. Dr. Huang is currently preparing a proposal to be submitted to the Project Advisory Committee and the AWWARF to request project continuation funding. Further data related to the occurrence survey for antibiotics will be conducted upon the approval of the continuation funding.

Sub-Task 3C: Data Analysis and Synthesis

No activities related to this sub-task are planned during this project period. After completion of the occurrence survey, data will be evaluated to identify trends meriting further study. Data will be compared with expectations based on physical/chemical properties of the compounds as well as results reported by other researchers. As indicated in the fifth progress report, we plan to prepare manuscripts describing results of the occurrence survey and the new

analytical methods developed during this project for measurement of antibiotics. Preparation of these manuscripts will begin during the eighth project period.

APPENDIX A: Summary of Data for Acidic Drugs and Beta-Blockers during the Sixth Project Period

Compound	Location	Date	Concentration (ppt)
Diclofenac	Blank	3/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	43,39
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	46,42
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	<10,<10
	Dublin/San Ramon Effluent: post chlorination	3/21/02	<10,<10
	Sweetwater, Pond Water	4/1/02	41,40
	Sweetwater, Shallow Well	4/1/02	36,24
	Sweetwater, Deep Well	4/1/02	<19,<19
	Santa Ana River, Above Prado Wetlands	4/6/02	<19,<19
	Santa Ana River, Within Prado Wetlands	4/6/02	<19,<19
	Santa Ana River, Below Prado Wetlands	4/6/02	<19,<19
Gemfibrozil	Blank	3/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	270, 110
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	310, 320
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	2800, 2300
	Dublin/San Ramon Effluent: post chlorination	3/21/02	1700, 1600
	Sweetwater, Pond Water	4/1/02	1300, 1600
	Sweetwater, Shallow Well	4/1/02	590, 500
	Sweetwater, Deep Well	4/1/02	<19, <19
	Santa Ana River, Above Prado Wetlands	4/6/02	<19, <19
	Santa Ana River, Within Prado Wetlands	4/6/02	22,19
	Santa Ana River, Below Prado Wetlands	4/6/02	<19, <19
Ibuprofen	Blank	3/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	<10,<10
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	<10,<10
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	200, 50
	Dublin/San Ramon Effluent: post chlorination	3/21/02	160, 180
	Sweetwater, Pond Water	4/1/02	170, 140
	Sweetwater, Shallow Well	4/1/02	220, 230
	Sweetwater, Deep Well	4/1/02	<9, <9
	Santa Ana River, Above Prado Wetlands	4/6/02	<9, <9
	Santa Ana River, Within Prado Wetlands	4/6/02	<9, <9
	Santa Ana River, Below Prado Wetlands	4/6/02	<9, <9
Indometacine	Blank	3/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	20, 18
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	21, 20
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	50, 32
	Dublin/San Ramon Effluent: post chlorination	3/21/02	29, 40
	Sweetwater, Pond Water	4/1/02	69, 62
	Sweetwater, Shallow Well	4/1/02	56, 41
	Sweetwater, Deep Well	4/1/02	<9, <9
	Santa Ana River, Above Prado Wetlands	4/6/02	<9, <9
	Santa Ana River, Within Prado Wetlands	4/6/02	<9, <9
	Santa Ana River, Below Prado Wetlands	4/6/02	<9, <9

* Recovery of labeled mecoprop (internal standard) added to samples at 1,000 ng/L.

Compound	Location	Date	Concentration (ppt)
Ketoprofen	Blank	3/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	<10, <10
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	<10, <10
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	<10, <10
	Dublin/San Ramon Effluent: post chlorination	3/21/02	<10, <10
	Sweetwater, Pond Water	4/1/02	<19, <19
	Sweetwater, Shallow Well	4/1/02	<19, 19
	Sweetwater, Deep Well	4/1/02	<19, <19
	Santa Ana River, Above Prado Wetlands	4/6/02	<19, <19
	Santa Ana River, Within Prado Wetlands	4/6/02	<19, <19
	Santa Ana River, Below Prado Wetlands	4/6/02	<19, <19
Naproxen	Blank	3/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	220, 160
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	200, 200
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	1900, 1500
	Dublin/San Ramon Effluent: post chlorination	3/21/02	1400, 1400
	Sweetwater, Pond Water	4/1/02	230, 170
	Sweetwater, Shallow Well	4/1/02	480, 340
	Sweetwater, Deep Well	4/1/02	<9, <9
	Santa Ana River, Above Prado Wetlands	4/6/02	<9, <9
	Santa Ana River, Within Prado Wetlands	4/6/02	<9, <9
	Santa Ana River, Below Prado Wetlands	4/6/02	<9, <9
Mecoprop*	Blank	3/26/02	4%
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	77%, 57%
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	71%, 62%
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	118%, 116%
	Dublin/San Ramon Effluent: post chlorination	3/21/02	82%, 78%
	Sweetwater, Pond Water	4/1/02	7%, 4%
	Sweetwater, Shallow Well	4/1/02	67%, 56%
	Sweetwater, Deep Well	4/1/02	57%, 64%
	Santa Ana River, Above Prado Wetlands	4/6/02	68%, 21%
	Santa Ana River, Within Prado Wetlands	4/6/02	33%, 16%
	Santa Ana River, Below Prado Wetlands	4/6/02	38%, 59%

* Recovery of labeled mecoprop (internal standard) added to samples at 1,000 ng/L.

Compound					Recovery (%)
Metoprolol	Blank	3/26/02	<10		76%
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	66		
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	72, 80		
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	<10, <10		
	Dublin/San Ramon Effluent: post chlorination	3/21/02	83, 59		
	Sweetwater, Pond Water	4/1/02	73, 110		
	Sweetwater, Shallow Well	4/1/02	25, 20		
	Sweetwater, Deep Well	4/1/02	14		67%
	Santa Ana River, Above Prado Wetlands	4/6/02	17, 17		
	Santa Ana River, Within Prado Wetlands	4/6/02	16, <10		
	Santa Ana River, Below Prado Wetlands	4/6/02	16, 17		
	Blank	4/6/02	< 10		60%
Propranolol	Blank	3/26/02	<10		65%
	San Jose/Santa Clara Effluent: pre chlorination	3/26/02	64		
	San Jose/Santa Clara Effluent: post chlorination	3/26/02	52, 54		
	Dublin/San Ramon Effluent: pre chlorination	3/21/02	<10, <10		
	Dublin/San Ramon Effluent: post chlorination	3/21/02	<10, <10		
	Sweetwater, Pond Water	4/1/02	16, 17		
	Sweetwater, Shallow Well	4/1/02	14, 12		
	Sweetwater, Deep Well	4/1/02	13		70%
	Santa Ana River, Above Prado Wetlands	4/6/02	12, 12		
	Santa Ana River, Within Prado Wetlands	4/6/02	12, <10		
	Santa Ana River, Below Prado Wetlands	4/6/02	<10, 13		
	Blank	4/6/02	< 10		54%

APPENDIX B: Summary of Data for Antibiotics during the Sixth Project Period

Compound	Location	Date	Concentration $(\mu g/L)^{(1)}$	Concentration $(\mu g/L)^{(2)}$	Spike Recovery
Sulfamethoxazole	South Cobb WWTP – Secondary	2/15/2002	<LOD, <LOD	NA	88%, 92%
	South Cobb WWTP – HOCl Final	2/15/2002	<LOD, <LOD	NA	56%, 63%
	DI Water Sample	2/15/2002	NA	NA	122%
	Sweetwater Recharge – Pond	2/17/2002	<LOD, <LOD	NA	29% [n]
	Sweetwater Recharge – Shallow Well	2/17/2002	<LOD, <LOD	NA	24%
	Sweetwater Recharge – Deep Well	2/17/2002	0.04, 0.03	NA	73%
	DI Water Sample	2/17/2002	NA	NA	64%
	Clayton WWTP – Secondary	3/19/2002	2.15, 2.02	1.47, 1.60	81%, 84%
	Clayton WWTP – UV Final	3/19/2002	2.27, 2.22	1.87, 2.14	84%, 79%
	DI Water Sample	3/19/2002	NA	NA	76%
	Sweetwater Recharge – Pond	4/1/2002	<LOD, <LOD	<LOD, <LOD	0% [n]
	Sweetwater Recharge – Shallow Well	4/1/2002	0.60	0.54	68%
	Sweetwater Recharge – Deep Well	4/1/2002	0.02, <LOD	0.06, 0.10	62%
	DI Water Sample	4/1/2002	NA	NA	65%
	Mt. View WWTP – Trickling Filter	4/9/2002	7.25, 6.10	3.40	68%
	Mt. View WWTP – Nitrification	4/9/2002	7.16, 1.08	1.85, 1.17	120%
	Mt. View WWTP – Wetland Influent	4/9/2002	0.35	0.26	57%
	Mt. View WWTP – Wetland Effluent	4/9/2002	0.59, <LOD	0.04, 0.63	65%
	FWH AWWTP – Primary	4/22/2002	0.85	0.62	76%
	FWH AWWTP – Secondary	4/22/2002	1.54, 1.47	1.97, 2.04	70%
	FWH AWWTP – GAC	4/22/2002	0.12, 0.12	0.08, 0.08	54%
	FWH AWWTP – O₃ Final	4/22/2002	<LOD, <LOD	<LOD, <LOD	76%
Sulfamethazine	South Cobb WWTP – Secondary	2/15/2002	<LOD, <LOD	NA	54%, 48%
	South Cobb WWTP – HOCl Final	2/15/2002	<LOD, <LOD	NA	67%, 71%
	DI Water Sample	2/15/2002	NA	NA	104%
	Sweetwater Recharge – Pond	2/17/2002	<LOD, <LOD	NA	20% [n]
	Sweetwater Recharge – Shallow Well	2/17/2002	0.03, 0.07	NA	0% [n]
	Sweetwater Recharge – Deep Well	2/17/2002	0.14, 0.30	NA	98%
	DI Water Sample	2/17/2002	NA	NA	102%
	Clayton WWTP – Secondary	3/19/2002	<LOD, <LOD	NA	23%, 31%
	Clayton WWTP – UV Final	3/19/2002	<LOD, <LOD	NA	69%, 76%
	DI Water Sample	3/19/2002	NA	NA	96%
	Sweetwater Recharge – Pond	4/1/2002	<LOD, <LOD	<LOD, <LOD	0% [n]
	Sweetwater Recharge – Shallow Well	4/1/2002	<LOD	<LOD	15% [n]
	Sweetwater Recharge – Deep Well	4/1/2002	<LOD, <LOD	0.05, 0.07	107%
	DI Water Sample	4/1/2002	NA	NA	93%
	Mt. View WWTP – Trickling Filter	4/9/2002	0.05, 0.80	0.46	55%
	Mt. View WWTP – Nitrification	4/9/2002	0.75, 0.08	0.31, 0.30	38% [n]
	Mt. View WWTP – Wetland Influent	4/9/2002	<LOD	<LOD	53%
	Mt. View WWTP – Wetland Effluent	4/9/2002	<LOD, <LOD	<LOD, <LOD	63%
	FWH AWWTP – Primary	4/22/2002	0.67	0.21	97%
	FWH AWWTP – Secondary	4/22/2002	1.02, 0.75	0.40, 0.60	55%
	FWH AWWTP – GAC	4/22/2002	1.27, 0.94	0.54, 0.37	98%
	FWH AWWTP – O₃ Final	4/22/2002	0.47, 0.40	0.12, 0.11	102%

Compound	Location	Date	Concentration $(\mu g/L)^{(1)}$	Concentration $(\mu g/L)^{(2)}$	Spike Recovery
Trimethoprim	South Cobb WWTP – Secondary	2/15/2002	1.32, 1.23	1.21	95%, 118%
	South Cobb WWTP – HOCl Final	2/15/2002	<LOD, <LOD	<LOD	145%, 165%
	DI Water Sample	2/15/2002	NA	NA	73%
	Sweetwater Recharge – Pond	2/17/2002	1.73, 1.44	0.47	154% [b]
	Sweetwater Recharge – Shallow Well	2/17/2002	0.14, <LOD	0.05	95%
	Sweetwater Recharge – Deep Well	2/17/2002	0.19, 0.19	0.03	130%
	DI Water Sample	2/17/2002	NA	NA	102%
	Clayton WWTP – Secondary	3/19/2002	1.61, 1.75	0.73, 0.68	93%, 150% [b]
	Clayton WWTP – UV Final	3/19/2002	3.85, 1.30	1.76, 0.38	156%, 103% [b]
	DI Water Sample	3/19/2002	NA	NA	93%
	Sweetwater Recharge – Pond	4/1/2002	3.43, 3.71	0.75, 0.76	91%
	Sweetwater Recharge – Shallow Well	4/1/2002	0.21	0.11	140% [b]
	Sweetwater Recharge – Deep Well	4/1/2002	<LOD, <LOD	0.03, 0.04	524% [b]
	DI Water Sample	4/1/2002	NA	NA	91%
	Mt.View WWTP – Trickling Filter	4/9/2002	4.61, 2.74	0.51, 0.47	135%
	Mt.View WWTP – Nitrification	4/9/2002	3.38, 0.51	0.59, 0.52	116%
	Mt.View WWTP – Wetland Influent	4/9/2002	0.58	0.14	323% [b]
	Mt.View WWTP – Wetland Effluent	4/9/2002	0.12, <LOD	0.05, 0.10	313% [b]
	FWH AWWTP – Primary	4/22/2002	0.40	0.50	40% [a]
	FWH AWWTP – Secondary	4/22/2002	3.40, 1.14	0.49, 3.29	86%
	FWH AWWTP – GAC	4/22/2002	3.00, 3.96	1.93, 1.14	118%
	FWH AWWTP – O₃ Final	4/22/2002	1.14, 2.21	<LOD, <LOD	226% [b]
Ciprofloxacin	South Cobb WWTP – Secondary	2/15/2002	0.10, 0.10	NA	87%, 103%
	South Cobb WWTP – HOCl Final	2/15/2002	<LOD, <LOD	NA	75%, 67%
	DI Water Sample	2/15/2002	NA	NA	103%
	Sweetwater Recharge – Pond	2/17/2002	<LOD, <LOD	NA	108%
	Sweetwater Recharge – Shallow Well	2/17/2002	<LOD, <LOD	NA	89%
	Sweetwater Recharge – Deep Well	2/17/2002	<LOD, <LOD	NA	99%
	DI Water Sample	2/17/2002	NA	NA	103%
	Clayton WWTP – Secondary	3/19/2002	0.37, 0.17	NA	106%, 140% [b]
	Clayton WWTP – UV Final	3/19/2002	<LOD, <LOD	NA	96%, 110%
	DI Water Sample	3/19/2002	NA	NA	106%
	Sweetwater Recharge – Pond	4/1/2002	0.61, 0.34	0.36, 0.19	88%
	Sweetwater Recharge – Shallow Well	4/1/2002	0.11	0.06	78%
	Sweetwater Recharge – Deep Well	4/1/2002	<LOD, <LOD	0.03, 0.02	101%
	DI Water Sample	4/1/2002	NA	NA	103%
	Mt.View WWTP – Trickling Filter	4/9/2002	0.68, 0.68	0.48, 0.51	102%
	Mt.View WWTP – Nitrification	4/9/2002	0.90, 0.15	0.12, 0.90	90%
	Mt.View WWTP – Wetland Influent	4/9/2002	<LOD	<LOD	93%
	Mt.View WWTP – Wetland Effluent	4/9/2002	0.15, <LOD	0.19, <LOD	99%
	FWH AWWTP – Primary	4/22/2002	0.54	0.13	124%
	FWH AWWTP – Secondary	4/22/2002	0.26, 0.35	0.18, 0.19	130%
	FWH AWWTP – GAC	4/22/2002	0.11, 0.17	0.12, 0.08	92%
	FWH AWWTP – O₃ Final	4/22/2002	0.04, 0.02	0.03, 0.01	161% [b]

Compound	Location	Date	Concentration $(\mu g/L)^{(1)}$	Concentration $(\mu g/L)^{(2)}$	Spike Recovery
Norfloxacin	South Cobb WWTP – Secondary	2/15/2002	<LOD, <LOD	NA	103%, 91%
	South Cobb WWTP – HOCl Final	2/15/2002	<LOD, <LOD	NA	53%, 61%
	DI Water Sample	2/15/2002	NA	NA	99%
	Sweetwater Recharge – Pond	2/17/2002	<LOD, <LOD	NA	114%
	Sweetwater Recharge – Shallow Well	2/17/2002	<LOD, <LOD	NA	98%
	Sweetwater Recharge – Deep Well	2/17/2002	<LOD, <LOD	NA	101%
	DI Water Sample	2/17/2002	NA	NA	100%
	Clayton WWTP – Secondary	3/19/2002	<LOD, <LOD	NA	116%, 110%
	Clayton WWTP – UV Final	3/19/2002	<LOD, <LOD	NA	110%, 109%
	DI Water Sample	3/19/2002	NA	NA	99%
	Sweetwater Recharge – Pond	4/1/2002	<LOD, <LOD	<LOD, <LOD	168% [b]
	Sweetwater Recharge – Shallow Well	4/1/2002	<LOD	<LOD	93%
	Sweetwater Recharge – Deep Well	4/1/2002	<LOD, <LOD	<LOD, <LOD	95%
	DI Water Sample	4/1/2002	NA	NA	101%
	Mt. View WWTP – Trickling Filter	4/9/2002	0.07, 0.03	0.13, 0.09	93%
	Mt. View WWTP – Nitrification	4/9/2002	0.09, 0.07	NA	100%
	Mt. View WWTP – Wetland Influent	4/9/2002	<LOD	<LOD	95%
	Mt. View WWTP – Wetland Effluent	4/9/2002	<LOD, <LOD	<LOD, <LOD	110%
	FWH AWWTP – Primary	4/22/2002	2.41	0.48	105%
	FWH AWWTP – Secondary	4/22/2002	0.18, 0.33	0.16, 0.23	81%
	FWH AWWTP – GAC	4/22/2002	0.07, 0.07	0.08, 0.07	115%
	FWH AWWTP – O₃ Final	4/22/2002	<LOD, <LOD	<LOD, <LOD	105%
Enrofloxacin	South Cobb WWTP – Secondary	2/15/2002	<LOD, <LOD	NA	89%, 87%
	South Cobb WWTP – HOCl Final	2/15/2002	<LOD, <LOD	NA	57%, 65%
	DI Water Sample	2/15/2002	NA	NA	101%
	Sweetwater Recharge – Pond	2/17/2002	<LOD, <LOD	NA	72%
	Sweetwater Recharge – Shallow Well	2/17/2002	<LOD, <LOD	NA	83%
	Sweetwater Recharge – Deep Well	2/17/2002	<LOD, <LOD	NA	99%
	DI Water Sample	2/17/2002	NA	NA	87%
	Clayton WWTP – Secondary	3/19/2002	<LOD, <LOD	NA	94%, 101%
	Clayton WWTP – UV Final	3/19/2002	<LOD, <LOD	NA	86%, 88%
	DI Water Sample	3/19/2002	NA	NA	100%
	Sweetwater Recharge – Pond	4/1/2002	0.26, <LOD	0.14, <LOD	73%
	Sweetwater Recharge – Shallow Well	4/1/2002	0.08	0.04	79%
	Sweetwater Recharge – Deep Well	4/1/2002	<LOD, <LOD	<LOD, <LOD	102%
	DI Water Sample	4/1/2002	NA	NA	103%
	Mt. View WWTP – Trickling Filter	4/9/2002	<LOD, <LOD	<LOD, <LOD	101%
	Mt. View WWTP – Nitrification	4/9/2002	0.24, <LOD	0.23, <LOD	92%
	Mt. View WWTP – Wetland Influent	4/9/2002	<LOD	<LOD	108%
	Mt. View WWTP – Wetland Effluent	4/9/2002	0.04, <LOD	0.08, <LOD	123%
	FWH AWWTP – Primary	4/22/2002	0.11	0.07	103%
	FWH AWWTP – Secondary	4/22/2002	0.06, 0.09	0.05, 0.06	146%
	FWH AWWTP – GAC	4/22/2002	0.08, 0.07	0.06, 0.05	98%
	FWH AWWTP – O₃ Final	4/22/2002	0.06, 0.02	0.05, 0.02	152% [b]

Compound	Location	Date	Concentration (µg/L)[1]	Concentration (µg/L)[2]	Spike Recovery
Ofloxacin	South Cobb WWTP – Secondary	2/15/2002	<LOD, <LOD	NA	138 %, 124 %
	South Cobb WWTP – HOCl Final	2/15/2002	<LOD, <LOD	NA	87 %, 98 %
	DI Water Sample	2/15/2002	NA	NA	101%
	Sweetwater Recharge – Pond	2/17/2002	<LOD, <LOD	NA	159%
	Sweetwater Recharge – Shallow Well	2/17/2002	<LOD, <LOD	NA	109%
	Sweetwater Recharge – Deep Well	2/17/2002	<LOD, <LOD	NA	115%
	DI Water Sample	2/17/2002	NA	NA	104%
	Clayton WWTP – Secondary	3/19/2002	0.26, 0.26	NA	134%, 123%
	Clayton WWTP – UV Final	3/19/2002	0.21, 0.20	NA	125%, 116%
	DI Water Sample	3/19/2002	NA	NA	99%
	Sweetwater Recharge – Pond	4/1/2002	1.14, 1.34	0.54, 0.66	113%
	Sweetwater Recharge – Shallow Well	4/1/2002	<LOD, <LOD	<LOD, <LOD	105%
	Sweetwater Recharge – Deep Well	4/1/2002	<LOD, <LOD	<LOD, <LOD	106%
	DI Water Sample	4/1/2002	NA	NA	103%
	Mt. View WWTP – Trickling Filter	4/9/2002	0.59, 0.28	0.18, 0.20	162% [b]
	Mt. View WWTP – Nitrification	4/9/2002	0.48, 0.34	0.35, 0.26	161% [b]
	Mt. View WWTP – Wetland Influent	4/9/2002	<LOD	0.02	124%
	Mt. View WWTP – Wetland Effluent	4/9/2002	<LOD, <LOD	<LOD, <LOD	133%
	FWH AWWTP – Primary	4/22/2002	1.45	0.10	203% [b]
	FWH AWWTP – Secondary	4/22/2002	0.37, 0.47	0.13, 0.13	138%
	FWH AWWTP – GAC	4/22/2002	0.08, 0.08	0.04, 0.04	100%
	FWH AWWTP – O₃ Final	4/22/2002	0.04, <LOD	0.01, 0.03	172% [b]

Note: The reported concentrations were not corrected by recoveries. Unless specified, spike concentration is 1.0 µg/L. Spike recoveries based on internal standard quantification.

(1) Quantification based on internal standard method

(2) Quantification based on standard addition method

(a) Compound quantification hampered by matrix interference
(b) Recovery overestimated due to problems differences with the internal standard in the amount of signal suppression

APPENDIX C: Analytical Method for Antibiotics

Sample Preparation:

Wastewater samples were collected in 1-L amber glass bottles. The samples were then filtered through 0.5-μm glass fiber filters (Pall, Ann Arbor, MI). 0.1 M of NaCl was added. The samples were then acidified to pH 2.5.

Solid Phase Extraction:

Each 1-L sample was extracted through a 500-mg anion exchanger (Isolute, Mid Glamorgan, U.K.) stacked on top of a 500-mg hydrophilic-lipophilic balance HLB cartridge (Waters, Taunton, MA). Both cartridges were pre-conditioned with 6 mL of methanol followed by 6 mL of 4.38 mM H_3PO_4. The 1-L samples were extracted at a rate of ~6mL/min. The HLB cartridges were eluted with 10 mL of 95% methanol/5% 4.38 mM H_3PO_4. The analytes were eluted into high-density polyethylene conical test tubes (Becton Dickinson, Franklin Lakes, NJ). 10 μL of 100 mg/L of both sulfamerazine and lomefloxacin were added to the analytes. The analytes were then blown down in a water bath at 30 °C using nitrogen. The analytes were reconstituted in 1 mL of 20% methanol /80% 4.38 mM H_3PO_4. One unspiked sample from each matrix was divided into two parts after reconstitution. For one half of the sample, a known amount of each antibiotic was added. This sample was used for standard addition quantification. No antibiotics were added to the other half. All samples were then transferred into amber vials for LC/MS analysis.

LC/MS Analysis:

Analytes were injected into an Agilent 1100 series HPLC (Palo Alto, CA). A 2.1x150 mm 5-micron Zorbax SB-C18 column (Agilent, Palo Alto, CA) was used to separate the analytes. The column temperature was set at 30 °C. Two mobile phases were used. Mobile phase A contained 0.002% glacial acetic acid and 10% acetonitrile. Mobile phase B was 100% acetonitrile. A flowrate of 0.25 mL/min was used. The mobile phase gradient for this method is shown in Table C.1. After the gradient was complete, the column was flushed with 100% B for 10 minutes. A post-time of 15 minutes was used to allow the column to equilibrate before the next sample injection.

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Table C.1. Mobile Phase gradient for LC/MS analytical method.

Time (min)	B (%)
0	0
2	0
8	8.5
20	18
25	50
30	100

Ions of the analytes were detected using a HP1100 Series MSD (Agilent, Palo Alto, CA). Positive mode electrospray ionization and selected ion-monitoring were used. In order to avoid excessive cleaning of the capillary, the MSD was only operated in the period of 6 minutes to 27 minutes after sample injection. Analytes were detected at a fragmentor voltage of 85 V and 120 V. The run at the higher fragmentor voltage provided additional confirmation of the presence of the antibiotics. The retention times, molecular ions and confirming ions for the antibiotics are shown in Table C.2. The relative abundance of the ions at the two fragmentor voltages used are shown in Table C.3. Several additional MSD parameters are shown in Table C.4.

The antibiotics are quantified using the standard addition method or the internal standard method. The molecular ion of each antibiotic is used in both quantification techniques.

Table C.2. The retention time, molecular ion and fragment ions of antibiotics.

Antibiotic	Retention Time (min)	[MH]+ ion	Confirming ion 2	Confirming ion 3
Norfloxacin	16.3	320	302	276
Ciprofloxacin	17.4	332	314	288
Ofloxacin	16.3	362	318	261
Enrofloxacin	20.4	360	342	316
Lomefloxacin	18.1	352	334	
Sulfamerazine	9.7	265	156	
Sulfamethazine	12.4	279	156	
Trimethoprim	14	291	261	
Sulfamethoxazole	18.4	254	156	

Table C.3. The relative abundance of molecular and confirming ions for the two fragmentor voltages.

	[MH]+ ion Relative Abundance (%)		Confirming ion 2 Relative Abundance (%)		Confirming ion 3 Relative Abundance (%)	
	Fragmentor = 85 V	Fragmentor =120 V	Fragmentor = 85 V	Fragmentor =120 V	Fragmentor = 85 V	Fragmentor =120 V
Norfloxacin	100	97	5	100	13	90
Ciprofloxacin	100	100	4	71	9	73
Ofloxacin	100	92	10	100	1	33
Enrofloxacin	100	100	1	36	9	90
Lomefloxacin	100	100	2	25		
Sulfamerazine	100	52	15	100		
Sulfamethazine	100	100	5	71		
Trimethoprim	100	100	1	9		
Sulfamethoxazole	100	37	41	100		

Table C.4. Additional MSD Parameters.

Drying Gas Flowrate (mL/min)	10
Drying Gas Temperature (°C)	350
Nebulizer Pressure (psig)	30
Capillary Voltage (V)	3500

APPENDIX D: QA/QC Plan for Antibiotics

Sample Collection: Grab samples will be collected in 1-L amber glass bottles with Teflon-lined screw caps. Each bottle will be kept in an individual polyethylene bag. Prior to sampling, bottles will be cleaned in our laboratory with Micro brand laboratory detergent, rinsed with water followed by methanol and deionized water between each analysis. Bottles will be shipped to participants in coolers with blue ice packs.

For samples collected from wastewater treatment plants or water treatment plants using chlorine for disinfection, $Na_2S_2O_3$ will be added to the samples bottle as a preservative. Each set of samples will be shipped with a field blank, which will be analyzed with the samples. Samples will be collected by field personnel who are familiar with trace organic sampling protocols. Field personnel will wear polyethylene gloves when handling bottles and will be instructed to minimize the amount of time that the bottle is kept uncapped outside of the cooler. Sampling times, locations and personnel will be recorded on a log sheet that will accompany each set of samples. Samples will be shipped in the cooler via overnight mail. Upon arrival at Georgia Tech, samples will be visually inspected and stored in a 4°C refrigerator. Samples will be extracted as soon as practical and within no more than 48 hours after arrival.

Sample Extraction and Analysis: Each set of ten samples will be analyzed in a batch that contains appropriate QA/QC standards. The following samples will be included with each set of samples.

(1) Field blank (1 L of deionized water that travels to and from the field site);
(2) Matrix recovery sample (1 sample from the site spiked with each analyte at a concentration of 1.0 μg/L);
(3) Duplicate sample;
(4) Auxiliary standard consisting of a mixture of the antibiotic analytes prepared in our laboratory.

After SPE extraction and blowdown, samples will be spiked with the internal standard lomefloxacin and sulfamerazine for the purpose of evaluating the LC/MS performance in each run. The antibiotics will be analyzed using the method described in the Appendix C. Antibiotics

will be identified by its chromatographic retention time, molecular ion and confirming fragment ions. The relative abundance of ions needs to agree with the correct ratio without exceeding 15% in difference.

The antibiotics will be quantified by the standard addition method and the calibration standards will be run to confirm the linearity of calibration curves. The calibration curve will be checked every ten samples by running a blank and a reslope standard from the middle of the calibration curve. If the calibration standard disagrees with the standard curve by more than 25%, the samples in the following section will be rerun. The target for recoveries will be 60-120%. If acceptable recoveries are not obtained, the data will be reported with permanent qualifiers.

APPENDIX F: Responses to PAC Comments on the Fifth Periodic Report

In general the PAC was very pleased with the progress of the project for both
Method development in field sampling analysis.

There are no outstanding new developments in this report but at this stage of the project this can
not be expected and as also shown in this report the most important task is to consolidate and
apply the existing methods. Nevertheless, the search for suitable surrogates should be intensified
to assure analytical QC.

Suggestion: An index of all the acronyms that are used would be helpful and should be included
in all reports.

Specific comments:

Page #	Comment
11	• Just a reminder that the internal standard need to be added at the very beginning of the analysis (in the sample bottle) or at the end (after blow-down) and not in the middle of the sample prep. If it is added in the middle, it must state so and it is really not functioning as an internal standard but as a surrogate for the steps that follow. Just a concern. *Response:* This will be taken into account in the future and the internal standard will be added at the end of analysis (i.e., after blow-down).
12	• The USGS uses acidified methanol for some of the extractions. A known amount of acid is placed directly in the methanol for use. You may want to try this and eliminate the methanol/water mix since the water increases the blow-down time. *Response:* We will attempt to use this technique in the extraction of antibiotics in the future. • Table 1: Please provide the relative abundance of the fragment ions.

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	Response: Relative abundances are provided in the Appendix C of the 6th report.
13	• "... , the rest wastewater samples yielded recoveries ranging from 49% to 121% and ..." Such large variations of the recoveries may be problematic! Please address reliability aspects more deeply. Is this just a semi-quantitative approach? *Response:* As discussed in this progress report, the addition of NaCl decreased the standard deviation of fluoroquinolone recoveries. The standard addition method has been used for quantification in this project period. The standard addition method provides a better quantification than the internal standard method. Quantification were conducted by both methods and are compared in Appendix B.
15	• I really have some concerns about using norfloxacin as internal standard as it may also appear in environmental samples. Your conclusions do not sound very logical as you first mention that you included norfloxacin because it might be present in wastewater and later you state that it might be used as internal standard in surface water. So you could only use this compound as IS when you are sure that the surface water is not influenced by wastewater? That does not appear to be a practical approach that might be transferred to other labs even if you do not consider incidental spills of norfloxacin. Your second approach (lomefloxacin) sounds much more promising but I still have concerns as it is also (less frequently) applied. *Response:* We agree with the PAC's concern and thus did not use norfloxacin as an internal standard in our study. We will use lomefloxacin instead. In the future, we will analyze samples without internal standard addition for each set of sampling in order to ensure that none of the internal standards are present in the samples. • last paragraph: The shift of the retention times also demonstrates the benefits (necessity?) of MS/MS analysis! *Response:* The shift of retention times occurs only for fluoroquinolones in very high

	DOC samples. Although the retention times were shifted, the difference in retention time between the analytes do not change significantly. To identify the antibiotics, we relied upon the molecular ions and two confirming ions at the correct relative abundance (as always for all fluoroquinolones). The standard addition method also helped confirm the new retention times and thus the antibiotics.
16	• The researchers may want to try using cartridge type SPE devices instead of discs. We have found discs do not have the sampling capacity of the cartridges. This may be why the sampling in the effluents is giving lower recoveries than in finished water. The other suggestion is to extract less sample using the discs and inject more of what is processed onto the LC/MS. This may return the recoveries to acceptable levels. *Response:* We have concluded that the tandem SPE and LC/MS method is most reliable and efficient and thus has been used in the preliminary occurrence survey of antibiotics. No further method development will be conducted on the cation-exchange SPE discs for fluoroquinolone extraction. • Question: If the recoveries of the sulfonamides decrease significantly (dramatically?) at lower spiking levels, how reliable are the trace-level results of the analyzed samples? *Response:* The recoveries for sulfonamides have been significantly improved at a spiking level of 1 μg/L during this progress period after using NaCl addition prior to solid-phase extraction. 1 μg/L is within the range of concentrations which we have found sulfamethoxazole and trimethoprim.
25	• (" Concentrations of acidic drugs decrease when the wastewater passes through the nitrification system. Concentrations of beta-blockers are unaffected by nitrification."): I do not believe that the small number of investigated samples is representative to verify this assumption! I don't even believe that this is true for all

	acidic drugs! Of course, the concentrations for diclofenac and indometacine are lower in the effluent after nitrification but why are they higher again in the plant effluent? Aren't these variations random fluctuations in the effluents or just within the analytical standard deviations? *Response:* We agree with the PAC that it is inappropriate to draw sweeping conclusions based upon a limited number of samples. We included the comments to demonstrate the fact that nothing dramatic (e.g. 95% removal) occurs during nitrification. Hopefully, completion of a second round of sampling and the analysis of samples from a number of locations will allow us to draw some general conclusions based upon trends observed at the sampled sites.
28	• (conc. of beta blockers were higher in the effluents than in the influents!): Again, I have some concerns that the samples may not be representative to give such assumptions. This was only a single sampling. How did you assure that the individual residence times of the waters collected at the different stages of the STP have accurately been accounted for? This is vital to fade out temporal fluctuations of the concentrations of the PhACs in the wastewater. *Response:* We will refrain from making such statement in future reports. Since we analyzed grab samples and concentrations of PhACs can fluctuate greatly, we cannot draw any conclusions about parcels of water.
31	• (sub-task 3c, publications): Good idea, good choice!

OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Seventh Progress Report

September 15, 2002

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SUMMARY

During the sixth project period, we completed method development activities and analyzed additional samples as part of the occurrence survey. We also began our analysis of the data in preparation of manuscripts for publication in peer-reviewed scientific journals.

Final adjustment of the methods for antibiotic analysis was conducted during this project period. These minor adjustments helped to streamline the method and improve its reliability. Careful analysis of internal standards indicated that good recoveries were obtained except for one sample that unexpectedly contained a chlorine residual that was not quenched prior to analysis.

Samples collected as part of the occurrence survey provided additional information on the concentrations of PhACs in municipal wastewater effluent and in water produced by advanced treatment facilities. In addition, analysis of samples collected before and after chlorine disinfection of wastewater effluent and laboratory studies conducted in the wastewater effluent matrix indicated that several of the PhACs are removed during chlorine disinfection.

Analysis of data also was performed to assess the sources and fate of PhACs and their potential presence in water supplies. Comparison of measured PhAC concentrations in wastewater effluent with predictions based upon prescription data indicated that effluent from conventional wastewater treatment plants typically contains PhACs at concentrations between one to two orders of magnitude below those predicted in sewage. Removal of PhACs occurs in systems typically employed for water reuse. The most effective treatment system appears to be microfiltration coupled with reverse osmosis, which lowers concentrations of PhACs below method detection limits. Less common advanced treatment technologies, such as GAC coupled with ozonation also remove PhACs effectively. Soil aquifer treatment removes most, but not all of the PhACs. Engineered treatment wetlands may remove some of the PhACs, but the removal is much less effective than SAT.

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PROGRESS THIS PERIOD

TASK 1: LITERATURE REVIEW

The objective of this task is to identify compounds to be studied during the occurrence survey. The selection criteria for compounds in the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and availability of suitable analytical methods. Progress on the literature review was presented in the first and second progress reports. The literature review has been completed and will be updated prior to preparation of the final report. No additional progress related to task one was made during the current project period.

Sub-Task 1A: Drugs Used in Human Therapy

In the first progress report, we reviewed data on prescription drugs likely to be present in wastewater effluent in the United States. Using data on the number of prescriptions for the top 200 drugs and information on prescription formulations and metabolism, we estimated ranges of concentrations of 136 drugs expected to be present in wastewater effluent. In addition, we reviewed published occurrence data to identify other compounds of interest that did not appear among the common prescription drugs. A total of eleven drugs were identified for further consideration.

During the current project period no activity was conducted in association with this subtask.

Sub-Task 1B: Drugs Used in Animal Husbandry

In the second progress report, we reviewed data on antibiotics used in livestock both therapeutically to treat diseases and sub-therapeutically as feed additives to promote growth. We focused our review on antibiotics used in animal feeding operations, because these sources could result in concentrated discharges of antibiotics. We also reviewed fate and transport properties and available analytical methods. On the basis of these results and our review of antibiotics used in human therapy, we identified that sulfonamide and fluoroquinolone antibiotics as the most probable water contaminants, followed by macrolide antibiotics. Among these antibiotic classes, we identified five antibiotics that merit further consideration.

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During the current project period no activity was conducted in association with this sub-task

TASK 2: ANALYTICAL METHOD DEVELOPMENT AND TESTING

The objective of this task was to identify and test analytical methods that will be used during the occurrence survey. As part of the task, analytical methods for extraction, cleanup and derivitization were tested by adding drugs to deionized water and to samples collected from sites being considered for inclusion in the occurrence survey. Analysis of antibiotics was included as a separate task from the other pharmaceutically active compounds (PhACs) because the analytical methods for antibiotics (e.g., HPLC/MS) are fundamentally different from the gas chromatography methods used for the other compounds.

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the first progress report, we described our preliminary efforts to develop suitable analytical methods for eleven compounds identified as part of the literature survey. We used solid phase extraction followed by derivitization and GC/MS/MS to quantify pharmaceuticals in deionized water and in samples collected from several of the candidate sampling sites. To quantify recoveries and matrix effects, all samples also were analyzed after addition of known quantities of analytes.

During the second project period, we focused our attention on improving the accuracy and precision of the analytical methods by analyzing samples from three sites being considered for inclusion in the occurrence survey. As a result of analytical difficulties, we did not analyze three of the compounds (i.e., carbamazepine, nabumetone and nadolol) during the second project period. For the remaining eight compounds, analysis of duplicate samples indicated that, with the exception of two beta-blockers, results were reproducible (i.e., we observed an average of 26% error between duplicate samples). Analysis of blank samples indicated that sample carryover and cross contamination were not significant problems. Analysis of spike recovery samples indicated variable recoveries, with values as low as 30% for some analytes. All of the drugs were detected in one or more of the unspiked wastewater effluent samples.

During the third project period, we continued to improve the analytical methods by identifying steps where analytes were lost during analysis. For the acidic drugs, we changed the

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solid phase extraction technique and added labeled mecoprop as an internal standard. As a result of the new SPE method, spike recoveries improved significantly. For the beta-blockers, we increased the time of the drying step to improve the efficiency of derivitization, but this only had a minor effect on spike recoveries. We also eliminated the use of PFE-lined containers, which resulted in losses of beta-blockers during storage. A QA/QC plan also was submitted to the PAC.

During the fourth project period, we attempted to resolve the remaining issues associated with the analytical methods. Attempts to use labeled propranolol as an internal standard for the beta-blockers failed because the labeled compound could not be discriminated from the unlabeled compounds. Alternative surrogates for beta-blockers could be derivitized and analyzed, but were too polar to be retained during solid phase extraction. We also evaluated the variability in method performance for acidic drugs and beta-blockers by analyzing a total of 18 samples from two surface waters and an advanced wastewater treatment plant. Analysis of surface water samples from a site that was subjected to chlorine disinfection indicated that several of the analytes were lost in the presence of free chlorine. We also tested a GC/MS/MS technique for analysis of carbamazepine. The compound could be detected easily at high concentrations. However, sensitivity decreased significantly at low concentrations, possibly as the result of losses in the injection port of the GC.

During the fifth project period we evaluated the possible use of epinephrine and deoxyepinephrine as internal standards in the analysis of beta-blockers. While it was possible to analyze the derivatives, they were lost during the extraction, solvent transfer and blow-down steps to a much greater degree than the other compounds. Therefore, we decided to limit our assessment of recovery to matrix spike recovery measurements with both beta-blockers for each set of samples.

During the fifth project period we also attempted to improve the recovery of carbamazepine by modifying the SPE method and by conditioning the injection port liner and replacing it after each set of analyses. Results of our analyses indicated that carbamazepine was present at a concentration around 1,000 ng/L in the effluent of the Mt. View WWTP and in the pond at the Sweetwater recharge facility. However, we were unable to obtain reproducible, linear standard curves because the injection port liners could only be used for a few samples before they had to be replaced. Given the numerous analytical challenges associated with the

analysis of carbamazepine and the need to complete the occurrence survey, we decided to forgo any further attempt to analyze this compound.

No further activities associated with method development were conducted during the seventh project period.

Sub-Task 2B: Antibiotics

Our analysis of antibiotics focused on fluoroquinolone and sulfonamide antibiotics. Ciprofloxacin, sulfamethoxazole and sulfamethazine were selected as target analytes of occurrence analysis. In the second and third progress reports, we reported our efforts to develop suitable analytical methods for these compounds. A dual-cartridge solid phase extraction (SPE) method was developed to extract antibiotics from water samples. Antibiotics were analyzed using LC/MS and LC/FLD (fluorescence detection).

During the fourth progress report, we attempted to improve the analytical methods. We identified the steps where analytes were lost and made changes to minimize the losses. High-density polyethylene conical tubes were shown to yield the smallest losses of fluoroquinolones during the blow-down step and were used in all the later experiments. We determined that acidifying and storing samples in amber glass bottles best preserves analytes prior to solid-phase extraction and yields the smallest losses of analytes through adsorption to the container walls. Sample extracts were better preserved at 0° C rather than at 5° C when analysis by LC/MS could not be conducted immediately. To achieve better results, separate LC/MS methods were developed for sulfonamide and fluoroquinolone antibiotics respectively. LC/MS conditions were modified to reduce matrix effects and increase sensitivity. We investigated several structurally related sulfonamide and fluoroquinolone antibiotics as internal standards. Our studies indicated that sulfamerazine and enrofloxacin can serve as appropriate internal standards. These research efforts improved the method recovery to above 60% and also increased sensitivity in detection.

Near the end of the fourth project period, the cation-exchange extraction method for fluoroquinolones was found to work successfully in reagent water after finding a proper cation-exchanger (high-density, mixed-phase cation-exchange discs, 3M) and avoided errors in sample preparation. The cation-exchange extraction followed by HPLC/fluorescence detection is a simple and sensitive method that can be easily performed in most existing water utility labs and can also be used to independently confirm the analysis by the dual-cartridge SPE followed by

LC/MS. Therefore, it was concluded that the cation-exchange SPE merited further investigation with wastewater matrices.

During the fifth project period, we examined the accuracy and precision of the dual-cartridge SPE and LC/MS methods in several more wastewater samples and included two additional antibiotics, trimethoprim and norfloxacin, in the analysis. The results indicated consistent recoveries meeting the QA/QC criteria for the fluoroquinolones (norfloxacin and ciprofloxacin). However, the recoveries for sulfonamides and trimethoprim were still below our target values. Further studies on the cation-exchange SPE followed by LC/fluorescence detection method indicated that the method is adversely affected by the organic matter encountered in more complicated wastewater matrices, rendering consistent performance difficult. It was concluded that the method is only suitable for qualitative screening purposes for fluoroquinolones and may be suitable for compound quantitation in relatively clean water samples.

In the sixth project period, the analytical method was improved further. The fluoroquinolones ofloxacin and enrofloxacin were added to the analysis, rendering a total of four fluoroquinolones in the occurrence survey. Lomefloxacin was added as an internal standard for the fluoroquinolones. The recoveries for sulfonamides and trimethoprim were greatly improved after using salt addition prior to the SPE step. For more accurate quantification, the standard addition method was used as an alternative quantification technique and was compared to the internal standard method (the methods were described in details in the 6th report). Analytical efficiency was enhanced after combining the two LC/MS methods for fluoroquinolones and sulfonamides, respectively. Finally, the LC/MS sensitivity was improved by lowering the eluent buffer concentrations while still maintaining sufficient buffering capacity.

During this project period, the developed analytical method was used for more sample analyses and the standard addition method was used to assess recoveries in spiked samples in addition to the internal standard method (lomefloxacin for fluoroquinolones and sulfamerazine for sulfonamides). Recoveries of reagent water spike and matrix spikes for 15 samples collected from seven sampling sites are summarized in the Appendix B. Fluctuation of recoveries was observed. Low recoveries were obtained for sulfamethoxazole and sulfamethazine in the West Basin (5/22/2002) microfiltration influent and effluent samples. In this analysis, the samples were acidified to pH 3.0 (higher than the pH 2.5 used previously) as part of an attempt to allow

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the anion exchangers to extract a greater amount of organic matter in the matrices. This pH change probably caused the recoveries to decrease. Low recoveries were observed for all antibiotics in the reverse osmosis effluent sample. After checking with the plant operator, we learned that the West Basin reverse osmosis uses chlorine to prevent membrane fouling and thus the RO effluent contains approximately 1-2 mg/L chlorine. Since we did not anticipate the presence of chlorine in these samples, sodium thiosulfate was not added to the samples. The near zero recovery for all antibiotics in the RO effluent is likely resulted from reactions of antibiotics with chlorine at the acidified pH ranges. Our laboratory experiments have confirmed that all of the antibiotics selected in this study react readily with chlorine. As a result, the RO effluent data form the West Basin site cannot be used in the occurrence survey.

Due to experimental errors, recoveries could not be accurately determined for the F. Wayne Hill samples (7/17/2002). For these samples, insufficient amount of antibiotic stock was added by mistake to the spiked extracts in assessing spike recoveries using the standard addition method and the internal standard method was not conducted. Some LC/MS instrument difficulty was also encountered during these sample runs, which led to little sample extract leftover for repeating the standard addition approach. The recoveries for sulfamethoxazole and sulfamethazine were also lower than the QA/QC criteria (Appendix D of the 6th report) for the Clayton samples (8/20/2002). This was likely caused by fast elution of sulfonamides from the SPE cartridges. Later analyses indicated that slow elution of antibiotics from the SPE cartridges (approx. one drop per second) yielded better recoveries. These experiences show that acidifying the samples to near pH 2.5 and slowly eluting compounds from the SPE cartridges are critical to maintain good recoveries for sulfonamides.

Overall, the recoveries were better for fluoroquinolones and trimethoprim than for sulfonamides. Excluding the West Basin reverse osmosis effluent samples (5/22/2002), the average recovery for all matrices for ciprofloxacin, norfloxacin, enrofloxacin, ofloxacin, and trimethoprim were 68±27%, 82±21%, 94±37%, 88±33% and 105±19%, respectively. Excluding the West Basin samples (5/22/2002), the average recovery for all matrices for sulfamethoxazole and sulfamethazine were 51±15% and 49±11%, respectively. Good recoveries were obtained for all four DI water spiked samples based on the internal standard method. However, the standard addition method yielded higher recoveries. This is probably caused by poor peak shape resulted from low buffer concentration in the LC mobile phases. In the future, we plan to increase

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slightly the buffer concentration in the LC mobile phases to improve the peak shape. In addition, none of the field blank samples for the seven sampling sites was detected with any antibiotics.

The method development is considered to be complete. Much of the data collected during this project period meets the QA/QC criteria for the occurrence survey. The data are discussed under Task 3.

TASK 3: OCCURRENCE SURVEY

Sub-Task 3A: Site Selection

Because the most important source of PhACs is believed to be the discharge of municipal wastewater effluent, we focused our efforts on sampling of municipal wastewater effluent and water recycling systems that serve as important barriers to the entry of wastewater-derived contaminants into drinking water sources. In the first stage of the site selection process, we identified representative sites and made arrangements with utilities to obtain samples.

As part of the site selection process, preliminary samples were collected during the first three project periods from sites that we considered for inclusion in the occurrence survey. The preliminary data helped us to assess the suitability of sites to be included in the occurrence survey. In several cases, sites that we had intended to sample were eliminated because changes had occurred at the sites. For example:

-The Dublin/San Ramon Advanced Wastewater Treatment Plant was taken out of service during Spring 2001 because the utility district put the project on indefinite hold.

-We were informed that the Witchita Falls Pilot Advanced Water Treatment Plant treats water that mainly originates from agricultural runoff.

-The Rio Hondo Recharge Facility was eliminated because construction prevented us from obtaining samples.

The sites that were eliminated due to the considerations described above were replaced by comparable sites as they were identified.

A final list of sites sampled during the occurrence survey is included in Table 1. The selected sites included a total of eight conventional wastewater treatment plants, three advanced wastewater treatment plants, two engineered treatment wetlands and two background sites. Two additional background sites were added later to account for baseline conditions in the Southeastern United States. Each of the sites is described briefly in the following section.

R.M. Clayton Wastewater Treatment Plant (Atlanta, GA): The R.M. Clayton municipal wastewater treatment plant is a 5.26 m^3 s^{-1} (120 MGD) facility. The plant is equipped with primary screening and clarification, followed by activated sludge treatment with three-stage biological phosphorous removal in activated sludge reactors. Following clarification, the wastewater undergoes ultraviolet disinfection.

Table 1. Summary of sample collection sites in the occurrence survey.

Location	Description	Dates Sampled
Conventional WWTPs		
Clayton	BNR, UV	3/19/02, 8/20/02
Dublin/San Ramon	AS, Cl_2	3/21/02
Hyperion	AS, Cl_2	9/18/01, 5/22/02
Mt. View	AS, biotower, UV	9/4/01, 4/9/02, 8/21/02
Roger Road	AS, Cl_2	2/17/02, 4/1/02
San Jose/Santa Clara	BNR, effluent filtration, Cl_2	3/26/02, 6/26/02
South Cobb	AS, Cl_2	2/15/02, 6/12/02
Southeast San Francisco	O_2-AS, Cl_2	7/1/02
Advanced Treatment Plants		
F. Wayne Hill	Activated carbon, ozone, UV	4/22/02, 7/17/02
OCWD	Microfiltration, RO, UV	9/18/01
West Basin	Microfiltration, RO, UV	9/18/01, 5/22/02
Groundwater Recharge		
Sweetwater Recharge Facility	Secondary effluent recharge	2/17/02, 4/1/02
Engineered Wetlands		
Mt. View	Tertiary effluent, RT ~7 days	9/4/01, 4/9/02, 7/12/02
Prado	Effluent-dominated river water	4/6/02
Background		
MWD Water	Los Angeles water supply	9/18/01
Russian River	Marin County, CA	5/14/01, 6/11/01, 8/13/01
Lake Altoona	Intake for Wyckoff WTP, GA	9/6/02
Flint River Reservoir	Intake for Smith WTP, GA	9/6/02

Notes:

WWTP = conventional municipal wastewater treatment plant; AS = Activated sludge; UV = ultraviolet disinfection; Cl_2 = chlorine disinfection; RT = hydraulic retention time; OCWD = Orange County Water District; MWD = Metropolitan (CA) Water District

Dublin/San Ramon Advanced Wastewater Treatment Plant (Dublin, CA): The Dublin/San Ramon Services municipal wastewater treatment plant is a 0.50 m³ s⁻¹ (12 MGD) facility. The plant is equipped with primary screening and clarification, followed by activated sludge treatment and chlorine disinfection.

Hyperion Wastewater Treatment Plant (Los Angeles, CA): The Hyperion municipal wastewater treatment plant treats a total of 15.7 m³ s⁻¹ (358 MGD) of municipal wastewater effluent with advanced primary treatment or secondary treatment. The water sampled during the occurrence study originated in the secondary treatment plant, which treats 8.45 m³ s⁻¹ (193 MGD) of wastewater effluent. The secondary treatment plant is equipped with primary screening and clarification followed by pure oxygen activated sludge treatment, clarification and chlorine disinfection. The samples analyzed as part of the occurrence survey were collected at the West Basin AWWTP, which treats the secondary effluent from the Hyperion treatment plant.

Mt. View Wastewater Treatment Plant (Martinez, CA): The Mt. View municipal wastewater treatment plant is a 0.06 m³ s⁻¹ (1.5 MGD) facility equipped with primary screening and clarification followed by a trickling filter for secondary treatment and a biotower for ammonia removal. The effluent is subjected to ultraviolet disinfection prior to being discharged to an engineered treatment wetland.

Roger Road Wastewater Treatment Plant (Tuscon, AZ): The Roger Road municipal wastewater treatment plant is a 1.4 m³ s⁻¹ (31 MGD) facility equipped with primary screening and clarification, followed by activated sludge treatment and chlorine disinfection. The treatment plant discharges directly to an infiltration pond that recharges an aquifer at the Sweetwater recharge facility. During the occurrence survey, samples collected from the infiltration pond were assumed to be representative of the effluent from Roger Road treatment plant.

San Jose/Santa Clara Wastewater Treatment Plant (San Jose, CA): The San Jose municipal wastewater treatment plant is a 7.3 m³ s⁻¹ (170 MGD) facility equipped with primary screening and clarification, followed by activated sludge treatment with three-stage biological phosphorous removal in activated sludge reactors. Following clarification, the wastewater undergoes mixed media filtration and chlorine disinfection.

South Cobb Wastewater Treatment Plant (Cobb County, GA): The South Cobb municipal wastewater treatment plant is a 1.8 m^3 s^{-1} (40 MGD) facility equipped with primary treatment and aerated activated sludge treatment followed by chlorine disinfection.

The Southeast San Francisco Wastewater Treatment Plant (San Francisco, CA): The Southeast San Francisco municipal wastewater treatment plant is a 6.6 m^3 s^{-1} (150 MGD) facility equipped with primary screening and clarification, followed by pure oxygen activated sludge treatment and chlorine disinfection.

F. Wayne Hill Advanced Wastewater Treatment Plant (Gwinnett County, GA): The F. Wayne Hill facility is a 0.88 m^3 s^{-1} (20 MGD) advanced wastewater treatment plant. The facility consists of primary treatment followed by activated sludge treatment in a reactor operated for biological nutrient removal. After the secondary clarification, the wastewater undergoes lime addition and recarbonation followed by dual-media filtration, pre-ozonation, granular activated carbon filtration and ozonation.

West Central Basin Municipal Water Advanced Wastewater Treatment Plant (Los Angeles, CA): The West Basin treatment plant is an advanced wastewater treatment plant consisting of two treatment trains. The first treatment train uses lime coagulation followed by cellulose acetate membranes while the second train uses microfiltration followed by reverse osmosis with thin-film composite membranes. The two trains are combined prior to ultraviolet disinfection in the presence of added hydrogen peroxide to enhance the removal of organic contaminants. As part of the occurrence survey samples were collected from the second treatment train.

Orange County Water District (OCWD) Advanced Treatment Plant (Fountain Valley, CA): The OCWD operates an advanced wastewater treatment plant as part of the Talbert Barrier seawater intrusion project. The treatment plant, collectively referred to as Water Factory 21, consists of two treatment trains that treat wastewater effluent form Orange County Sanitation District's adjacent municipal wastewater treatment plant. During the occurrence survey, samples were collected from the treatment train that consists of microfiltration, reverse osmosis with thin-film composite membranes and ultraviolet disinfection in the presence of hydrogen peroxide.

Sweetwater Recharge Facility (Tuscon AZ): The Sweetwater groundwater recharge site consists of an infiltration pond that receives wastewater effluent from the Roger Road wastewater treatment plant. The underlying aquifer is equipped with an extensive network of

monitoring wells. As a part of the occurrence survey, groundwater was collected from two downgradient wells: (1) a shallow well screened at 5.1 meters; and, (2) a deep well screened at approximately 30.5 m. According to tracer data collected at the site the water sampled from both wells consists entirely of wastewater effluent (i.e., there is no dilution with local groundwater) and has a residence time in the aquifer of approximately 2.5 and 15 days, respectively.

Mt. View Engineered Treatment Wetland (Martinez, CA): The Mt. View engineered treatment wetlands consist of a series of five ponds in series connected by weirs and underground piping. The wetland ponds are approximately 1.5 meters deep and are extensively vegetated along the edges with cattails duckweed. The mean hydraulic residence time of the wetland is approximately 7 days.

Prado Engineered Treatment Wetlands (Orange County, CA): The Prado Engineered Treatment wetlands treat water from the Santa Ana River. During summertime, most of the water in the Santa Ana River originates at Riverside and San Bernardino tertiary wastewater treatment plants located approximately 20 km upstream. During other times of the year, the river receives a combination of stormwater runoff and wastewater discharge from the upstream watershed. The wetland consists of a series of treatment cells vegetated with cattail and duckweed.

Sub-Task 3B: Sample Collection and Analysis

While the quality of some of the data collected before completion of method develop was useful to our analysis, the preliminary samples were not analyzed using all of the steps ultimately incorporated into the analytical method described in the QA/QC plan. As a result, the preliminary results are only useful for screening purposes. During the fourth, fifth and sixth project periods, we analyzed samples from selected sites using the accepted analytical methods for acidic drugs and beta-blockers. Method development activities for antibiotics were completed during the sixth project period and samples from five sites were analyzed for the selected compounds using the final methods.

During the seventh project period we collected and analyzed additional samples for acidic drugs, beta-blockers and antibiotics from several of the sites (see Appendices A and B for details). Results from those analyses are presented in the following paragraphs.

To further investigate the potential for removal of PhACs during chlorine disinfection, samples were collected from the San Jose/Santa Clara and Southeast San Francisco WWTPs. Although both treatment plants use chlorine for disinfection, the forms of chlorine differ between the two facilities. The San Jose/Santa Clara WWTP is equipped with biological nutrient removal, which results in very low concentrations of ammonia in the water after secondary treatment. However, the treatment plant applies monochloramine before filtration to prevent biological growth on the filters and while minimizing the potential formation of disinfection byproducts. As a result, we cannot assume that all of the chlorine used for disinfection consists of HOCl and OCl⁻. The Southeast San Francisco WWTP does not practice nitrification. As a result, much of the chlorine added during disinfection reacts with ammonia to form monochloramine (NH_2Cl).

Results of measurements made before and after the chlorine contact basins at the two WWTPs are depicted on a logarithmic scale in Figure 1. A quick inspection of these data suggests that the concentrations of acidic drugs and β-blockers are unaffected by disinfection. However, closer analysis suggests that several compounds react with free chlorine. To highlight the potential importance of transformation reactions that occur during chlorine disinfection, the same data are plotted as the ratio of concentrations after disinfection to the concentrations entering the disinfection process (Figure 2). Data for ibuprofen and indometacine from both treatment plants and propranolol from Southeast San Francisco are not plotted because the concentration entering the disinfection system was below the detection limit. The data from the San Jose/Santa Clara WWTP (black bars) suggest that approximately half of the diclofenac, ketoprofen and naproxen were removed by reactions during disinfection while gemfibrozil, metoprolol and propranolol did not appear to react. The data from the Southeast WWTP are more difficult to interpret due to analytical variability. However, they suggest that the pharmaceuticals were much less reactive with monochloramine.

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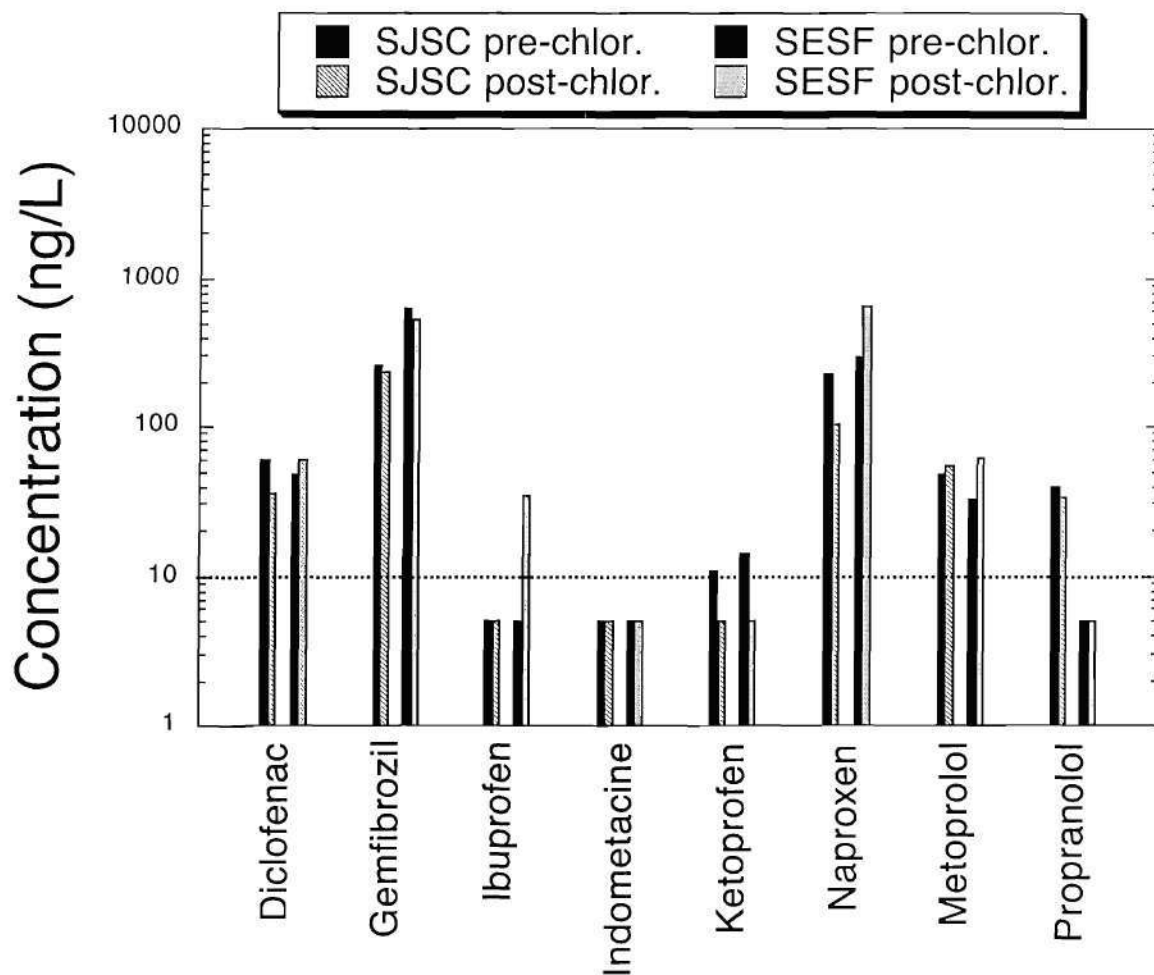
Figure 1: Concentrations of acidic drugs and beta-blockers measured before and after chlorine disinfection at the San Jose/Santa Clara (SJSC) and the Southeast San Francisco (SESF) Municipal Wastewater Treatment Plants. The dashed line indicates the limit of quantification. Samples in which analyte concentrations were below the detection limit are plotted at half the limit of quantification, as indicated by the dotted line.

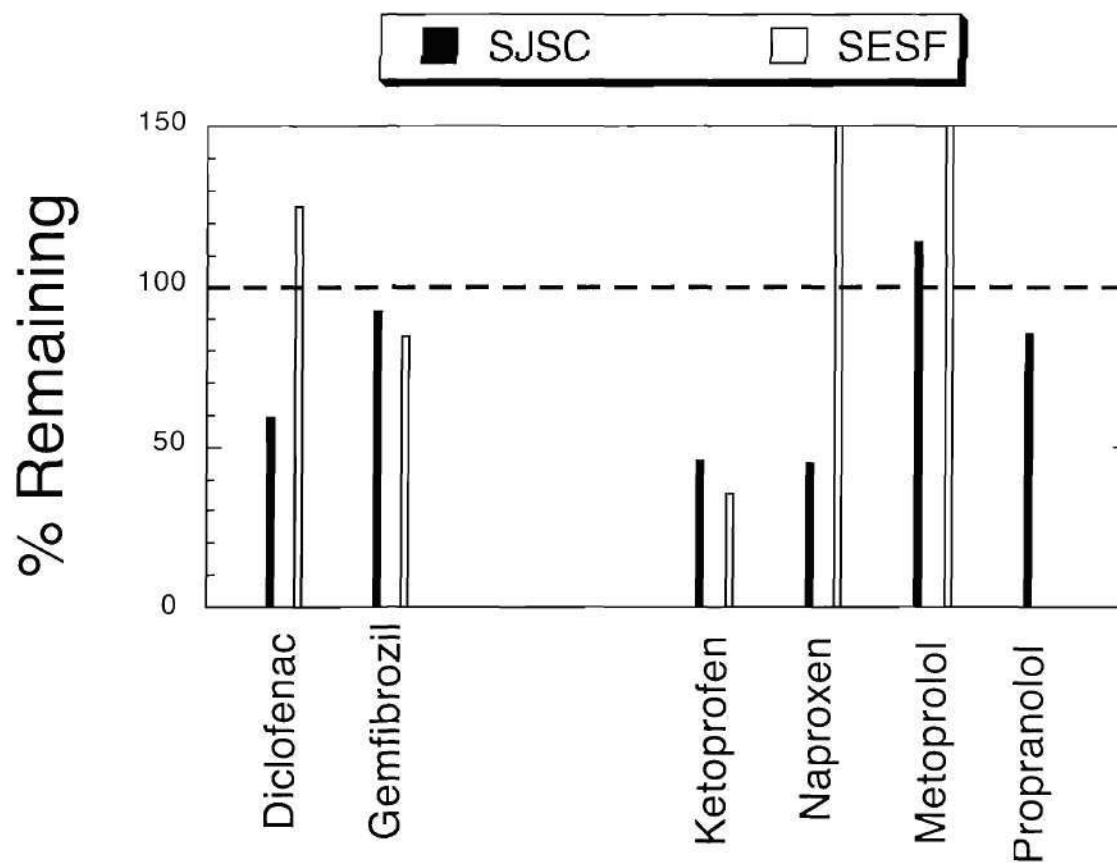Figure 2: Ratios of concentrations of pharmaceuticals before and after disinfection at the San Jose/Santa Clara (SJSC) and the Southeast San Francisco (SESF) Municipal Wastewater Treatment Plants. Ratios for naproxen and metoprolol at the SESF facility are greater than 150% due to variability in wastewater composition.

Variability in wastewater composition and the operation of the disinfection process make it extremely difficult to evaluate relatively modest changes in pharmaceutical concentrations during disinfection without resorting to composite sampling. To gain further insight into the potential importance of reactions that occur during chlorine disinfection, we conducted an experiment using wastewater effluent collected before disinfection at the San Jose/Santa Clara and Southeast San Francisco WWTPs. As part of this experiment, we added 1,000 ng/L of each of the target analytes to the wastewater prior to addition of a low dose (i.e., 0.14 mM or 10 mg/L as Cl_2) and a high dose (i.e., 0.86 mM or 60 mg/L as Cl_2) of chlorine. After one hour, the chlorine was quenched by addition of an excess of sodium thiosulfate.

Results from the experiment (Figure 3) provide further evidence that chlorine disinfection removes some of the pharmaceuticals from wastewater that does not contain high concentrations of ammonia. Consistent with their disappearance at the San Jose/Santa Clara WWTP and the results of our previous recovery studies inadvertently performed in the presence of chlorine, diclofenac and naproxen were almost completely removed by the low and high concentrations of free chlorine. Ketoprofen was not removed when free chlorine was added to the San Jose/Santa Clara wastewater effluent. Therefore the apparent decrease in concentrations of ketoprofen observed at the San Jose/Santa Clara WWTP may be attributable to uncertainty in measurements near the method detection limit (i.e., the concentration of ketoprofen entering the disinfection system was only about twice the method detection limit). Ibuprofen and propranolol also reacted with chlorine in the experiments performed with San Jose/Santa Clara secondary effluent. However, the transformation was not complete during the one-hour contact time at either dose. These data suggest that the 10-20% decrease in the concentration of these two compounds observed at the San Jose/Santa Clara WWTP may be attributable to transformation rather than variability in wastewater composition. The laboratory data also suggest that diclofenac, naproxen and metoprolol react with monochloramine. However, the rates of reaction are relatively slow and probably will not be important under most conditions encountered at municipal wastewater treatment plants. The slower reactions could result in losses of the compounds during long contact times with monochloramine, as would be encountered in a water distribution system that uses monochloramine as a residual disinfectant.
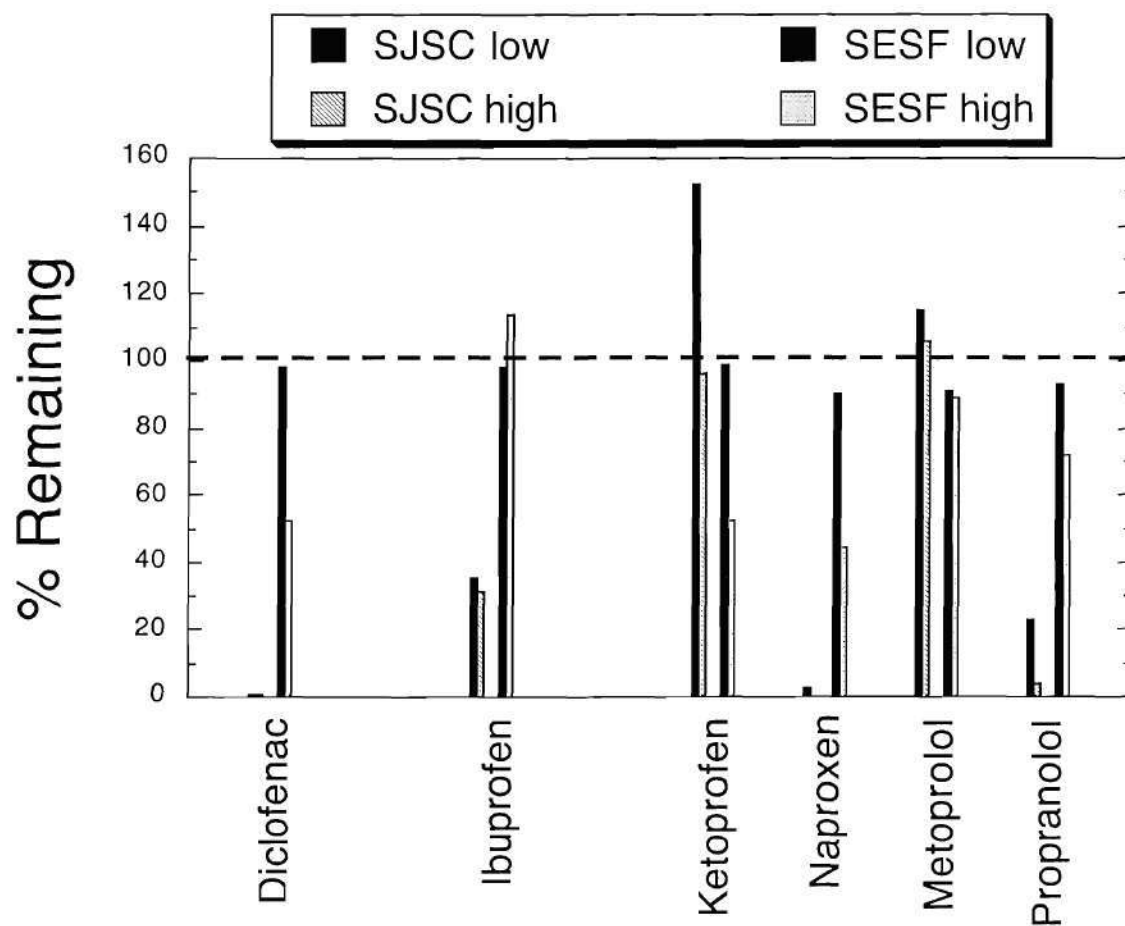
Figure 3: Results from laboratory experiments involving the addition of low (10 mg/L as Cl_2) and high (60 mg/L as Cl_2) doses of chlorine to secondary effluent samples from the San Jose/Santa Clara (SJSC) and the Southeast San Francisco (SESF) Municipal Wastewater Treatment Plants.

Antibiotics were analyzed from five wastewater treatment plants (Clayton WWTP, South Cobb WWTP, F. Wayne Hill AWWTP, Hyperion WWTP, and West Basin AWWTP) and two background sites (Lake Altoona and the Flint River Reservoir). Lake Altoona is located north of Atlanta and serves as the raw intake for the Hugh Wyckoff WTP and the Flint River Reservoir is the main catchment of storm runoff south of Atlanta and serves as the raw intake for the J. W. Smith WTP. The corresponding finished drinking water for these two surface waters were also analyzed.

Samples analyzed for antibiotics were collected and analyzed using the method and the QA/QC plan described in the 6th report except that a larger volume (i.e., 3-4 L) was extracted for the surface and drinking water. 1.0 μg/L of each target antibiotic was added to the samples prior to extraction to assess matrix spike recoveries. The occurrence results are shown in Figures 4-8 and are summarized in the Appendix B. The antibiotic concentrations included in the figures were determined by standard addition method and were not corrected for recovery.

For the samples analyzed in this project period, antibiotics were detected mainly in the secondary wastewater effluent samples. Sulfamethoxazole (160-640 ng/L), trimethoprim (20-1220 ng/L), and ofloxacin (140-760 ng/L) were detected in all secondary effluent samples. Ciprofloxacin (80-560 ng/L) was detected in the secondary effluent from the Hyperion and Clayton WWTPs. A low concentration of norfloxacin (40-60 ng/L) was detected in the South Cobb secondary effluent. The veterinary antibiotics sulfamethazine and enrofloxacin were not detected in any of the samples. The concentrations of all antibiotics were below the detection limits (about 10 ng/L) in the background samples. Antibiotics also were not detected in the finished drinking water samples.

Comparisons among wastewater treatment processes yield conclusions in agreement with previous observations. The results from the West Basin AWWTP confirm earlier observations that microfiltration does not remove PhACs. The data from the South Cobb WWTP suggest that chlorination removes antibiotics. The data from the F. Wayne Hill results indicate that granular activated carbon and ozonation can effectively reduce the concentrations of antibiotics.

Figure 4. Occurrence results of ciprofloxacin during the 7th project period. The line indicates the method detection limit.
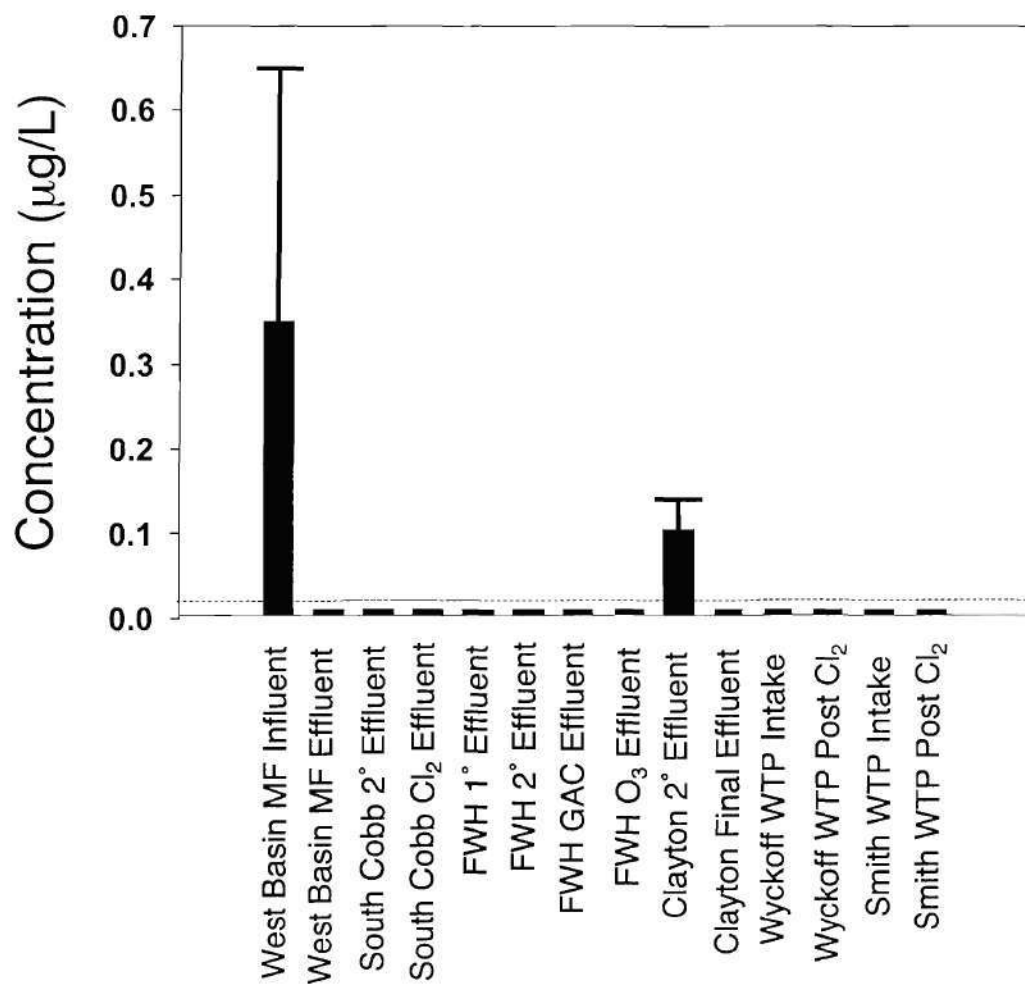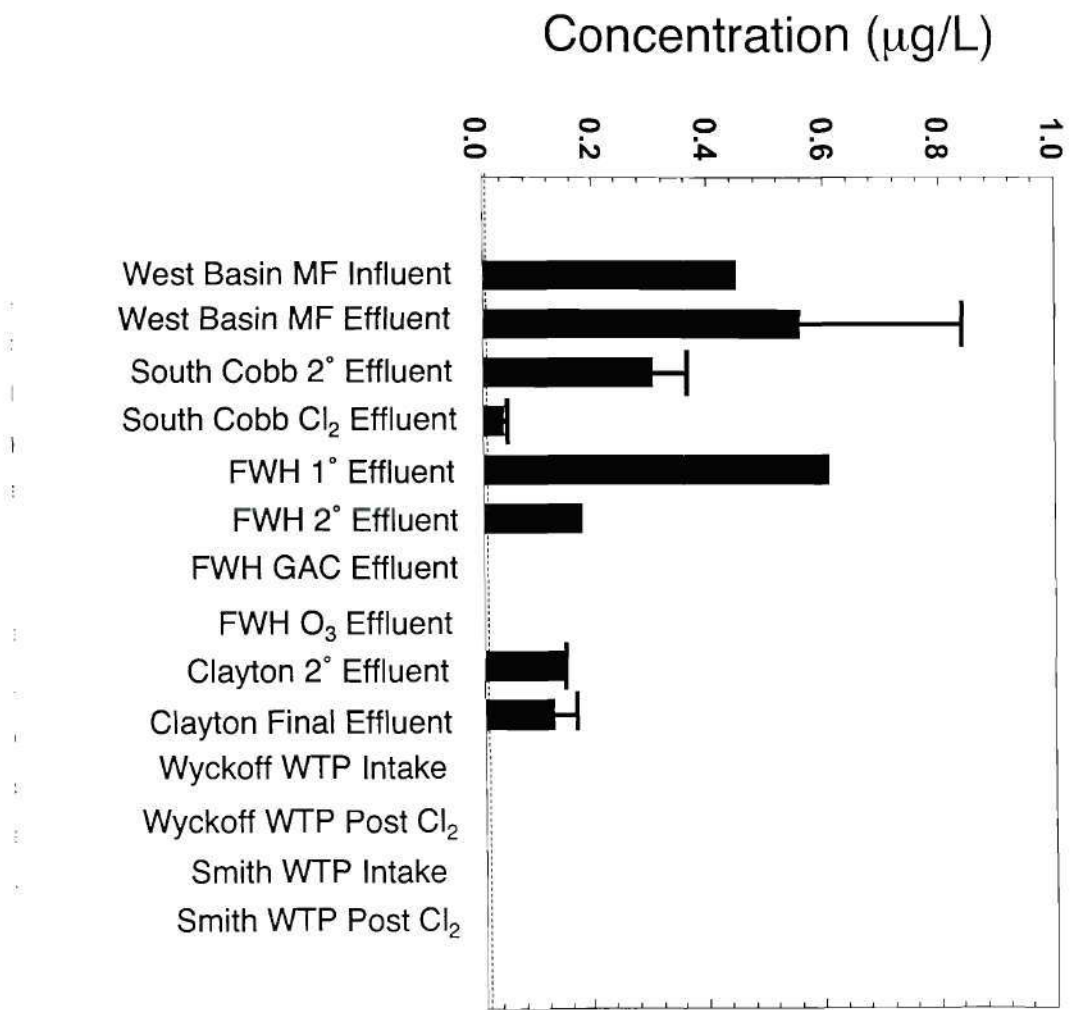
Figure 5. Occurrence results of ofloxacin during the 7th project period. The line indicates the method detection limit.

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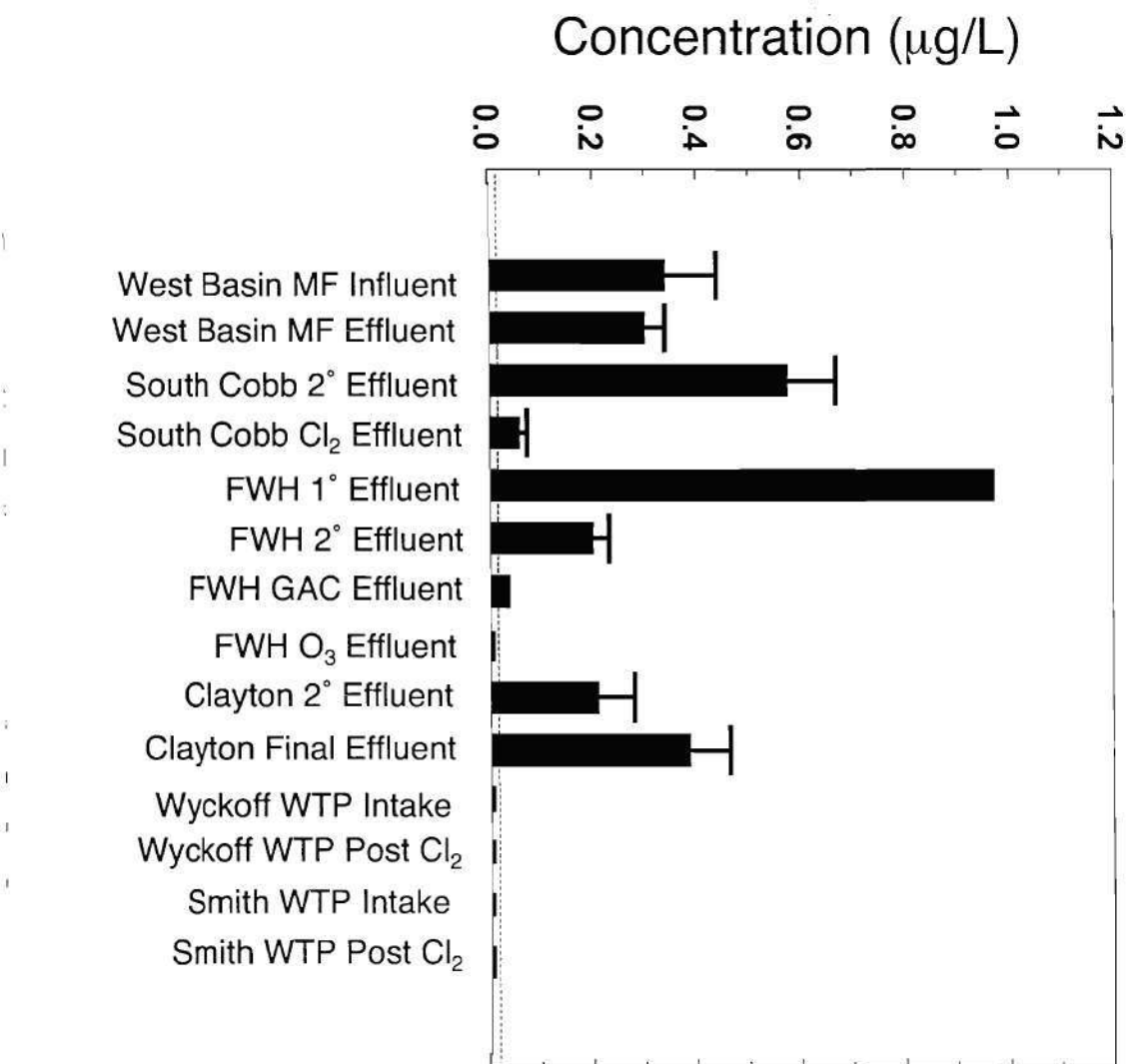
Figure 6: Occurrence results of sulfamethoxazole during the 7th project period. The line indicates the method detection limit.
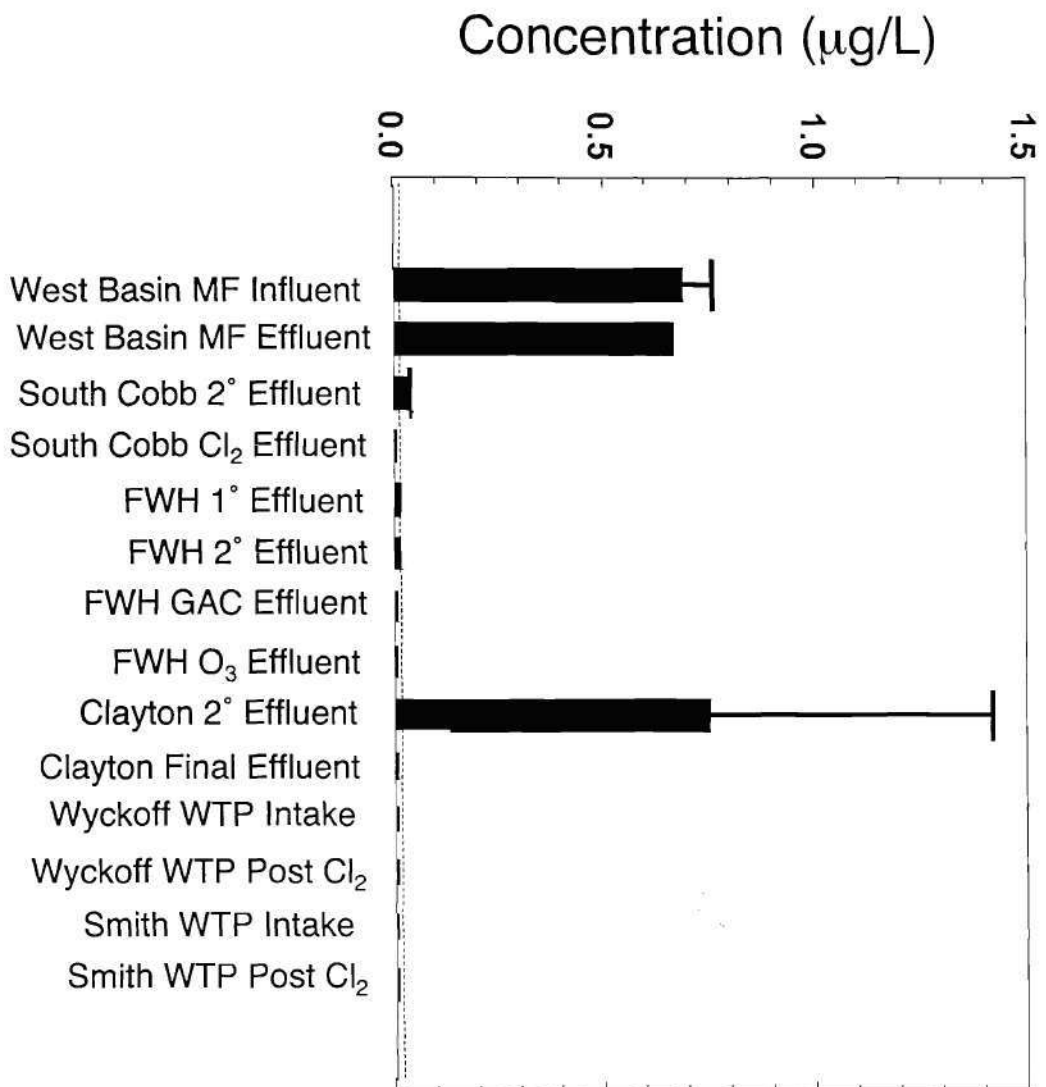
Figure 7. Occurrence results of trimethoprim during the 7th project period. The line indicates the method detection limit.

Sub-Task 3C: Data Analysis and Synthesis

As discussed in the sixth project report, we are in the process of writing a manuscript summarizing the results of the occurrence survey. The manuscript will present details of the analytical methods, the data and a discussion of the implications of the data for the water industry. Although we have not yet completed our interpretation of the data, we have been considering the most effective way to summarize the data and to describe the implications of the data. Our preliminary approach is summarized in the following paragraphs to provide the PAC with an opportunity to provide us with advice on alternative approaches.

Measurements to date of acidic drugs and β-blockers are summarized in Figure 8. The thick black bars depict the range of concentrations measured in the six wastewater treatment plants and the line indicates the median observed concentration. These data are compared with the predicted concentrations in raw sewage (indicated with the double lines above the bars) as presented in the first progress report. The data also are compared to the median and range of concentrations in German wastewater treatment plants as reported by Ternes (1998) (indicated with the thin black bars and line). Although this figure is complicated, it allows us to summarize a significant amount of information and draw preliminary conclusions about the data.

Comparing the median concentrations detected in wastewater effluent with the medians detected by Ternes (1998) suggests that the concentrations of PhACs are comparable or occur at lower concentrations in the United States compared to Germany. Two of the compounds (i.e., gemfibrozil and naproxen) had similar or slightly higher median concentrations in the United States while median concentrations of the remaining compounds were approximately an order of magnitude lower than those observed in Germany. The lower concentrations usually detected in US effluent samples could be due to more dilution of wastewater in the United States (i.e., higher per capita wastewater production in the US), lower per capita drug use in the US or better removal during treatment by the US treatment plants. Among the compounds studied in the occurrence survey, gemfibrozil and naproxen were the only two compounds detected in all wastewater effluent samples.

Comparing the range of concentrations detected in wastewater effluent with concentrations predicted for sewage indicates that the estimates provide a reasonable upper-bound concentration possible in wastewater effluent. This observation is significant because it
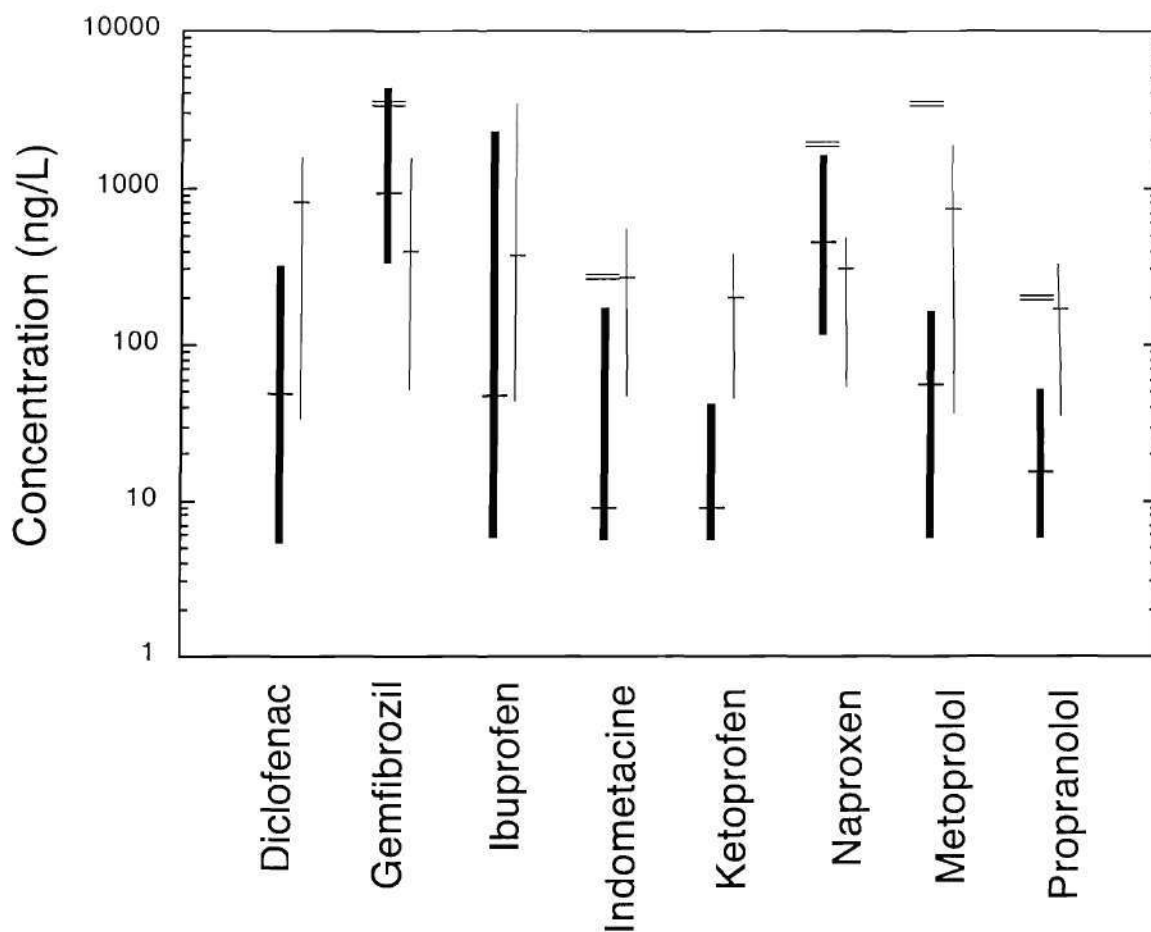
Figure 8: Comparison of concentrations of acidic drugs and beta-blockers with predicted concentrations in sewage and concentrations measured in wastewater effluent in Germany. The thick bars indicate the range of concentrations measured during the occurrence survey and the single line represents the median concentration. The thin bars indicate the range and median concentrations reported by Ternes (1998). The double lines (=) indicate the estimated concentrations in sewage in the US, as described in the first progress report. Non-detects are plotted at half the limit of quantification.

demonstrates that prescription data may be a reasonable way of prioritizing concerns about PhACs and estimating maximum concentrations that could be released to the environment.

Estimates of the percent of each compound removed during conventional wastewater treatment can be made using predicted concentrations in sewage and median concentrations measured in effluent during the occurrence survey (Table 2). The estimated removals range from 73-99.9%, which is consistent with data from Ternes (1998) as well as predictions made with fugacity models (Khan and Ongerth, 2002).

As discussed in the previous section, data from samples collected before and after disinfection at the SJSC WWTP and laboratory experiments suggest that several compounds are removed during chlorine disinfection of nitrified wastewater effluent. Using data from the measurements from the SJSC WWTP, estimated removals range from 0 to 60% (Table 2). Diclofenac, and naproxen and to a lesser degree, ibuprofen and propranolol will be removed to an appreciable degree when chlorine disinfection is practiced in the absence of excess ammonia. When ammonia is present during disinfection, only those compounds that react with chloramines (i.e., naproxen and propranolol) will be transformed and the extent of transformation is limited.

Table 2: Estimated removal of acidic drugs and beta-blockers by conventional and advanced treatment processes.

Compound	Estimated Removal (%)				
	WWTP	HOCl	MF/RO	SAT	Wetland
Diclofenac	NA	40	>91	>53	
Gemfibrozil	73	10	>99.5	>98	8.3
Ibuprofen	99.9			>94	>50
Indometacine	NA			>86	
Ketoprofen	NA		>86		
Naproxen	82	60	>96	>95	86
Metoprolol	98	0	>85	85	79
Propranolol	94	20	>84	21	64

Acidic drugs and beta-blockers are effectively removed in advanced treatment plants equipped with reverse osmosis processes. Data collected at the OCWD and West Basin advanced treatment plants indicate that concentrations of PhACs are unaffected by microfiltration. After reverse osmosis treatment, concentrations of PhACs are below the method detection limits. Estimated the lower bounds of the removal efficiency of the compounds is summarized in Table 2. For the compound detected at the highest concentration after microfiltration treatment (i.e., gemfibrozil) we estimate >99.5% removal efficiency. Because the other compounds tested have similar structures to gemfibrozil, it is likely that the removal efficiency of the other compounds is at least as high as that measured for gemfibrozil.

The results observed at the Sweetwater groundwater recharge facility indicate that soil aquifer treatment effectively removes acidic drugs (Table 2). The removal of beta-blockers was incomplete, with measured removals of 85% and 21% for metoprolol and propranolol, respectively. These results are similar to results presented by Drewes et al. (2002), who reported excellent removal of most of the same compounds but not primidone and carbamazepine at this site.

The removal of acidic drugs and beta-blockers in the engineered treatment wetland was limited. Based upon a comparison of concentrations in the influent and effluent of the wetland, it is possible that some transformation occurred (Table 2). However, fluctuations in the composition of the wastewater effluent and mixing in the wetland cells make it difficult to quantify removals accurately. The incomplete removal of pharmaceuticals in the wetland is comparable to results of a more detailed study conducted at the same site to detect the removal of 17β-estradiol and ethinyl estradiol (Gray and Sedlak 2002).

The occurrence data of antibiotics in the secondary effluent from six different municipal wastewater treatment plants are summarized in Figure 9 and 10. Among the antibiotics, sulfamethoxazole, trimethoprim, ciprofloxacin and ofloxacin are the most frequently detected compounds. The veterinary antibiotics sulfamathazine and enrofloxacin were only detected occasionally and usually at lower concentrations than the other antibiotics. Norfloxacin was detected at lower frequency and concentrations probably because of its less common usage compared to ciprofloxacin and ofloxacin for human therapy in the United States according to the published prescription data (RxList). Variation in antibiotic concentrations exits among different treatment plants as well as sampling dates.
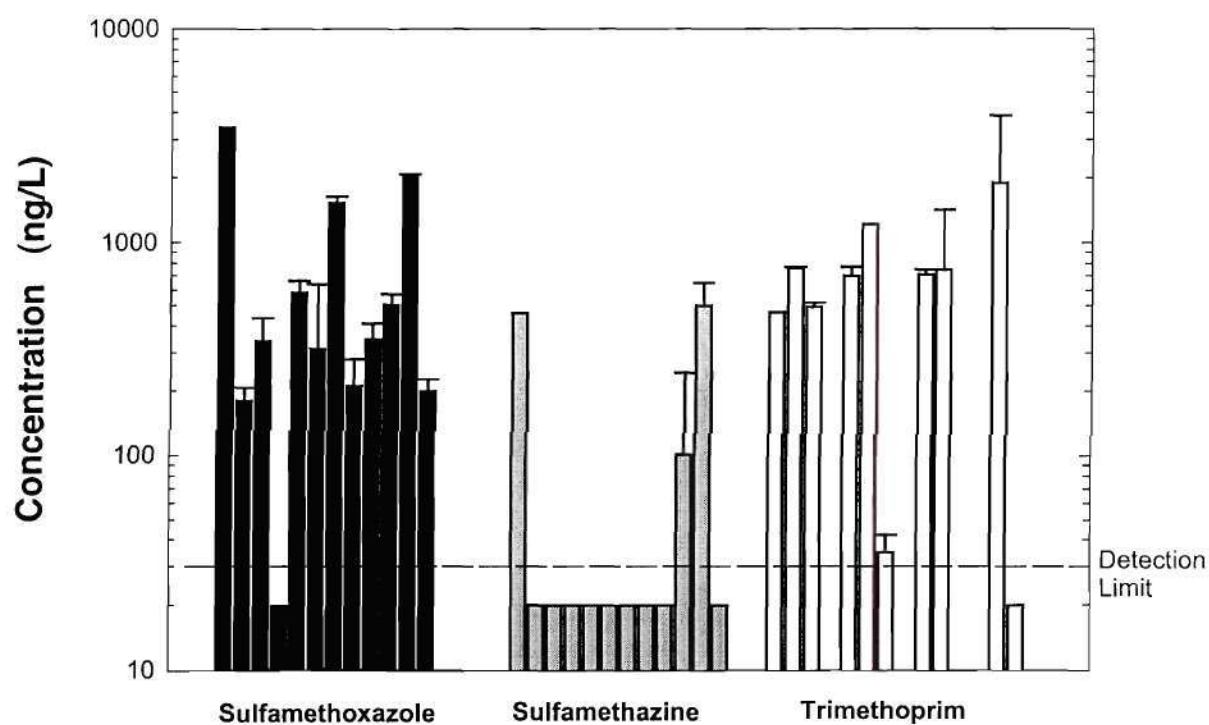
Figure 9. Occurrence of sulfamethoxazole, sulfamethazine and trimethoprim in secondary wastewater effluent (*i.e.*, after activated sludge or biological trickling filter treatment). The samples were collected from a total of six wastewater treatment plants.
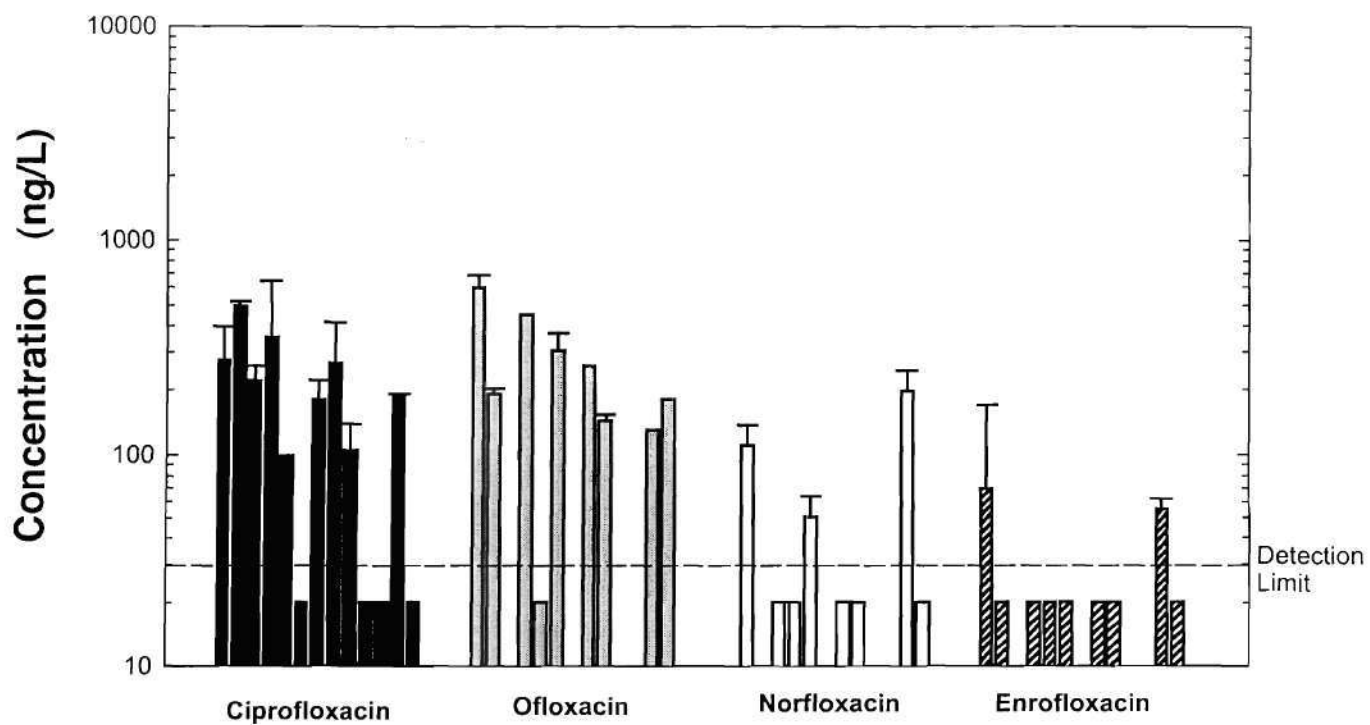
Figure 10. Occurrence of fluoroquinolone antibiotics in secondary wastewater effluent (*i.e.*, after activated sludge or biological trickling filter treatment). The samples were collected from a total of six wastewater treatment plants.

Among the antibiotics, sulfamethoxazole, trimethoprim, ciprofloxacin and ofloxacin are the most frequently detected compounds. Compared to the predicted influent concentrations for these antibiotics, the measured median concentrations measured in secondary wastewater effluent were approximately an order of magnitude lower than the predicted concentrations in sewage, with the exception of ofloxacin, which occurred at approximately half of the predicted concentration. Overall, the experimental observations indicate that the estimates based upon prescription information provide a reasonable upper-bound concentration possible in wastewater effluent. In contrast to the human health antibiotics, the veterinary antibiotics sulfamethazine and enrofloxacin were only detected occasionally, and always at lower concentrations than the other antibiotics.

The occurrence data of antibiotics obtained in this study are also compared to the occurrence data in several previous studies conducted in Europe (Table 3). Golet et al. (2001) reported concentrations of ciprofloxacin and norfloxacin in primary and tertiary wastewater effluent (i.e., after effluent filtration). Although direct comparison of secondary effluent results is not available, the concentration range for ciprofloxacin in secondary effluent is similar to the concentration rage reported by Golet et al. (2001). The median concentration of ciprofloxacin in secondary effluent is between the reported median concentrations of ciprofloxacin in primary and tertiary effluent. In contrast to the results by Golet et al. (2001), significantly lower concentrations of norfloxacin were observed in our study. This is likely due to the difference in antibiotic use patterns between the U.S. and Switzerland.

The concentration rages for sulfamethoxazole and trimethoprim in secondary effluent are comparable to those reported by Hirsh et al. (1999) and Hartig et al. (1999), although the maximum detected concentrations for both antibiotics were higher in this study. Hirsh et al. (1999) determined concentrations of antibiotics in sewage treatment plant effluent and Hartig et al. (1999) measured the concentrations of sulfamethoxazole in secondary effluent. The median concentration of sulfamethoxazole is lower than that reported by Hirsh et al. (1999) while the median concentration of trimethoprim is comparable to that reported by Hirsh et al. (1999). Similar to most of our observations, Hirsh et al. (1999) did not detect significant amounts of sulfamethazine in sewage treatment plant effluent.

Table 3. Comparisons of occurrence of antibiotics in wastewater effluent.

Compound	Predicted influent conc. (including metabolism) (ng/L)	This Study[a]			Other Studies			
		Max. (ng/L)	Min. (ng/L)	Median (ng/L)	Max. (ng/L)	Min. (ng/L)	Median (ng/L)	Reference
Ciprofloxacin	1400 (970-1900)	495	<30	180	405 ± 32^b 108 ± 10^c	249 ± 3^b 45 ± 3^c	291^b 66^c	Golet et al. (2001)
Ofloxacin	350 (250-490)*	600	<30	190	-	-	-	-
Norfloxacin	-	195	<30	<30	367 ± 15^b 120 ± 12^c	270 ± 11^b 48 ± 2^c	295^b 73^c	Golet et al. (2001)
Enrofloxacin	-	70	<30	<30	-	-	-	-
Sulfamethoxazole	3200 (1400-7200)	3400	<30	343	2000^d 1500 ± 320^a	400^d 300 ± 12^a	900^d -	Hirsch et al. (1999) Hartig et al. (1999)
Sulfamethazine	-	500	<30	<30	<20	<20	-	Hirsch et al. (1999)
Trimethoprim	1500 (450-4700)	1890	<30	698	660	320	620	Hirsch et al. (1999)

Note: *Prediction based on levoflcxain, a chiral isomer of ofloxacin; a: Data are for secondary effluent from municipal wastewater treatment plants; b: primary effluent; c: tertiary effluent; d: Data are for effluent from several sewage treatment plants.

Table 4. Occurrence of antibiotics in wastewater effluent after secondary, UV disinfection or chlorination treatment.

Compound	Secondary			UV			Cl₂		
	Max. (ng/L)	Min. (ng/L)	Median (ng/L)	Max. (ng/L)	Min. (ng/L)	Median (ng/L)	Max. (ng/L)	Min. (ng/L)	Median (ng/L)
Ciprofloxacin	495	<30	180	<20	<20	<20	<20	<20	-
Ofloxacin	600	<30	190	205	130	168	45	<20	-
Norfloxacin	195	<30	<30	<20	<20	<20	<20	<20	-
Enrofloxacin	70	<30	<30	<20	<20	<20	<20	<20	-
Sulfamethoxazole	3400	<30	343	2005	323	385	60	<20	-
Sulfamethazine	500	<30	<30	<20	<20	<20	<20	<20	-
Trimethoprim	1890	<30	698	1070	<20	545	<20	<20	-

Note: (i) The secondary treatment includes activated sludge or tricking filter treatment. (ii) The secondary effluent results included 8-13 samples, the UV effluent results included three samples, and the chlorination effluent results included two samples from a total of six wastewater treatment plants.

Table 5. Occurrence of antibiotics in wastewater effluent after advanced treatment.

Compound	Microfiltration			Reverse Osmosis*			GAC			O₃		
	Max. (ng/L)	Min. (ng/L)	Median (ng/L)	Max. (ng/L)	Min. (ng/L)	Median (ng/L)	Max. (ng/L)	Min. (ng/L)	Median (ng/L)	Max. (ng/L)	Min. (ng/L)	Median (ng/L)
Ciprofloxacin	265	<10	-	<10	<10	-	100	<10	<10	20	<10	<10
Ofloxacin	760	360	-	<10	<10	-	40	<10	-	20	<10	-
Norfloxacin	<10	<10	-	<10	<10	-	75	<10	-	<10	<10	-
Enrofloxacin	<10	<10	-	<10	<10	-	55	<10	-	35	<10	-
Sulfamethoxazole	295	195	-	<10	<10	-	545	40	60	40	<10	<10
Sulfamethazine	<10	<10	-	<10	<10	-	455	<10	<10	115	<10	<10
Trimethoprim	670	670	-	<10	<10	-	1535	<10	-	<10	<10	-

Note: The microfiltration and reverse osmosis effluent results included two samples and the GAC and O₃ effluent results included four samples. The MF/RO and GAC/O₃ treatment was used in two separate advanced wastewater treatment plants. *The reverse osmosis process also used chlorine to prevent membrane fouling. This information was not obtained prior to the sampling and thus quenching the residual chlorine was not performed on these samples prior to the analytical procedure.

The concentration range and median concentration of antibiotics measured in effluent of disinfection processes and in advanced treatment processes are summarized in Table 4 and 5. The results suggest that ofloxacin, sulfamethazine and trimethorpin are removed during chlorination. To confirm the susceptibility of antibiotics to reaction with chlorine, we conducted chlorination experiments for the antibiotics in secondary effluent collected from the F. Wayne Hill WWTP. The F. Wayne Hill WWTP utilizes activated sludge treatment operated for biological nutrient removal. The secondary effluent was filtered by glass fiber filters and spiked with antibiotics at 0.1 µg/L and 0.5-1 µg/L for fluoroquinolones and sulfonamides, respectively. Chlorine was then added in the form of NaOCl at 2 mg/L for the experiments with fluoroquinolones and at 10 mg/L for the experiments with sulfonamides and trimethoprim. It is possible, though, that some amounts of chloramine may have formed due to the presence of ammonia in the secondary effluent.

Fast reaction with chlorine was observed for all antibiotics. Figure 11 summarizes the percentage reduction of antibiotic concentration after 30 minute of chlorination contact time, indicating that the antibiotics are removed by 46 to 99%. Although chlorination of ofloxacin was not examined in secondary effluent, chlorination of ofloxacin in river water matrix at similar antibiotic and chlorine concentrations yielded 95% of removal (data not shown). The fast reaction and significant removal of antibiotics by chlorine in complicated matrices suggest that elimination of antibiotics may occur during the chlorination process in wastewater treatment plants. The estimated percentage of removal by chlorination based upon the occurrence data is summarized in Table 6.
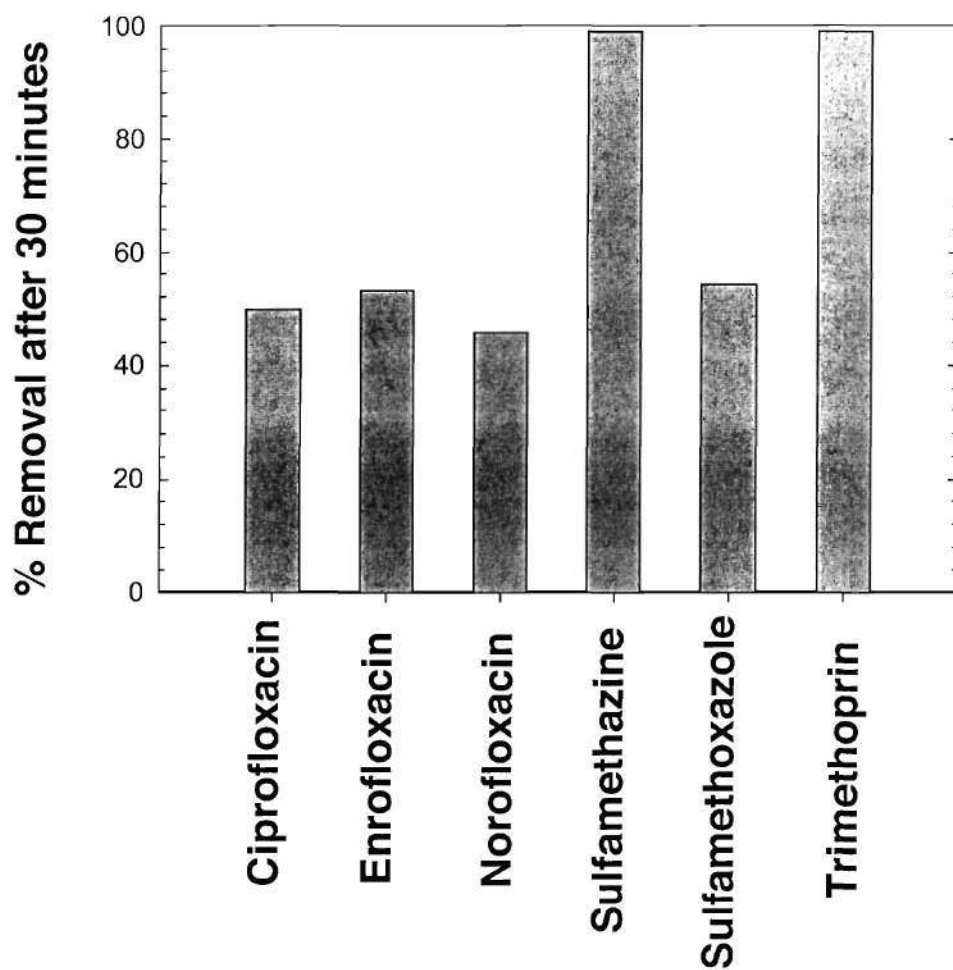
Figure 11. Removal of antibiotics by chlorination in secondary wastewater effluent. Antibiotic concentrations: 0.1 µg/L for fluoroquinolones and 0.5-1 µg/L for sulfonamides and trimethoprim. Chlorine dosages: 2 mg/L for fluoroquinolones and 10 mg/L for sulfonamides and trimethoprim in the form of NaOCl.

Table 6. Removal of antibiotics by wastewater treatment processes and soil aquifer treatment (SAT) and in an engineered wetland.

Compound	Estimated Removal (%)					
	UV[a]	Cl$_2$[a]	MF/RO[a]	GAC/O$_3$[a]	SAT	Wetland
Ciprofloxacin	>81	80	>95	>89	91	-
Ofloxacin	>10	85	98	>85	98	-
Norfloxacin	-	>60	-	>95	-	-
Enrofloxacin	-	-	-	-	93	-
Sulfamethoxazole	0	90	>94	>92	94	0
Sulfamethazine	-	-	-	>77	-	-
Trimethoprim	0-97[b]	98	99	>99	95	46

a: The removal was calculated in comparison with the secondary effluent concentrations; The concentrations of norfloxacin, enrofloxacin and sulfamethazine were often too low to determine the percentage of removal; b: Based upon two sampling events, one indicated no removal and the other indicated near 97% of removal.

The occurrence results suggests that UV disinfection eliminates greater than 80% of ciprofloxacin but removes ofloxacin and sulfamethoxazole poorly (0-10%) (Tables 4 and 5). Based upon the results of two sampling events, very different removals (0 vs. 97%) by UV disinfection were observed for trimethoprim and more analyses are necessary before a conclusion can be made. The removal of ciprofloxacin during UV treatment is consistent with its observed reactivity with sunlight.

Advanced treatment processes, microfiltration followed by reverse osmosis and granular activated carbon filtration followed by ozonation, effectively reduce the concentrations of antibiotics to below the detection limit (Table 5). The removals by the advanced treatment processes are 90% or higher for most antibiotics (Table 6). However, caution needs to be exercised for the conclusion of reverse osmosis. As mentioned earlier, the residual chlorine in the RO effluent may contribute to the reduction of antibiotic concentrations. Thus more analyses with quenching the residual chlorine are necessary to eliminate any potential experimental artifacts and obtain accurate results. However, since chlorine is used within the RO process, it

may be possible that both chlorine and membrane filtration remove the antibiotics and the two mechanisms may be difficult to distinguish.

The removal percentage by the soil aquifer treatment is estimated by comparing the concentrations of antibiotics in the infiltration basin at the Sweetwater groundwater recharge site with the concentration of antibiotics in the deep monitoring well (30.5 meter). The removals were greater than 90% for most of the antibiotics (Table 6). Only one set of occurrence data have been obtained thus far for the Mt. View engineered wetland. Comparing the concentrations of antibiotics in the wetland influent to those in the wetland effluent, the removal was estimated to be negligible for sulfamethoxazole and about 46% for trimethoprim. The concentrations of fluoroquinolones were too low to determine the removal percentage. We plan to conduct further analyses at the Mt. View wetland to obtained clearer conclusions.

PLANS FOR NEXT PERIOD

Task 1: Literature Review

Sub-Task 1A: Drugs Used in Human Therapy

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Sub-Task 1B: Drugs Used in Animal Husbandry

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Task 2: Analytical Method Development and Testing

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

During the next project period no new research is planned is association with this task.

Sub-Task 2B: Antibiotics

During the next project period no new research is planned is association with this task.

Task 3: Occurrence Survey

Sub-Task 3A: Site Selection

No further activity is planned in association with this task.

Sub-Task 3B: Sample Collection and Analysis

As described in the previous project report, we prepared a proposal for continuation funding to support further sample collection and analysis by Dr. Huang at Georgia Tech. However, funding was not available from AWWARF. Therefore, we will not expand the scope of Dr. Huang's participation significantly. During the next project period, we will collect nd analyze samples from two or three additional sites to confirm results described in the previous sections.

Sub-Task 3C: Data Analysis and Synthesis

As described in this project report, we are making progress in the analysis of our data and plan to write a manuscript detailing the results of the occurrence survey. A draft of the manuscript will be shared with the PAC prior to submission.

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APPENDIX A: Summary of Data for Acidic Drugs and Beta-Blockers during the Seventh Project Period

Compound	Location	Date	Concentration (ppt)
Diclofenac	Blank	6/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	60
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	30,41
	Southeast San Francisco: pre chlorination	7/1/02	48
	Southeast San Francisco: post chlorination	7/1/02	60
Gemfibrozil	Blank	6/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	250
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	230,240
	Southeast San Francisco: pre chlorination	7/1/02	640
	Southeast San Francisco: post chlorination	7/1/02	540
Ibuprofen	Blank	6/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	<10
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	<10
	Southeast San Francisco: pre chlorination	7/1/02	<10
	Southeast San Francisco: post chlorination	7/1/02	<10
Indometacine	Blank	6/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	<10
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	<10
	Southeast San Francisco: pre chlorination	7/1/02	<10
	Southeast San Francisco: post chlorination	7/1/02	<10
Ketoprofen	Blank	6/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	11
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	<10
	Southeast San Francisco: pre chlorination	7/1/02	14
	Southeast San Francisco: post chlorination	7/1/02	<10
Naproxen	Blank	6/26/02	<10
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	230
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	41,160
	Southeast San Francisco: pre chlorination	7/1/02	290
	Southeast San Francisco: post chlorination	7/1/02	640
Mecoprop*	Blank	6/26/02	
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	66%
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	67%,76%
	Southeast San Francisco: pre chlorination	7/1/02	98%
	Southeast San Francisco: post chlorination	7/1/02	101%

* Recovery of labeled mecoprop (internal standard) added to samples at 1,000 ng/L.

Compound					Recovery (%)
Metoprolol	Blank	6/26/02	<10		47%
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	61		
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	51,57		
	Southeast San Francisco: pre chlorination	7/1/02	33		71%,68%
	Southeast San Francisco: post chlorination	7/1/02	100,20		
	Southeast San Francisco: post chlorination	8/14/02	42		
	Mt. View Final Effluent	8/21/02	9		
Propranolol	Blank	6/26/02	<10		41%
	San Jose/Santa Clara Effluent: pre chlorination	6/26/02	36		
	San Jose/Santa Clara Effluent: post chlorination	6/26/02	35,33		
	Southeast San Francisco: pre chlorination	7/1/02	<10		55%, 53%
	Southeast San Francisco: post chlorination	7/1/02	<10		
	Southeast San Francisco: post chlorination	8/14/02	20		
	Mt. View Final Effluent	8/21/02	5		

APPENDIX B: Summary of Occurrence Data for Antibiotics during the Seventh Project Period

Compound	Location	Date	Concentration (μg/L)[1]	Concentration (μg/L)[2]	Spike Recov.[1]	Spike Recov.[2]
Sulfamethoxazole	West Basin WWTP – MF Influent	5/22/2002	0.41, 0.27	0.10	15% [b]	8% [b]
	West Basin WWTP – MF Effluent	5/22/2002	0.27, 0.32	0.10	2% [b]	4% [b]
	West Basin WWTP – RO Effluent	5/22/2002	<LOD, <LOD	<LOD	0% [b]	0% [b]
	DI Water Sample	5/22/2002	NA	NA	67%	16%
	South Cobb WWTP - Secondary	6/12/02	0.64, 0.51	0.13	34%	7%
	South Cobb WWTP - Final (HOCl)	6/12/02	0.07, 0.05	0.02	57%	15%
	FWH AWWTP - Primary	7/17/02	0.97	NA	NA	NA
	FWH AWWTP - Secondary	7/17/02	0.22, 0.18	NA	NA	NA
	FWH AWWTP - GAC	7/17/02	0.04	NA	NA	NA
	FWH AWWTP - Final (O₃)	7/17/02	<LOD	NA	NA	NA
	Clayton WWTP, GA - Secondary	8/20/02	0.16, 0.26	0.11	NA	26% [b]
	Clayton WWTP, GA - Final (UV)	8/20/02	0.44, 0.33	0.18, 0.14	NA	5% [b]
	Hugh Wyckoff WTP – Intake	8/29/02	<LOD, <LOD	<LOD, <LOD	69%	77%
	Hugh Wyckoff WTP - Final (HOCl)	8/29/02	<LOD	<LOD	NA	NA
	J. W. Smith WTP - Intake	9/6/02	<LOD, <LOD	<LOD, <LOD	44%	50%
	J. W. Smith WTP - Final (HOCl)	9/6/02	<LOD	<LOD	NA	NA
	DI Water Sample	9/6/02	NA	NA	48%	35%
	DI Water Sample	9/6/02	NA	NA	48%	37%
Sulfamethazine	West Basin WWTP – MF Influent	5/22/2002	<LOD, <LOD	<LOD	0% [b]	0% [b]
	West Basin WWTP – MF Effluent	5/22/2002	<LOD, <LOD	<LOD	0% [b]	0% [b]
	West Basin WWTP – RO Effluent	5/22/2002	<LOD, <LOD	<LOD	0% [b]	0% [b]
	DI Water Sample	5/22/2002	NA	NA	NA	104%
	South Cobb WWTP - Secondary	6/12/02	<LOD, <LOD	<LOD	39%	51%
	South Cobb WWTP - Final (HOCl)	6/12/02	<LOD, <LOD	<LOD	48%	66%
	FWH AWWTP - Primary	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Secondary	7/17/02	<LOD, <LOD	NA	NA	NA
	FWH AWWTP - GAC	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Final (O₃)	7/17/02	<LOD	NA	NA	NA
	Clayton WWTP, GA - Secondary	8/20/02	<LOD, <LOD	<LOD	NA	0% [b]
	Clayton WWTP, GA - Final (UV)	8/20/02	<LOD, <LOD	<LOD, <LOD	NA	4% [b]
	Hugh Wyckoff WTP – Intake	8/29/02	<LOD, <LOD	<LOD, <LOD	64%	92%
	Hugh Wyckoff WTP - Final (HOCl)	8/29/02	<LOD	<LOD	NA	NA
	J. W. Smith WTP - Intake	9/6/02	<LOD, <LOD	<LOD, <LOD	44%	70%
	J. W. Smith WTP - Final (HOCl)	9/6/02	<LOD	<LOD	NA	NA
	DI Water Sample	9/6/02	NA	NA	104%	61%
	DI Water Sample	9/6/02	NA	NA	104%	65%

Compound	Location	Date	Concentration (μg/L)[1]	Concentration (μg/L)[2]	Spike Recov.[1]	Spike Recov.[2]
Trimethoprim	West Basin WWTP – MF Influent	5/22/2002	0.74, 0.64	0.14, 0.13	97%	170%
	West Basin WWTP – MF Effluent	5/22/2002	0.67, 0.67	0.09, 0.11	83%	120%
	West Basin WWTP – RO Effluent	5/22/2002	<LOD, <LOD	<LOD, <LOD	0% [b]	0% [b]
	DI Water Sample	5/22/2002	NA	NA	97%	87%
	South Cobb WWTP - Secondary	6/12/02	0.03, 0.04	0.04	101%	37%
	South Cobb WWTP - Final (HOCl)	6/12/02	<LOD, <LOD	<LOD	98%	35%
	FWH AWWTP - Primary	7/17/02	0.02	NA	NA	NA
	FWH AWWTP - Secondary	7/17/02	0.02, 0.02	NA	NA	NA
	FWH AWWTP - GAC	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Final (O₃)	7/17/02	<LOD	NA	NA	NA
	Clayton WWTP, GA - Secondary	8/20/02	1.22, 0.27	0.69	NA	168%
	Clayton WWTP, GA - Final (UV)	8/20/02	<LOD, <LOD	<LOD, <LOD	NA	282% [a]
	Hugh Wyckoff WTP – Intake	8/29/02	<LOD, <LOD	<LOD, <LOD	111%	605% [a]
	Hugh Wyckoff WTP - Final (HOCl)	8/29/02	<LOD	<LOD	NA	NA
	J. W. Smith WTP - Intake	9/6/02	<LOD, <LOD	<LOD, <LOD	139%	553% [a]
	J. W. Smith WTP - Final (HOCl)	9/6/02	<LOD	<LOD	NA	NA
	DI Water Sample	9/6/02	NA	NA	109%	64%
	DI Water Sample	9/6/02	NA	NA	69%	67%
Ciprofloxacin	West Basin WWTP – MF Influent	5/22/2002	0.56, 0.14	0.41	29%	116%
	West Basin WWTP – MF Effluent	5/22/2002	<LOD, <LOD	<LOD	74%	93%
	West Basin WWTP – RO Effluent	5/22/2002	0.02, <LOD	<LOD	7% [b]	5% [a]
	DI Water Sample	5/22/2002	NA	NA	96%	67%
	South Cobb WWTP - Secondary	6/12/02	<LOD, <LOD	<LOD	94%	20%
	South Cobb WWTP - Final (HOCl)	6/12/02	<LOD, <LOD	<LOD	40%	62%
	FWH AWWTP - Primary	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Secondary	7/17/02	<LOD, <LOD	NA	NA	NA
	FWH AWWTP - GAC	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Final (O₃)	7/17/02	<LOD	NA	NA	NA
	Clayton WWTP, GA - Secondary	8/20/02	0.13, 0.08	0.05	NA	68%
	Clayton WWTP, GA - Final (UV)	8/20/02	<LOD, <LOD	<LOD, <LOD	NA	74%
	Hugh Wyckoff WTP – Intake	8/29/02	<LOD, <LOD	<LOD, <LOD	84%	90%
	Hugh Wyckoff WTP - Final (HOCl)	8/29/02	<LOD	<LOD	NA	NA
	J. W. Smith WTP - Intake	9/6/02	<LOD, <LOD	<LOD, <LOD	86%	80%
	J. W. Smith WTP - Final (HOCl)	9/6/02	<LOD	<LOD	NA	NA
	DI Water Sample	9/6/02	NA	NA	146%	104%
	DI Water Sample	9/6/02	NA	NA	167%	111%

Compound	Location	Date	Concentration (μg/L)[1]	Concentration (μg/L)[2]	Spike Recov.[1]	Spike Recov.[2]
Norfloxacin	West Basin WWTP – MF Influent	5/22/2002	<LOD, <LOD	<LOD	106%	65%
	West Basin WWTP – MF Effluent	5/22/2002	<LOD, <LOD	<LOD	53%	43%
	West Basin WWTP – RO Effluent	5/22/2002	<LOD, <LOD	<LOD	3% [b]	0% [b]
	DI Water Sample	5/22/2002	NA	NA	109%	67%
	South Cobb WWTP - Secondary	6/12/02	0.06, 0.04	0.02	85%	58%
	South Cobb WWTP - Final (HOCl)	6/12/02	<LOD, <LOD	<LOD	60%	25%
	FWH AWWTP - Primary	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Secondary	7/17/02	<LOD, <LOD	NA	NA	NA
	FWH AWWTP - GAC	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Final (O₃)	7/17/02	<LOD	NA	NA	NA
	Clayton WWTP, GA - Secondary	8/20/02	<LOD, <LOD	<LOD	NA	95%
	Clayton WWTP, GA - Final (UV)	8/20/02	<LOD, <LOD	<LOD, <LOD	NA	91%
	Hugh Wyckoff WTP – Intake	8/29/02	<LOD, <LOD	<LOD, <LOD	96%	78%
	Hugh Wyckoff WTP - Final (HOCl)	8/29/02	<LOD	<LOD	NA	NA
	J. W. Smith WTP - Intake	9/6/02	<LOD, <LOD	<LOD, <LOD	90%	70%
	J. W. Smith WTP - Final (HOCl)	9/6/02	<LOD	<LOD	NA	NA
	DI Water Sample	9/6/02	NA	NA	156%	99%
	DI Water Sample	9/6/02	NA	NA	174%	109%
Enrofloxacin	West Basin WWTP – MF Influent	5/22/2002	<LOD, <LOD	<LOD	98%	82%
	West Basin WWTP – MF Effluent	5/22/2002	<LOD, <LOD	<LOD	76%	91%
	West Basin WWTP – RO Effluent	5/22/2002	<LOD, <LOD	<LOD	8% [b]	6% [b]
	DI Water Sample	5/22/2002	NA	NA	112%	92%
	South Cobb WWTP - Secondary	6/12/02	<LOD, <LOD	<LOD	77%	27%
	South Cobb WWTP - Final (HOCl)	6/12/02	<LOD, <LOD	<LOD	43%	12%
	FWH AWWTP - Primary	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Secondary	7/17/02	<LOD, <LOD	NA	NA	NA
	FWH AWWTP - GAC	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Final (O₃)	7/17/02	<LOD	NA	NA	NA
	Clayton WWTP, GA - Secondary	8/20/02	<LOD, <LOD	<LOD	NA	102%
	Clayton WWTP, GA - Final (UV)	8/20/02	<LOD, <LOD	<LOD, <LOD	NA	101%
	Hugh Wyckoff WTP – Intake	8/29/02	<LOD, <LOD	<LOD, <LOD	148%	107%
	Hugh Wyckoff WTP - Final (HOCl)	8/29/02	<LOD	<LOD	NA	NA
	J. W. Smith WTP - Intake	9/6/02	<LOD, <LOD	<LOD, <LOD	119%	69%
	J. W. Smith WTP - Final (HOCl)	9/6/02	<LOD	<LOD	NA	NA
	DI Water Sample	9/6/02	NA	NA	116%	58%
	DI Water Sample	9/6/02	NA	NA	151%	68%

Compound	Location	Date	Concentration (μg/L)[1]	Concentration (μg/L)[2]	Spike Recov.[1]	Spike Recov.[2]
Ofloxacin	West Basin WWTP – MF Influent	5/22/2002	0.45	0.83	33%	87%
	West Basin WWTP – MF Effluent	5/22/2002	0.76, 0.36	0.55	76%	111%
	West Basin WWTP – RO Effluent	5/22/2002	<LOD, <LOD	<LOD	2% [b]	0% [b]
	DI Water Sample	5/22/2002	NA	NA	115%	80%
	South Cobb WWTP - Secondary	6/12/02	0.26, 0.35	0.21	83%	84%
	South Cobb WWTP - Final (HOCl)	6/12/02	0.05, 0.04	0.01	102%	80%
	FWH AWWTP - Primary	7/17/02	0.61	NA	NA	NA
	FWH AWWTP - Secondary	7/17/02	0.18	NA	NA	NA
	FWH AWWTP - GAC	7/17/02	<LOD	NA	NA	NA
	FWH AWWTP - Final (O$_3$)	7/17/02	<LOD	NA	NA	NA
	Clayton WWTP, GA - Secondary	8/20/02	0.14, 0.15	0.14	NA	112%
	Clayton WWTP, GA - Final (UV)	8/20/02	0.16, 0.10	0.15, 0.09	NA	113%
	Hugh Wyckoff WTP – Intake	8/29/02	<LOD, <LOD	<LOD, <LOD	100%	107%
	Hugh Wyckoff WTP - Final (HOCl)	8/29/02	<LOD	<LOD	NA	NA
	J. W. Smith WTP - Intake	9/6/02	<LOD, <LOD	<LOD, <LOD	132%	117%
	J. W. Smith WTP - Final (HOCl)	9/6/02	<LOD	<LOD	NA	NA
	DI Water Sample	9/6/02	NA	NA	127%	107%
	DI Water Sample	9/6/02	NA	NA	130%	107%

Note: The reported concentrations were not corrected by recoveries. Spike concentrations were 1.0 μg/L.

Approximate Method Levels of Detection (LOD): 5-10 ng/L for drinking water and DI water; 10 ng/L for advanced treatment process (RO, GAC and O$_3$) effluent and surface water, 20 ng/L for disinfection (chlorination and UV disinfection) effluent; 20-70 ng/L for secondary wastewater effluent depending upon sample matrices (most secondary effluent has LOD around 20-30 ng/L).

(1) Quantification based on standard addition method
(2) Quantification based on internal standard method
(a) Recovery overestimated due to differences with the internal standard in the amount of signal suppression
(b) Low recovery due to problems with the extraction step

APPENDIX C: Responses to PAC Comments on the Sixth Periodic Report

As you can see from the limited number of comments, everyone is pleased with the progress of the study. The report is well written, and it accurately reflects the work effort and the quality of the work. The work on method development is well documented and well presented.

Specific Comments

Page 9...still below target values. The QA/QC program listed in the appendix needs to be referenced. This will allow the reader to know that the QA/QC plan is in the document. *Response:* Will do so in the final report.

The search for suitable surrogate standards to enable the accurate quantification of antibiotics seems to be one of the major tasks in the future. Dr. Heberer and his research team are currently discussing the issue in terms of their projects. They are trying to acquire several radio-labeled compounds. This effort is conducted with several other working groups to save money and make the analytical methods and results more comparable. This will also become a matter of interlaboratory tests.

As long as no suitable surrogates are available, the approach using standard addition seems to be superior. Nevertheless, the strong suppression of the signals observed for the sulfonamides for the samples collected from the shallow wells and the pond at the Sweetwater Recharge Facility (figure 9, p.21) may be problematic, especially, because it doesn't appear to be very reproducible. Thus, similar samples collected at different dates (figure 5, p.16) resulted in acceptable to no recovery! In view of such results, how can it be assured that the recovery of a spiked sample is comparable to that of an original sample?

Response: The exceptionally high amount of organic matter in the Sweetwater pond samples caused strong signal suppression for sulfonamides and thus significantly decrease the method sensitivity. This reduced sensitivity is mostly responsible for the inability to accurately determine recoveries. For exceptionally dirty samples, accurate assessment of recovery will be difficult unless more selective cleanup methods can be used to remove the interfering organic matter.

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It is stated that the only thing the DOC does is suppress signal (Page 22), it could also enhance it. USGS and Dupont studies have shown that there may be some kind of electrochemical reactivity going, as the droplets get smaller, that cause signal enhancement. Some of the figures presented (p. 15, 18) show recovery greater than 125%. This could be some of that problem.

Response: Although signal enhancement is a possibility, signal enhancement was rarely observed in our analyses. In our matrix effect assessment, all antibiotics show susceptibility to signal suppression in sample matrices (Figure 8 and 9). Therefore, we attribute the high recoveries to the errors caused by the internal standard method.

In that context, it will be helpful to have data provided (again perhaps for the final report), summarizing all of the DOC data and perhaps interpreting the data in relation to DOC. For example, could the greater concentration of a chemical in WWTP effluent compared to influent be due to matrix effects.

Response: A good idea. We have measured UV_{254} absorbance for all the collected samples and will assess whether these information can assist data interpretation.

On page 25, new investigations are described to explain the possible loss of the analytes from chlorinated samples. What is the final outcome of these investigations? Does this loss only appear during sample storage without adding sodium thiosulfate? It is stated that no losses were observed in the facilities (contact time 1 hour). How long and at which temperatures can the samples be stored without any losses?

Response: The results of these analyses are presented in the current project report. Some compounds appear to be removed under conditions encountered in wastewater disinfection systems. Failure to add a quenching agent to samples that contain a chlorine residual would likely lead to artifacts in samples measurement.

Figures 10 and 11 (p.26, 27): It looks rather confusing when "concentrations below the detection limit are plotted at half of the detection limit"! Which concentrations are meant?

These compounds have not been detected in the samples, thus, no concentrations can be assigned to these samples! In a nutshell, does it make sense to provide concentrations for results that are below the limits of quantification even if they are above the limit of detection? Certainly not. Therefore it would make sense to add dotted lines to the bar charts to indicate the limits of quantification. These considerations are also important to interpret the possible attenuation of the analytes in the sub-soil!

Response: Dotted lines have been added to the bar graphs to indicate that the values reported are below the method detection limit. We believe this is preferable to not plotting any data because that might imply that the analysis was not performed.

Graphs on pages 28-34 have no legend. It is difficult to determine what the bars represent.
Response: The black and grey bars represent results from duplicate analyses.

For the journal articles that are going to be written and for another appendix (page 47), you may want to include a detailed explanation of your acceptance criteria for the fragmentation and parent ions. Do you just use the parent, do you ratio to the parent, etc.
Response: The acceptance criteria for the fragmentation and parent ions for antibiotics was briefly discussed in the QA/QC plan (Appendix D, the 6th report): "Antibiotics will be identified by its chromatographic retention time, molecular ion and confirming fragment ions. The relative abundance of ions needs to agree with the correct ratio without exceeding 15% in difference." For the future publication and final report, we will also include these information.

In appendix B, for both Sulfamethozole and Sulfamethazine at the Sweetwater Recharge the concentrations in the pond were undetected while levels in the shallow well and or the Deep well were higher. In the case of the first set of testing done for sulfamethoazine at this location the pond concentrations were undetectable, the levels at shallow and deep wells were increasingly higher. Any explanation? Does the standard impact these results? When the standard addition method was used instead of the internal standard, this did not occur.
Response: As mentioned earlier, the high amount of organic matter in the Sweetwater pond samples caused strong signal suppression and significantly decreased the method sensitivity for

both sulfonamides. Therefore, sulfamethoxazole and sulfamethazine could not be detected in the pond water. In the shallow well and particularly deep well samples, signal suppression is less because the amount of organic matter in the samples is less. The method sensitivity is considerably higher in shallow well and deep well samples and thus sulfamethoxazole and sulfamethazine could be detected. This change in limit of detection (LOD) is caused by the different amount of organic matter in samples and is not related to the quantification method (internal standard or standard addition method). Also, at the second time of analyses for the Sweetwater samples, the buffer concentration for the LC/MS mobile phases was decreased to improve the limit of detection.

General comments

With all of the different constituents, and different methods applied to the constituents it is somewhat difficult to follow the approach taken for each class of chemicals and the specific chemicals. At some point, perhaps the final report, I recommend a table, grouped by class (e.g. fluoroquinolones) that summarizes the different steps taken for quantification, summarizes the problems, and summarizes the final selected method along with appropriate QA/QC info (i.e. recoveries, etc.). This is important so that other investigators can build upon this effort and not follow the wrong path in the future.

Response: This table will be included in the final report.

The USGS study should be cited (March 15, 2002-ES&T) in literature survey to identify whether there was any overlap of methods or results.

Response: The USGS survey will be included in the updated literature review.

OCCURRENCE SURVEY OF PHARMACEUTICALLY ACTIVE COMPOUNDS

Eighth Progress Report

January 15, 2003

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SUMMARY

During the seventh project period, we analyzed samples from a variety of locations as part of the occurrence survey. We also continued our analysis of the data and continued to prepare manuscripts for publication in peer-reviewed scientific journals.

During the eighth project period, we completed the collection and analysis of samples for the occurrence survey, submitted a manuscript describing one of our analytical methods to a scientific journal and prepared an outline of a paper reporting the results of the occurrence survey.

Samples were collected as part of the occurrence survey from a conventional wastewater treatment plant, three advanced wastewater treatment plants, an engineered treatment wetland and a background site. Measurements of the concentrations of pharmaceuticals collected before and after chlorination at the conventional wastewater treatment plant confirmed our prior observations about the effect of chlorine disinfection on certain pharmaceuticals. Data collected at the advanced treatment plants confirmed earlier observations that reverse osmosis effectively removes all of the pharmaceuticals. Finally, data collected at the engineered treatment wetland and associated wastewater treatment plant suggested some removal of antibiotics occurs during UV disinfection and no attenuation occurs in an engineered treatment wetland.

In anticipation of the project completion, progress has been made in disseminating results of the study to the scientific community. One manuscript describing the development of new solid-phase extraction and HPLC/MS method was submitted to the *Journal of Chromatography A*. An outline for a second manuscript describing the results of the occurrence survey has been prepared and will be developed into a full manuscript during the next project period.

CONTENTS

FIGURES

TABLES

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PROGRESS THIS PERIOD

TASK 1: LITERATURE REVIEW

The objective of this task is to identify compounds to be studied during the occurrence survey. The selection criteria for compounds in the occurrence survey include their expected concentrations in water supplies, environmental fate, potential effects and availability of suitable analytical methods. Progress on the literature review was presented in the first and second progress reports. The literature review has been completed and will be updated prior to preparation of the final report. No additional progress related to task one was made during the current project period.

Sub-Task 1A: Drugs Used in Human Therapy

In the first progress report, we reviewed data on prescription drugs likely to be present in wastewater effluent in the United States. Using data on the number of prescriptions for the top 200 drugs and information on prescription formulations and metabolism, we estimated ranges of concentrations of 136 drugs expected to be present in wastewater effluent. In addition, we reviewed published occurrence data to identify other compounds of interest that did not appear among the common prescription drugs. A total of eleven drugs were identified for further consideration.

During the current project period no activity was conducted in association with this sub-task.

Sub-Task 1B: Drugs Used in Animal Husbandry

In the second progress report, we reviewed data on antibiotics used in livestock both therapeutically to treat diseases and sub-therapeutically as feed additives to promote growth. We focused our review on antibiotics used in animal feeding operations, because these sources could result in concentrated discharges of antibiotics. We also reviewed fate and transport properties and available analytical methods. On the basis of these results and our review of antibiotics used in human therapy, we identified that sulfonamide and fluoroquinolone antibiotics as the most probable water contaminants, followed by macrolide antibiotics. Among these antibiotic classes, we identified five antibiotics that merit further consideration.

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During the current project period no activity was conducted in association with this sub-task.

TASK 2: ANALYTICAL METHOD DEVELOPMENT AND TESTING

The objective of this task was to identify and test analytical methods that will be used during the occurrence survey. As part of the task, analytical methods for extraction, cleanup and derivitization were tested by adding drugs to deionized water and to samples collected from sites being considered for inclusion in the occurrence survey. Analysis of antibiotics was included as a separate task from the other pharmaceutically active compounds (PhACs) because the analytical methods for antibiotics (e.g., HPLC/MS) are fundamentally different from the gas chromatography methods used for the other compounds.

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

In the first progress report, we described our preliminary efforts to develop suitable analytical methods for eleven compounds identified as part of the literature survey. We used solid phase extraction followed by derivitization and GC/MS/MS to quantify pharmaceuticals in deionized water and in samples collected from several of the candidate sampling sites. To quantify recoveries and matrix effects, all samples also were analyzed after addition of known quantities of analytes.

During the second project period, we focused our attention on improving the accuracy and precision of the analytical methods by analyzing samples from three sites being considered for inclusion in the occurrence survey. As a result of analytical difficulties, we did not analyze three of the compounds (i.e., carbamazepine, nabumetone and nadolol) during the second project period. For the remaining eight compounds, analysis of duplicate samples indicated that, with the exception of two beta-blockers, results were reproducible (i.e., we observed an average of 26% error between duplicate samples). Analysis of blank samples indicated that sample carryover and cross contamination were not significant problems. Analysis of spike recovery samples indicated variable recoveries, with values as low as 30% for some analytes. All of the drugs were detected in one or more of the unspiked wastewater effluent samples.

During the third project period, we continued to improve the analytical methods by identifying steps where analytes were lost during analysis. For the acidic drugs, we changed the

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solid phase extraction technique and added labeled mecoprop as an internal standard. As a result of the new SPE method, spike recoveries improved significantly. For the beta-blockers, we increased the time of the drying step to improve the efficiency of derivitization, but this only had a minor effect on spike recoveries. We also eliminated the use of PFE-lined containers, which resulted in losses of beta-blockers during storage. A QA/QC plan also was submitted to the PAC.

During the fourth project period, we attempted to resolve the remaining issues associated with the analytical methods. Attempts to use labeled propranolol as an internal standard for the beta-blockers failed because the labeled compound could not be discriminated from the unlabeled compounds. Alternative surrogates for beta-blockers could be derivitized and analyzed, but were too polar to be retained during solid phase extraction. We also evaluated the variability in method performance for acidic drugs and beta-blockers by analyzing a total of 18 samples from two surface waters and an advanced wastewater treatment plant. Analysis of surface water samples from a site that was subjected to chlorine disinfection indicated that several of the analytes were lost in the presence of free chlorine. We also tested a GC/MS/MS technique for analysis of carbamazepine. The compound could be detected easily at high concentrations. However, sensitivity decreased significantly at low concentrations, possibly as the result of losses in the injection port of the GC.

During the fifth project period we evaluated the possible use of epinephrine and deoxyepinephrine as internal standards in the analysis of beta-blockers. While it was possible to analyze the derivatives, they were lost during the extraction, solvent transfer and blow-down steps to a much greater degree than the other compounds. Therefore, we decided to limit our assessment of recovery to matrix spike recovery measurements with both beta-blockers for each set of samples.

During the fifth project period we also attempted to improve the recovery of carbamazepine by modifying the SPE method and by conditioning the injection port liner and replacing it after each set of analyses. Results of our analyses indicated that carbamazepine was present at a concentration around 1,000 ng/L in the effluent of the Mt. View WWTP and in the pond at the Sweetwater recharge facility. However, we were unable to obtain reproducible, linear standard curves because the injection port liners could only be used for a few samples before they had to be replaced. Given the numerous analytical challenges associated with the

analysis of carbamazepine and the need to complete the occurrence survey, we decided to forgo any further attempt to analyze this compound.

No further activities associated with method development were conducted during the seventh or eighth project periods.

Sub-Task 2B: Antibiotics

Our analysis of antibiotics has focused on fluoroquinolone and sulfonamide antibiotics. Ciprofloxacin, sulfamethoxazole and sulfamethazine are the selected target analytes of occurrence analysis. In the second and third progress reports, we reported our efforts to develop suitable analytical methods for these compounds. A dual-cartridge solid phase extraction (SPE) method was developed to extract antibiotics from water samples. Analysis of antibiotics was conducted by LC/MS and LC/FLD (fluorescence detection).

During the fourth progress report, several efforts were made to improve the analytical methods. We identified the steps where analytes were lost and made changes to minimize the loses. High-density polyethylene conical tubes were shown to yield the minimum loses of fluoroquinolones during the blow down step and were used in all the later experiments. We determined that acidifying and storing samples in amber glass bottles best preserve analytes prior to solid-phase extraction and yield minimum losses of analytes through adsorption to the container walls. Sample extracts were also found to be better preserved at 0 C than at 5 C when analysis by LC/MS could not be conducted immediately. To achieve better results, separate LC/MS methods were developed for sulfonamide and fluoroquinolone antibiotics, respectively. LC/MS conditions were modified to reduce matrix effect and increase sensitivity. We investigated several structurally related sulfonamide and fluoroquinolone antibiotics as internal standards. Our studies indicate that sulfamerazine and enrofloxacin serve as appropriate internal standards. These research efforts have improved the method recovery to be above 60% and have also increased sensitivity in detection.

Near the end of the fourth project period, the cation-exchange extraction method for fluoroquinolones was found to work successfully in reagent water after finding a proper cation-exchanger (high-density, mixed-phase cation-exchange discs, 3M) and avoided errors in sample preparation. The cation-exchange extraction followed by HPLC/fluorescence detection is a simple and sensitive method that can be easily performed in most existing water utility labs and

can also be used to independently confirm the analysis by the dual-cartridge SPE followed by LC/MS. Therefore, it was concluded that the cation-exchange SPE merited further investigation with wastewater matrices.

During the fifth project period, we examined the accuracy and precision of the dual-cartridge SPE and LC/MS methods in several more wastewater samples and included two additional antibiotics, trimethoprim and norfloxacin, in the analysis. The results provided consistent recoveries meeting the QA/QC criteria for the fluoroquinolones (norfloxacin and ciprofloxacin). However, the recoveries for sulfonamides and trimethoprim were still below our target values. Further studies on the cation-exchange SPE followed by LC/fluorescence detection method indicated that the method is adversely affected by the organic matters encountered in more complicated wastewater matrices, yielding inconsistent performance. It was concluded that the method is only suitable for qualitative screening purposes for fluoroquinolones and may be suitable for compound quantitation in relatively clean finished wastewater effluent.

In the sixth project period, significant improvements were made to the analytical method. The fluoroquinolones ofloxacin and enrofloxacin were added to the analysis, rendering a total of four fluoroquinolones (in addition to ciprofloxacin and norfloxacin) in the occurrence survey. Lomefloxacin was used as an internal standard for the fluoroquinolones. The recoveries for sulfonamides and trimethoprim were greatly improved after addition of salt prior to the SPE step. For more accurate quantification, the standard addition method was used as an alternative quantification technique and was compared to the internal standard method (the methods were described in details in the 6th report). Analytical efficiency was enhanced after combining the two LC/MS methods for fluoroquinolones and sulfonamides, respectively. Finally, the LC/MS sensitivity was improved by lowering the eluent buffer concentrations while still maintaining sufficient buffering capacity. The improvements have resulted in a robust and sensitive method (see Appendix C of the 6th report) and thus the method development was near completion.

During the seventh project period, the standard addition method was used to assess recoveries in spiked samples in addition to the internal standard method (lomefloxacin for fluoroquinolones and sulfamerazine for sulfonamides). It was discovered that chlorine residue in the West Basin reverse osmosis effluent caused low recoveries of antibiotics and quenching of the residual chlorine would be necessary in the future. Laboratory experiments confirmed that

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all of the antibiotics selected in this study react readily with chlorine at conditions similar to those at water and wastewater treatment plants. Analyses also found that acidifying the samples to near pH 2.5 and slowly eluting compounds from the SPE cartridges are critical to maintain good recoveries for sulfonamides. From some of the LC/MS chromatograms and experimental results, it was also concluded that slight increase in the buffer concentration of the LC mobile phases could be used to improve peak shape. Overall, the recoveries were better for fluoroquinolones and trimethoprim than for sulfonamides (see Appendix B of the 7th progress report). Excluding the West Basin reverse osmosis effluent samples, the average recoveries for ciprofloxacin, norfloxacin, enrofloxacin, and ofloxacin were 68±27%, 82±21%, 94±37% and 88±33%, respectively. The average recoveries for sulfamethoxazole, sulfamethazine and trimethoprim were 51±15%, 49±11% and 105±19%, respectively. Much of the data collected during the 7th project period met the QA/QC criteria for the occurrence survey. The method development was completed in the 7th project period.

During the current project period no activity was conducted in association with this sub-task.

TASK 3: OCCURRENCE SURVEY

Sub-Task 3A: Site Selection

Because the most important source of PhACs is believed to be the discharge of municipal wastewater effluent, we focused our efforts on sampling of municipal wastewater effluent and water recycling systems that serve as important barriers to the entry of wastewater-derived contaminants into drinking water sources. In the first stage of the site selection process, we identified representative sites and made arrangements with utilities to obtain samples.

As part of the site selection process, preliminary samples were collected during the first three project periods from sites that we considered for inclusion in the occurrence survey. The preliminary data helped us to assess the suitability of sites to be included in the occurrence survey. In several cases, sites that we had intended to sample were eliminated because changes had occurred at the sites. For example:

-The Dublin/San Ramon Advanced Wastewater Treatment Plant was taken out of service during Spring 2001 because the utility district put the project on indefinite hold.

-We were informed that the Witchita Falls Pilot Advanced Water Treatment Plant treats water that mainly originates from agricultural runoff.

-The Rio Hondo Recharge Facility was eliminated because construction prevented us from obtaining samples.

The sites that were eliminated due to the considerations described above were replaced by comparable sites as they were identified.

During the eighth project period an additional background sampling location was added at the intake of the James E. Quarles water treatment plant on the Chattahoochie River in Atlanta.

A final list of sites sampled during the occurrence survey is included in Table 1. The selected sites included a total of eight conventional wastewater treatment plants, three advanced wastewater treatment plants, two engineered treatment wetlands and three background sites. Each of the sites is described briefly in the text following the table.

Table 1. Summary of sample collection sites in the occurrence survey.

Location	Description	Dates Sampled
Conventional WWTPs		
Clayton	BNR, UV	3/19/02, 8/20/02
Dublin/San Ramon	AS, Cl_2	3/21/02
Hyperion	AS, Cl_2	9/18/01, 5/22/02
Mt. View	AS, biotower, UV	9/4/01, 4/9/02, 8/21/02
Roger Road	AS, Cl_2	2/17/02, 4/1/02
San Jose/Santa Clara	BNR, effluent filtration, Cl_2	3/26/02, 6/26/02
South Cobb	AS, Cl_2	2/15/02, 6/12/02
Southeast San Francisco	O_2-AS, Cl_2	7/1/02, 10/5/02
Advanced Treatment Plants		
F. Wayne Hill	Activated carbon, ozone, UV	4/22/02, 7/17/02
OCWD	Microfiltration, RO, UV	9/18/01
West Basin	Microfiltration, RO, UV	9/18/01, 5/22/02, 9/10/02
Groundwater Recharge		
Sweetwater Recharge Facility	Secondary effluent recharge	2/17/02, 4/1/02
Engineered Wetlands		
Mt. View	Tertiary effluent, RT ~7 days	9/4/01, 4/9/02, 7/12/02, 10/15/02
Prado	Effluent-dominated river	4/6/02
Background		
MWD Water	Los Angeles water supply	9/18/01
Russian River	Marin County, CA	5/14/01, 6/11/01, 8/13/01
Chatahoochie River	Intake for Quarles WTP, GA	12/12/02, 12/19/02
Lake Altoona	Intake for Wyckoff WTP, GA	9/6/02
Flint River Reservoir	Intake for Smith WTP, GA	9/6/02

Notes: WWTP = conventional municipal wastewater treatment plant; WTP = water treatment plant; AS = Activated sludge; UV = ultraviolet disinfection; Cl_2 = chlorine disinfection; RT = hydraulic retention time; OCWD = Orange County Water District; MWD = Metropolitan (CA) Water District.

R.M. Clayton Wastewater Treatment Plant (Atlanta, GA): The R.M. Clayton municipal wastewater treatment plant is a 5.26 m^3 s^{-1} (120 MGD) facility. The plant is equipped with primary screening and clarification, followed by activated sludge treatment with three-stage biological phosphorous removal in activated sludge reactors. Following clarification, the wastewater undergoes ultraviolet disinfection.

Dublin/San Ramon Advanced Wastewater Treatment Plant (Dublin, CA): The Dublin/San Ramon Services municipal wastewater treatment plant is a 0.50 m^3 s^{-1} (12 MGD) facility. The plant is equipped with primary screening and clarification, followed by activated sludge treatment and chlorine disinfection.

Hyperion Wastewater Treatment Plant (Los Angeles, CA): The Hyperion municipal wastewater treatment plant treats a total of 15.7 m^3 s^{-1} (358 MGD) of municipal wastewater effluent with advanced primary treatment or secondary treatment. The water sampled during the occurrence study originated in the secondary treatment plant, which treats 8.45 m^3 s^{-1} (193 MGD) of wastewater effluent. The secondary treatment plant is equipped with primary screening and clarification followed by pure oxygen activated sludge treatment, clarification and chlorine disinfection. The samples analyzed as part of the occurrence survey were collected at the West Basin AWWTP, which treats the secondary effluent from the Hyperion treatment plant.

Mt. View Wastewater Treatment Plant (Martinez, CA): The Mt. View municipal wastewater treatment plant is a 0.06 m^3 s^{-1} (1.5 MGD) facility equipped with primary screening and clarification followed by a trickling filter for secondary treatment and a biotower for ammonia removal. The effluent is subjected to ultraviolet disinfection prior to being discharged to an engineered treatment wetland.

Roger Road Wastewater Treatment Plant (Tuscon, AZ): The Roger Road municipal wastewater treatment plant is a 1.4 m^3 s^{-1} (31 MGD) facility equipped with primary screening and clarification, followed by activated sludge treatment and chlorine disinfection. The treatment plant discharges directly to an infiltration pond that recharges an aquifer at the Sweetwater recharge facility. During the occurrence survey, samples collected from the infiltration pond were assumed to be representative of the effluent from Roger Road treatment plant.

San Jose/Santa Clara Wastewater Treatment Plant (San Jose, CA): The San Jose municipal wastewater treatment plant is a 7.3 m^3 s^{-1} (170 MGD) facility equipped with primary

screening and clarification, followed by activated sludge treatment with three-stage biological phosphorous removal in activated sludge reactors. Following clarification, the wastewater undergoes mixed media filtration and chlorine disinfection.

South Cobb Wastewater Treatment Plant (Cobb County, GA): The South Cobb municipal wastewater treatment plant is a 1.8 m^3 s^{-1} (40 MGD) facility equipped with primary treatment and aerated activated sludge treatment followed by chlorine disinfection.

The Southeast San Francisco Wastewater Treatment Plant (San Francisco, CA): The Southeast San Francisco municipal wastewater treatment plant is a 6.6 m^3 s^{-1} (150 MGD) facility equipped with primary screening and clarification, followed by pure oxygen activated sludge treatment and chlorine disinfection.

F. Wayne Hill Advanced Wastewater Treatment Plant (Gwinnett County, GA): The F. Wayne Hill facility is a 0.88 m^3 s^{-1} (20 MGD) advanced wastewater treatment plant. The facility consists of primary treatment followed by activated sludge treatment in a reactor operated for biological nutrient removal. After the secondary clarification, the wastewater undergoes lime addition and recarbonation followed by dual-media filtration, pre-ozonation, granular activated carbon filtration and ozonation.

West Central Basin Municipal Water Advanced Wastewater Treatment Plant (Los Angeles, CA): The West Basin treatment plant is an advanced wastewater treatment plant consisting of two treatment trains. The first treatment train uses lime coagulation followed by cellulose acetate membranes while the second train uses microfiltration followed by reverse osmosis with thin-film composite membranes. The two trains are combined prior to ultraviolet disinfection in the presence of added hydrogen peroxide to enhance the removal of organic contaminants. As part of the occurrence survey samples were collected from the second treatment train.

Orange County Water District (OCWD) Advanced Treatment Plant (Fountain Valley, CA): The OCWD operates an advanced wastewater treatment plant as part of the Talbert Barrier seawater intrusion project. The treatment plant, collectively referred to as Water Factory 21, consists of two treatment trains that treat wastewater effluent form Orange County Sanitation District's adjacent municipal wastewater treatment plant. During the occurrence survey, samples were collected from the treatment train that consists of microfiltration, reverse osmosis with thin-film composite membranes and ultraviolet disinfection in the presence of hydrogen peroxide.

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Sweetwater Recharge Facility (Tuscon AZ): The Sweetwater groundwater recharge site consists of an infiltration pond that receives wastewater effluent from the Roger Road wastewater treatment plant. The underlying aquifer is equipped with an extensive network of monitoring wells. As a part of the occurrence survey, groundwater was collected from two downgradient wells: (1) a shallow well screened at 5.1 meters; and, (2) a deep well screened at approximately 30.5 m. According to tracer data collected at the site the water sampled from both wells consists entirely of wastewater effluent (i.e., there is no dilution with local groundwater) and has a residence time in the aquifer of approximately 2.5 and 15 days, respectively.

Mt. View Engineered Treatment Wetland (Martinez, CA): The Mt. View engineered treatment wetlands consist of a series of five ponds in series connected by weirs and underground piping. The wetland ponds are approximately 1.5 meters deep and are extensively vegetated along the edges with cattails duckweed. The mean hydraulic residence time of the wetland is approximately 7 days.

Prado Engineered Treatment Wetlands (Orange County, CA): The Prado Engineered Treatment wetlands treat water from the Santa Ana River. During summertime, most of the water in the Santa Ana River originates at Riverside and San Bernardino tertiary wastewater treatment plants located approximately 20 km upstream. During other times of the year, the river receives a combination of stormwater runoff and wastewater discharge from the upstream watershed. The wetland consists of a series of treatment cells vegetated with cattail and duckweed.

Sub-Task 3B: Sample Collection and Analysis

While the quality of some of the data collected before completion of method develop was useful to our analysis, the preliminary samples were not analyzed using all of the steps ultimately incorporated into the analytical method described in the QA/QC plan. As a result, the preliminary results are only useful for screening purposes. During the fourth through seventh project periods, we analyzed samples from selected sites using the accepted analytical methods for acidic drugs and beta-blockers. Method development activities for antibiotics were completed during the sixth project period and samples from five sites were analyzed for the selected compounds using the final methods during the sixth and seventh project periods.

During the eighth project period we collected and analyzed additional samples for acidic drugs, beta-blockers and antibiotics from several of the sites (see Appendices A and B for details). Results from those analyses are presented in the following paragraphs.

To investigate the potential for removal of PhACs during chlorine disinfection, samples were collected from the San Jose/Santa Clara and Southeast San Francisco WWTPs during the seventh project period. In addition to measuring the concentrations of pharmaceuticals before and after chlorine disinfection, several samples were spiked with a mixture of acidic drugs and beta-blockers and subjected to chloramination in the laboratory. Results from those experiments suggested that, in the absence of ammonia, most of the compounds were transformed by chlorine. In the presence of ammonia, when the predominant form of chlorine was monochloramine, most of the compounds were not transformed by chlorine. However, some of the results were ambiguous and analytical problems prevented analysis of data for gemfibrozil and indometacine.

To verify the results from the chlorination experiment, a similar experiment was conducted in a secondary effluent sample from the Southeast San Francisco wastewater treatment plant. Concentrations of acidic drugs and beta-blockers measured prior to spiking the samples (solid bars in Figure 1) were comparable to those measured previously. Results from the new chlorination experiment were consistent with previous results. After the addition of 10 mg/L as Cl_2 of NaOCl (grey bars in Figure 1) the concentrations of gemfibrozil, ibuprofen, indometacine, metoprolol and propranolol decreased by 20-50%. These results suggest that disinfection with chloramines probably will not have a significant effect on concentrations of these pharmaceuticals. In contrast, the addition of 350 mg/L as Cl_2 of NaOCl (clear bars in Figure 1) resulted in the complete removal of all of the pharmaceuticals except ibuprofen and ketoprofen. Under these conditions, the chlorine exists as HOCl/OCl⁻ because the ammonia has been removed by breakpoint chlorination. Although these are extreme conditions compared to those encountered in disinfection systems, these results further confirm the potential importance of free chlorine disinfection in the removal of pharmaceuticals.
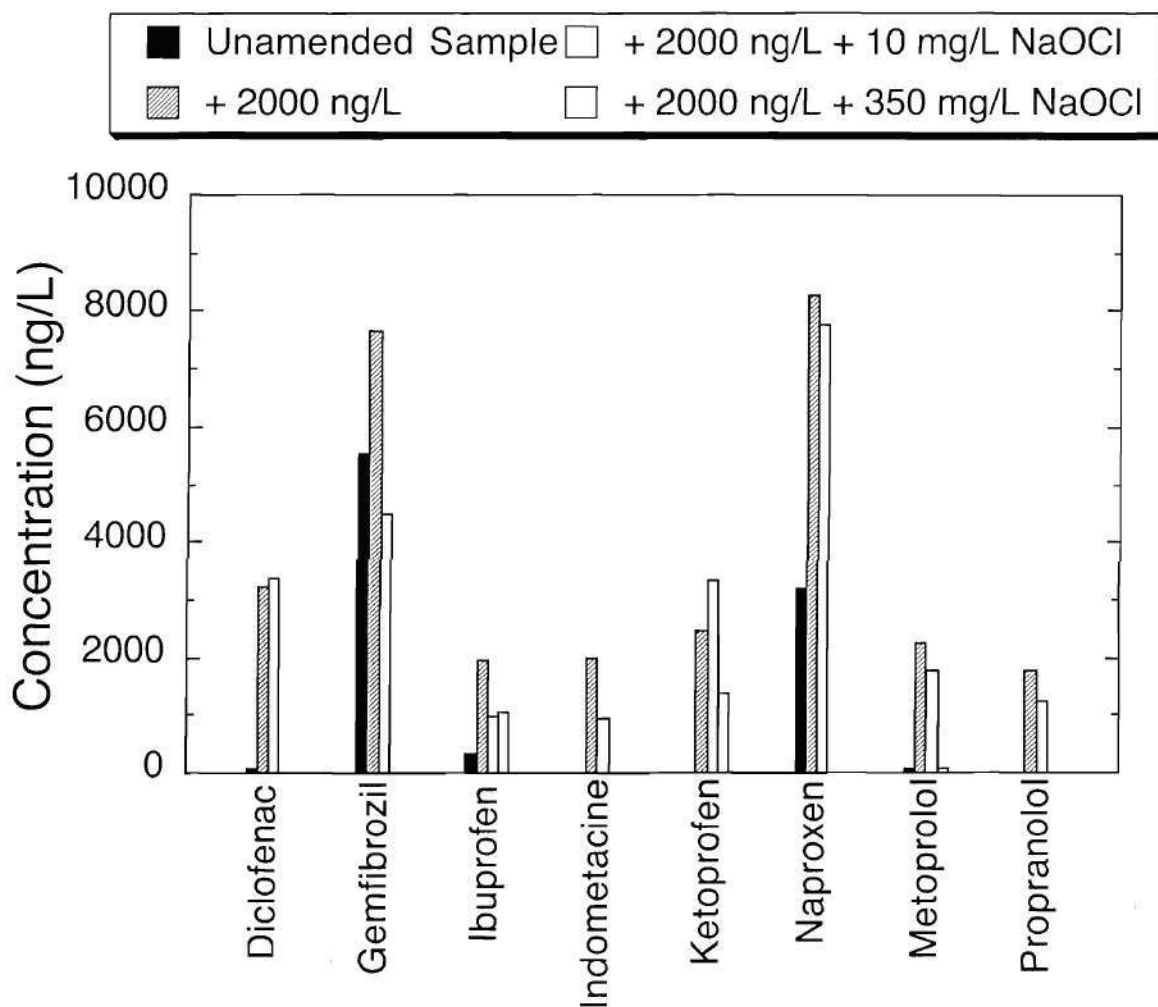
Figure 1: Results from laboratory experiments involving the addition of low (10 mg/L as Cl_2) and high (350 mg/L as Cl_2) doses of chlorine for one hour to secondary effluent samples from the Southeast San Francisco (SESF) Municipal Wastewater Treatment Plant. Samples were amended with 2000 ng/L of each pharmaceutical prior to chlorination.

Samples also were collected and analyzed for acidic drugs and beta-blockers at two advanced treatment plants (Figure 2). Consistent with previous measuremnts, results indicate that all of the pharmaceuticals were removed in the advanced treatment plants. At Water Factory 21, little change was observed in concentrations of pharmaceuticals during microfiltration. No pharmaceuticals were detected after reverse osmosis. At the F. Wayne Hill treatment plant, concentrations entering prior to treatment typically were lower than those observed at Water Factory 21. All pharmaceuticals were removed during GAC treatment. It should be noted that the recovery of the surrogate standard for acidic drugs (i.e., mecoprop) was below our quality control criteria for those samples collected after reverse osmosis (0% recovery), GAC (37% recovery) and ozone treatment (11% recovery).
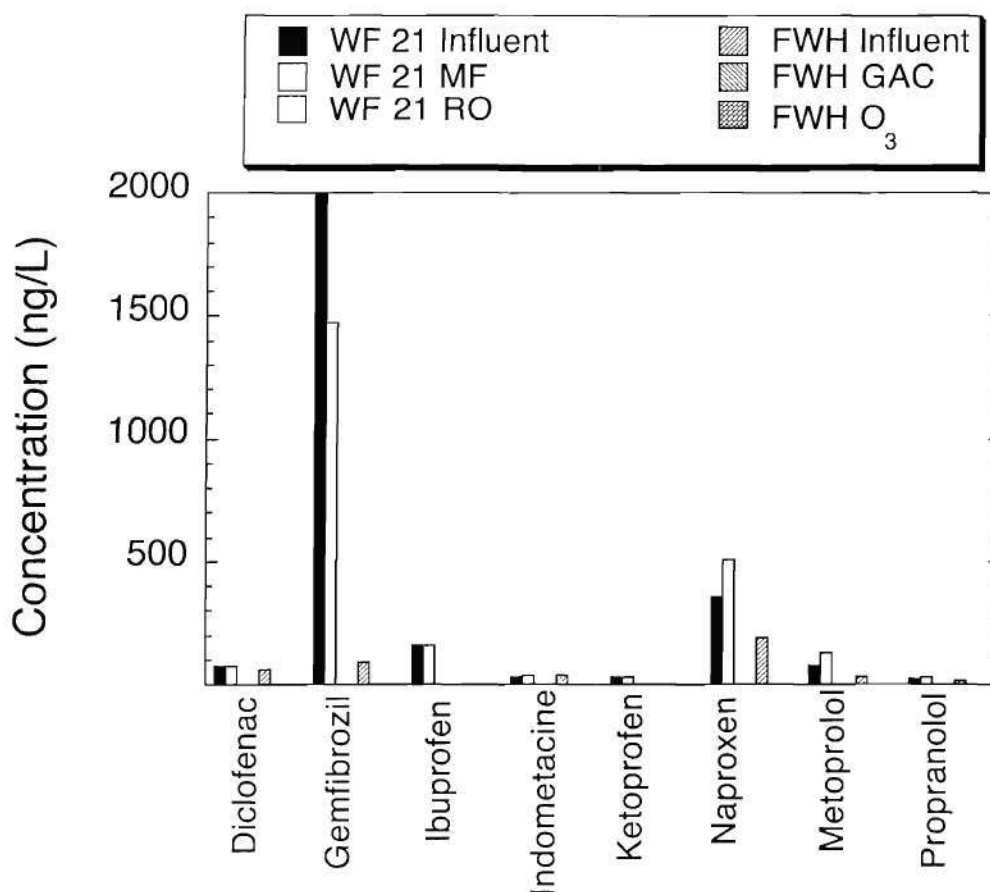

Figure 2: Concentrations of acidic drugs and beta-blockers measured at OCWD's Water Factory 21 and the F. Wayne Hill advanced treatment plants.

During the 8th project period, wastewater samples were collected and analyzed from the Hyperion WWTP, West Basin AWWTP, Mt. View WWTP and Mt. View wetland. Background samples from the Chattahoochee River and the finished drinking water from the Quarles WTP were sampled twice. Samples collection and analysis followed the analytical method and the QA/QC plan developed for this study (see Appendices C and D in the 6th report) except that a larger volume of 2-4 L was extracted for the surface and drinking water. 1.0 µg/L of each target antibiotic was added to the samples prior to extraction to assess matrix spike recoveries. The occurrence results are summarized in the Appendix B. The antibiotic concentrations were determined by internal standard method and by standard addition method and were not corrected by recovery.

The results from the West Basin samples in this project period agree with earlier findings. Ciprofloxacin, ofloxacin, sulfamethoxazole and trimethoprim were detected in the microfiltration (MF) influent and secondary wastewater effluent samples. The concentrations of ofloxacin and trimethoprim decreased negligibly by the MF process while the concentration of ciprofloxacin decreased by about 35%. Significant matrix interference was encountered for sulfamethoxazole in the MF influent samples and thus the removal of sulfamethoxazole by MF process could not be evaluated. Since it was found out in the last project period that West Basin AWWTP adds chlorine to their RO unit to prevent membrane fouling, 2 mg/L of sodium thiosulfate (in excess amount for reducing the residual chlorine) was added this time upon collecting the RO effluent samples. The sodium thiosulfate eliminated the interference of residual chlorine and yielded much improved recoveries. The recoveries were 91-116% for the fluoroquinolones, 93% for trimethoprim and 47-71% for the sulfonamides based upon quantification by internal standards. None of the antibiotics were detectable in the RO effluent. The antibiotics were likely removed by chlorine, by the RO membrane, or by both mechanisms.

The results from the Mt. View wastewater treatment plant and wetland samples also generally agree with earlier findings. Ciprofloxacin, ofloxacin, sulfamethoxazole and trimethoprim were the four antibiotics detected in all samples. Compared to the trickling filter effluent, nitrification did not reduce the concentrations of sulfamethoxazole or trimethoprim while reduced the concentrations of ciprofloxacin and ofloxacin by about 50%. Except for sulfamethoxazole, the concentrations of ciprofloxacin, ofloxacin and trimethoprim were considerably lower in the wetland influent and effluent samples. Mt. View WWTP utilizes UV

disinfection prior to discharge to the wetland. The low concentrations of ciprofloxacin, ofloxacin and trimethoprim are likely the result of photodegradation during UV disinfection or exposure to sunlight. The comparable concentrations of sulfamethoxazole in the wetland influent and effluent samples indicate that the wetland process does not remove this antibiotic. This observation agrees with the slow biodegradation, high photo-stability and low affinity to sediments for sulfamethoxazole as discussed in the previous project reports.

The Chattahoochee River and the drinking water from the Quarles WTP were analyzed twice in December 2002. None of the antibiotics were above the detection limits in all samples (20-70 ng/L for the surface water and 2-7 ng/L for the drinking water). The Chattahoochee River contained significant amounts of suspended solids and organic matter at both times of sampling with the second set of samples a little cleaner. Low recoveries were obtained for the first set of samples due to high degree of matrix interference. The recoveries were improved significantly for fluoroquinolones and trimethoprim in the second set of samples but were still low for sulfamethoxazole and sulfamethazine.

Sub-Task 3C: Data Analysis and Synthesis

As discussed in the seventh project report, we are in the process of writing a manuscript summarizing the results of the occurrence survey. The manuscript will present details of the analytical methods, the data and a discussion of the implications of the data for the water industry. Although we have not yet completed our interpretation of the data, we have been considering the most effective way to summarize the data and to describe the implications of the data. Our preliminary approach was summarized in the seventh report. An outline for a manuscript to be submitted to *Water Research* is summarized below:

I.   Introduction

    A. Background

        1. Detection of pharmaceuticals

            a. Germany/Switzerland (Heberer, Ternes, etc.)

            b. North America (USGS, others)

        2. Concerns about drinking water

            a. Indirect potable reuse

                i. SAT

    ii. Advanced treatment/reinjection

   b. Unplanned reuse

  B. Objectives

   1. Assess wastewater as a potential source

    a. Prediction based upon US prescriptions

    b. Measurements in representative WWTPs

    c. Comparison with data from other locations

   2. Conduct preliminary evaluation of treatment efficacy

    a. SAT

    b. Advanced treatment plants

    c. Engineered wetlands/effluent dominated surface waters

II. Materials and Methods

  A. Sample locations (summarize information in Table 1)

  B. Sample collection

   1. Grab samples, size, containers, etc.

   2. Shipping

  C. Solid phase extraction

   1. Acidic drugs

   2. Beta-blockers

   3. Antibiotics

  D. Analytical Methods

   1. Acidic drugs

   2. Beta-blockers

   3. Antibiotics

  E. QA/QC

   1. Blanks, duplicates, surrogates, recoveries

   2. Acceptance criteria

   3. Data not meeting criteria

  F. Approach for predicting pharmaceutical concentrations in sewage

III. Results

  A. Wastewater Effluent (n = 16)

        1. Final effluent

            a. Summary of data (similar to Fig 8 of progress report 7)

            b. Full data available in AWWA report

            c. Evaluate for trends by treatment plant type (AS vs. tr. filter)

        2. Removal during disinfection

            a. Chlorination experiments (similar to Fig. 3,11 in report 7)

            b. Removal in UV

    B. Removal in processes applied after conventional wastewater treatment

        1. SAT (figure showing pond, shallow and deep wells at Sweetwater)

        2. Advanced Treatment (MF/RO, GAC/O_3)

        3. Treatment Wetlands

IV.   Discussion

    A. Comparisons of concentrations in wastewater effluent

        1. Compare with predicted influent concentrations

        2. Compare with data from Europe and other countries

        3. Compare with USGS study and others in US

    B. Efficacy of Treatment methods

        1. Conventional wastewater treatment

        2. Wastewater (and water) disinfection

        3. Advanced treatment

        4. SAT

        5. Wetlands and surface waters

    C. Potential for exposure to pharmaceuticals in drinking water

        1. Indirect potable reuse

            a. Advanced treatment is effective

            b. SAT removes most but not all pharmaceuticals

        2. Unplanned reuse

            a. Not much removal by natural attenuation in surface waters

            b. Pharmaceutical presence in raw water determined mainly by dilution of wastewater effluent.

In addition, a manuscript describing our progress in developing new methods for analysis of antibiotics has been submitted to the *Journal of Chromatography A* and is currently under review. A copy of the manuscript is included with this progress report for the PAC to review. Comments will be considered and, if appropriate, included in the final version of the paper.

PLANS FOR NEXT PERIOD

Task 1: Literature Review
Sub-Task 1A: Drugs Used in Human Therapy

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Sub-Task 1B: Drugs Used in Animal Husbandry

No additional research is planned in association with this task. This section will be updated prior to preparation of the final report to include additional publications.

Task 2: Analytical Method Development and Testing

Sub-Task 2A: Drugs Used in Human Therapy (Excluding Antibiotics)

During the next project period no new research is planned is association with this task.

Sub-Task 2B: Antibiotics

During the next project period no new research is planned is association with this task.

Task 3: Occurrence Survey

Sub-Task 3A: Site Selection

No further activity is planned in association with this task.

Sub-Task 3B: Sample Collection and Analysis

No further activity is planned in association with this task.

Sub-Task 3C: Data Analysis and Synthesis

During the final project period, data will be synthesized and incorporated into a manuscript for publication. After completion of the manuscript, the final report will be prepared.

APPENDIX A: Summary of Data for Acidic Drugs and Beta-Blockers during the Eighth Project Period

Compound	Location	Date	Concentration (ppt)
Diclofenac	Southeast San Francisco secondary effluent	10/4/02	38,85
	OCWD influent	10/16/02	78
	OCWD microfiltration effluent	10/16/02	77,77
	OCWD RO effluent	10/16/02	<10
	F. Wayne Hill influent	10/17/02	60
	F. Wayne Hill GAC effluent	10/17/02	<10*
	F. Wayne Hill ozonation effluent	10/17/02	<10*
Gemfibrozil	Southeast San Francisco secondary effluent	10/4/02	4250, 6810
	OCWD influent	10/16/02	1,990
	OCWD microfiltration effluent	10/16/02	1860, 1470
	OCWD RO effluent	10/16/02	<10*
	F. Wayne Hill influent	10/17/02	92
	F. Wayne Hill GAC effluent	10/17/02	<10*
	F. Wayne Hill ozonation effluent	10/17/02	<10*
Ibuprofen	Southeast San Francisco secondary effluent	10/4/02	410, 230
	OCWD influent	10/16/02	160
	OCWD microfiltration effluent	10/16/02	140,180
	OCWD RO effluent	10/16/02	<10*
	F. Wayne Hill influent	10/17/02	<10
	F. Wayne Hill GAC effluent	10/17/02	<10
	F. Wayne Hill ozonation effluent	10/17/02	<10
Indometacine	Southeast San Francisco secondary effluent	10/4/02	<10, <10
	OCWD influent	10/16/02	33
	OCWD microfiltration effluent	10/16/02	41,44
	OCWD RO effluent	10/16/02	<10*
	F. Wayne Hill influent	10/17/02	36
	F. Wayne Hill GAC effluent	10/17/02	<10*
	F. Wayne Hill ozonation effluent	10/17/02	<10*
Ketoprofen	Southeast San Francisco secondary effluent	10/4/02	<10, <10
	OCWD influent	10/16/02	32
	OCWD microfiltration effluent	10/16/02	32, 37
	OCWD RO effluent	10/16/02	<10*
	F. Wayne Hill influent	10/17/02	<10
	F. Wayne Hill GAC effluent	10/17/02	<10
	F. Wayne Hill ozonation effluent	10/17/02	<10
Naproxen	Southeast San Francisco secondary effluent	10/4/02	2000, 4350
	OCWD influent	10/16/02	350
	OCWD microfiltration effluent	10/16/02	510, 510
	OCWD RO effluent	10/16/02	<10*
	F. Wayne Hill influent	10/17/02	190
	F. Wayne Hill GAC effluent	10/17/02	<10*
	F. Wayne Hill ozonation effluent	10/17/02	<10*
Mecoprop*	Southeast San Francisco secondary effluent	10/4/02	77%
	OCWD influent	10/16/02	79%
	OCWD microfiltration effluent	10/16/02	78%, 92%
	OCWD RO effluent	10/16/02	0%
	F. Wayne Hill influent	10/17/02	77%
	F. Wayne Hill GAC effluent	10/17/02	37%
	F. Wayne Hill ozonation effluent	10/17/02	11%

*Surrogate recovery outside of acceptable range.

Recovery based upon labeled mecoprop (internal standard) added to samples at 1,000 ng/L.

APPENDIX B: Summary of Occurrence Data for Antibiotics during the Eighth Project Period

Compound	Location	Date	Concentration (µg/L)[1]	Concentration (µg/L)[2]	Spike Recovery (%)[1]	Spike Recovery (%)[2]
Ciprofloxacin	West Basin AWWTP, CA – Microfiltration Influent	9/10/2002	0.90	0.31	182	124
	West Basin AWWTP, CA – Microfiltration Effluent	9/10/2002	0.58	0.14	208	191
	West Basin AWWTP, CA – Reverse Osmosis Effluent	9/10/2002	<MDL	<MDL[a]	109	210
	Mt. View WWTP, CA – Trickling Filter Effluent	10/15/2002	1.02	2.05	95	19[g]
	Mt. View WWTP, CA – Nitrification Effluent	10/15/2002	0.86	1.08	99	86
	Mt. View WWTP, CA – Wetland Influent	10/15/2002	<MDL	<MDL[b]	105	114
	Mt. View WWTP, CA – Wetland Effluent	10/15/2002	<MDL, <MDL	<MDL, <MDL[b]	86	94
	James E. Quarles TP, GA – Plant Intake[d]	12/12/2002	<MDL, <MDL	<MDL, <MDL[b]	53	18[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/12/2002	<MDL	<MDL[c]	NA	NA
	James E. Quarles TP, GA – Plant Intake[f]	12/19/2002	<MDL, <MDL	<MDL, <MDL[b]	123	177
	James E. Quarles TP, GA – Plant Effluent[e]	12/19/2002	<MDL	<MDL[c]	NA	NA
	DI Water Sample	12/19/2002	NA	NA	117	124
Enrofloxacin	West Basin AWWTP, CA – Microfiltration Influent	9/10/2002	<MDL	<MDL[a]	134	82
	West Basin AWWTP, CA – Microfiltration Effluent	9/10/2002	<MDL	<MDL[a]	102	66
	West Basin AWWTP, CA – Reverse Osmosis Effluent	9/10/2002	<MDL	<MDL[b]	108	143
	Mt. View WWTP, CA – Trickling Filter Effluent	10/15/2002	<MDL	<MDL[a]	78	36
	Mt. View WWTP, CA – Nitrification Effluent	10/15/2002	<MDL	<MDL[a]	61	94
	Mt. View WWTP, CA – Wetland Influent	10/15/2002	<MDL	<MDL[b]	110	89
	Mt. View WWTP, CA – Wetland Effluent	10/15/2002	<MDL, <MDL	<MDL, <MDL[b]	103	79
	James E. Quarles TP, GA – Plant Intake[d]	12/12/2002	<MDL, <MDL	<MDL, <MDL[b]	70	18[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/12/2002	<MDL	<MDL[c]	NA	NA
	James E. Quarles TP, GA – Plant Intake[f]	12/19/2002	<MDL, <MDL	<MDL, <MDL[b]	43	17[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/19/2002	<MDL	<MDL[c]	NA	NA
	DI Water Sample	12/19/2002	NA	NA	85	93

Compound	Location	Date	Concentration $(\mu g/L)^{(1)}$	Concentration $(\mu g/L)^{(2)}$	Spike Recovery $(\%)^{(1)}$	Spike Recovery $(\%)^{(2)}$
Norfloxacin	West Basin AWWTP, CA – Microfiltration Influent	9/10/2002	<MDL	<MDL[a]	159	85
	West Basin AWWTP, CA – Microfiltration Effluent	9/10/2002	<MDL	<MDL[a]	126	74
	West Basin AWWTP, CA – Reverse Osmosis Effluent	9/10/2002	<MDL	<MDL[b]	116	281
	Mt. View WWTP, CA – Trickling Filter Effluent	10/15/2002	<MDL	<MDL[a]	100	48
	Mt. View WWTP, CA – Nitrification Effluent	10/15/2002	<MDL	<MDL[a]	98	94
	Mt. View WWTP, CA – Wetland Influent	10/15/2002	<MDL	<MDL[b]	102	129
	Mt. View WWTP, CA – Wetland Effluent	10/15/2002	<MDL, <MDL	<MDL, <MDL[b]	103	97
	James E. Quarles TP, GA – Plant Intake[d]	12/12/2002	<MDL, <MDL	<MDL, <MDL[b]	47	12[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/12/2002	<MDL	<MDL[c]	NA	NA
	James E. Quarles TP, GA – Plant Intake[f]	12/19/2002	<MDL, <MDL	<MDL, <MDL[b]	96	110
	James E. Quarles TP, GA – Plant Effluent[e]	12/19/2002	<MDL	<MDL[c]	NA	NA
	DI Water Sample	12/19/2002	NA	NA	120	205
Ofloxacin	West Basin AWWTP, CA – Microfiltration Influent	9/10/2002	0.73	0.49	141	88
	West Basin AWWTP, CA – Microfiltration Effluent	9/10/2002	0.85	0.19	123	129
	West Basin AWWTP, CA – Reverse Osmosis Effluent	9/10/2002	<MDL	<MDL[b]	91	205
	Mt. View WWTP, CA – Trickling Filter Effluent	10/15/2002	1.81	0.72	121	40
	Mt. View WWTP, CA – Nitrification Effluent	10/15/2002	0.86	0.18	117	151
	Mt. View WWTP, CA – Wetland Influent	10/15/2002	0.17	0.26	112	84
	Mt. View WWTP, CA – Wetland Effluent	10/15/2002	0.12, 0.13	NA	116	28[g]
	James E. Quarles TP, GA – Plant Intake[d]	12/12/2002	<MDL, <MDL	<MDL, <MDL[b]	81	27[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/12/2002	<MDL	<MDL[c]	NA	NA
	James E. Quarles TP, GA – Plant Intake[f]	12/19/2002	<MDL, <MDL	<MDL, <MDL[b]	129	129
	James E. Quarles TP, GA – Plant Effluent[e]	12/19/2002	<MDL	<MDL[c]	NA	NA
	DI Water Sample	12/19/2002	NA	NA	117	160

Compound	Location	Date	Concentration (µg/L)[1]	Concentration (µg/L)[2]	Spike Recovery (%)[1]	Spike Recovery (%)[2]
Sulfamethoxazole	West Basin AWWTP, CA – Microfiltration Influent	9/10/2002	NA[g]	NA[g]	72	87
	West Basin AWWTP, CA – Microfiltration Effluent	9/10/2002	NA[g]	1.00	50	NA[g]
	West Basin AWWTP, CA – Reverse Osmosis Effluent	9/10/2002	<MDL	<MDL[b]	47	43
	Mt. View WWTP, CA – Trickling Filter Effluent	10/15/2002	0.69	0.33	57	55
	Mt. View WWTP, CA – Nitrification Effluent	10/15/2002	1.40	1.36	65	48
	Mt. View WWTP, CA – Wetland Influent	10/15/2002	0.91	1.05	30	25
	Mt. View WWTP, CA – Wetland Effluent	10/15/2002	1.04, 1.13	0.91, 1.12	12[g]	12[g]
	James E. Quarles TP, GA – Plant Intake[d]	12/12/2002	<MDL, <MDL	<MDL, <MDL[b]	0[g]	0[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/12/2002	<MDL	<MDL[c]	NA	NA
	James E. Quarles TP, GA – Plant Intake[f]	12/19/2002	<MDL, <MDL	<MDL, <MDL[b]	13[g]	18[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/19/2002	<MDL	<MDL[c]	NA	NA
	DI Water Sample	12/19/2002	NA	NA	41	39
Sulfamethazine	West Basin AWWTP, CA – Microfiltration Influent	9/10/2002	<MDL	<MDL[a]	24[g]	34[g]
	West Basin AWWTP, CA – Microfiltration Effluent	9/10/2002	<MDL	<MDL[a]	20[g]	32[g]
	West Basin AWWTP, CA – Reverse Osmosis Effluent	9/10/2002	<MDL	<MDL[b]	71	74
	Mt. View WWTP, CA – Trickling Filter Effluent	10/15/2002	<MDL	<MDL[a]	0[g]	11[g]
	Mt. View WWTP, CA – Nitrification Effluent	10/15/2002	<MDL	<MDL[a]	29	33
	Mt. View WWTP, CA – Wetland Influent	10/15/2002	<MDL	<MDL[b]	24	47
	Mt. View WWTP, CA – Wetland Effluent	10/15/2002	<MDL, <MDL	<MDL, <MDL[b]	0[g]	3[g]
	James E. Quarles TP, GA – Plant Intake[d]	12/12/2002	<MDL, <MDL	<MDL, <MDL[b]	33	9[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/12/2002	<MDL	<MDL[c]	NA	NA
	James E. Quarles TP, GA – Plant Intake[f]	12/19/2002	<MDL, <MDL	<MDL, <MDL[b]	8[g]	17[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/19/2002	<MDL	<MDL[c]	NA	NA
	DI Water Sample	12/19/2002	NA	NA	63	69

Compound	Location	Date	Concentration (μg/L)[1]	Concentration (μg/L)[2]	Spike Recovery (%)[1]	Spike Recovery (%)[2]
Trimethoprim	West Basin AWWTP, CA – Microfiltration Influent	9/10/2002	1.13	0.40	225	162
	West Basin AWWTP, CA – Microfiltration Effluent	9/10/2002	3.41	0.55	57	0[g]
	West Basin AWWTP, CA – Reverse Osmosis Effluent	9/10/2002	<MDL	<MDL[b]	93	69
	Mt. View WWTP, CA – Trickling Filter Effluent	10/15/2002	0.19	0.31	8[g]	5[g]
	Mt. View WWTP, CA – Nitrification Effluent	10/15/2002	0.21	0.46	93	48
	Mt. View WWTP, CA – Wetland Influent	10/15/2002	<MDL[b]	0.09	139	98
	Mt. View WWTP, CA – Wetland Effluent	10/15/2002	<MDL, <MDL[b]	0.02, 0.05	158	107
	James E. Quarles TP, GA – Plant Intake[d]	12/12/2002	<MDL, <MDL	<MDL, <MDL[b]	96	4[g]
	James E. Quarles TP, GA – Plant Effluent[e]	12/12/2002	<MDL	<MDL[c]	NA	NA
	James E. Quarles TP, GA – Plant Intake[f]	12/19/2002	<MDL, <MDL	<MDL, <MDL[b]	229	122
	James E. Quarles TP, GA – Plant Effluent[e]	12/19/2002	<MDL	<MDL[c]	NA	NA
	DI Water Sample	12/19/2002	NA	NA	78	92

Note: The reported concentrations were not corrected by recoveries; Spiked concentration is 1.0 μg/L.

(1) Quantification based on internal standard method

(2) Quantification based on standard addition method

Method Detection Levels:

(a) 30 to 90 ng/L (d) Concentration Factor = 3000

(b) 20 to 70 ng/L (e) Concentration Factor = 4000

(c) 2 to 7 ng/L (f) Concentration Factor = 2000

(g) Compound quantification hampered by matrix interferences