2005

Transport of Chemical and Microbial Compounds from Known Wastewater Discharges: Potential for Use as Indicators of Human Fecal Contamination

Susan Glassmeyer
U.S. Environmental Protection Agency

Edward Furlong
U.S. Geological Survey

Dana Kolpin
U.S. Geological Survey

Jeffrey Cahill
U.S. Geological Survey

Steven Zaugg
U.S. Geological Survey

See next page for additional authors

Environ. Sci. Technol. **2005**, *39,* 5157−5169

Transport of Chemical and Microbial Compounds from Known Wastewater Discharges: Potential for Use as Indicators of Human Fecal Contamination

SUSAN T. GLASSMEYER*
U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, 26 West Martin Luther King Drive, MS 564, Cincinnati, Ohio 45268

EDWARD T. FURLONG
U.S. Geological Survey, National Water Quality Laboratory, P.O. Box 25046, MS 407, Denver Federal Center, Building 95, Denver, Colorado 80225

DANA W. KOLPIN
U.S. Geological Survey, 400 S. Clinton Street, Room 269, Federal Building, Iowa City, Iowa 52244

JEFFERY D. CAHILL, STEVEN D. ZAUGG, AND STEPHEN L. WERNER
U.S. Geological Survey, National Water Quality Laboratory, P.O. Box 25046, MS 407, Denver Federal Center, Building 95, Denver, Colorado 80225

MICHAEL T. MEYER
U.S. Geological Survey, Organic Geochemistry Research Laboratory, 4821 Quail Crest Place, Lawrence, Kansas 66049

DAVID D. KRYAK
U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, D305-01, Research Triangle Park, North Carolina 27711

The quality of drinking and recreational water is currently (2005) determined using indicator bacteria. However, the culture tests used to analyze for these bacteria require a long time to complete and do not discriminate between human and animal fecal material sources. One complementary approach is to use chemicals found in human wastewater, which would have the advantages of (1) potentially shorter analysis times than the bacterial culture tests and (2) being selected for human-source specificity. At 10 locations, water samples were collected upstream and at two successive points downstream from a wastewaster treatment plant (WWTP); a treated effluent sample was also collected at each WWTP. This sampling plan was used to determine the persistence of a chemically diverse suite of emerging contaminants in streams. Samples were also collected at two reference locations assumed to have minimal human impacts. Of the 110 chemical analytes investigated in this project, 78 were detected at least once. The number of compounds in a given sample ranged from 3 at a reference location to 50 in a WWTP effluent sample. The total analyte load at each location varied from 0.018 μg/L at the reference location to 97.7 μg/L in a separate WWTP effluent sample. Although most of the compound concentrations were in the range of 0.01−1.0 μg/L, in some samples, individual concentrations were in the range of 5−38 μg/L. The concentrations of the majority of the chemicals present in the samples generally followed the expected trend: they were either nonexistent or at trace levels in the upstream samples, had their maximum concentrations in the WWTP effluent samples, and then declined in the two downstream samples. This research suggests that selected chemicals are useful as tracers of human wastewater discharge.

* Corresponding author phone: 513-569-7526; fax: 513-569-7757; e-mail: glassmeyer.susan@epa.gov.

Introduction

To protect public health, we need to monitor drinking and recreational water bodies to ensure that pathogens are not present. This objective, however, is not a straightforward task. Because of the large number of potential pathogens, indicator species are monitored when analyzing water samples for microorganisms of public health concern. The fact that some pathogenic organisms cannot be cultured makes direct analysis impractical. Ideally, indicator species are present when pathogens are present and are in sufficient concentrations so that they will not be difficult to detect; however, they should not grow and multiply in the aquatic environment. To provide a conservative level of protection, indicators are selected that are more environmentally stable and resistant to disinfectant stressors than the pathogens that they trace and that are easy to detect and identify (*1*, *2*). It is also desirable to differentiate between human and animal fecal material because people generally are more susceptible to pathogens from anthropogenic waste.

Currently in the United States, the total coliform test is required for screening drinking water samples for potential pathogen contamination (*3*). For recreational water, fecal coliforms have been the indicator of choice since the late 1960s (*4*). However, in 1986, the United States Environmental Protection Agency (USEPA) produced additional guidance asserting that monitoring both *E. coli* and enterococci instead of fecal coliform would provide improved public health protection because these organisms have shown strong relations to gastroenteric illnesses during epidemiological studies (*5*−*7*). Methods for these two indicators were promulgated in 2003 (*8*, *9*).

In their century of use, microbial indicators have been useful in protecting human health, but their disadvantages and limitations have become apparent. Most of the traditional biological assays require 18−48 h for the microorganisms to grow and be enumerated. In the time that it takes to go from sample collection to a positive test result, individuals can either consume or come into contact with the contaminated water. New microbial techniques, such as polymerase chain reaction (PCR), may reduce the time required to determine if pathogens are present, but at this time, these methods are not sufficiently robust to be practical for widespread implementation (*10*). In addition to being time-consuming, many microbial indicators also lack specificity; it is impossible to use these indicators to discriminate between human or animal sources or even determine if the presence of indicators results from fecal contamination (*11*−*14*). Such specificity is crucial to public health decision making. For example, if a watershed that tests positive for a pathogenic indicator contains a WWTP and a confined animal feeding operation, determining which operation (if either) is responsible for

10.1021/es048120k CCC: $30.25
Published on Web 06/11/2005

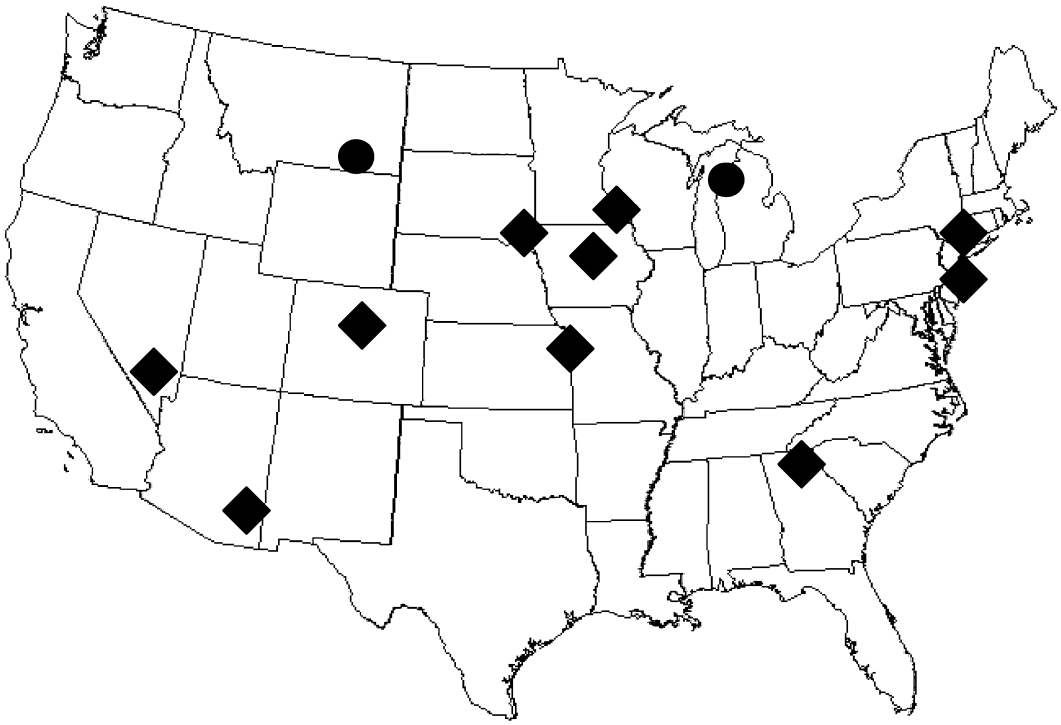

FIGURE 1. Sample collection locations. Diamonds indicate wastewaster treatment plants; circles designate the reference locations.

the contamination will save the other from making unnecessary and potentially costly "corrections" to their operation.

Recently, research has begun to determine the applicability of using chemical indicators of human fecal contamination to identify human sewage contamination in water bodies (*15*−*36*). Chemical indicator tests have an advantage over the current microbial tests because the time required for sample preparation and analysis can be substantially shorter than that required for culturing techniques and visualization of the colonies. Chemical indicators of human fecal contamination fall into several classes: those that are produced by humans, those that pass through humans, and those that are associated with the black water (sewage-contaminated) waste system.

The fecal sterol coprostanol was first suggested as an indicator of fecal pollution in the late 1960s (*37*). Coprostanol is a product of the bacterial degradation of cholesterol in the human gut (*38*). The rate of conversion of cholesterol to coprostanol is diet-dependent, but North Americans eating a typical mixed Western diet have high conversion rates (*39*). This conversion can be quantified by calculating the coprostanol-to-cholesterol ratio; this ratio has been found to range from 0.3 to greater than 15 in human fecal samples (*39*). In herbivores, the primary fecal sterol is 24-ethylcholestanol (*38*). Because the sterol composition in human wastes differs from those of other animals, the sterol composition provides the potential for discriminating between sources (*31*, *40*, *41*). Coprostanol and the other fecal sterols have been detected in surface-water (*16*, *24*−*28*, *31*, *40*, *41*) and sediment samples (*44*).

Other synthetic and natural organic compounds that are consumed and excreted by humans and domestic animals can be used to trace fecal sources. The chemical that has received the most interest as a sewage tracer is caffeine (*18*, *21*, *36*, *45*−*47*). Other human-derived contaminants, such as pharmaceuticals (*15*, *22*, *30*, *36*, *42*, *48*−*57*), also have the potential to serve as tracers of human waste.

In most of North America, black and gray wastewaters are combined when they leave homes, and graywater-derived compounds can also be exploited as indicators. Studies in England have shown that 16% of the volume of household waste comes from washing machines (*58*). The components of laundry detergents, surfactants (*42*, *59*, *60*), fluorescent whitening agents (*23*, *26*, *61*−*63*), and fragrances such as musks (*17*, *35*, *52*, *64*−*67*), have all been found in aquatic environments and may be useful as tracers of human waste.

To date, there has been no study that systematically examined multiple chemicals for their potential as tracers of human wastewater. This paper describes the results of research by the United States Geological Survey (USGS) and USEPA conducted in 2002. The study was designed to evaluate the utility of a suite of organic chemical compounds as specific indicators of human fecal contamination. The results are intended to determine which wastewater compounds are commonly found downstream from WWTPs and provide insight on their environmental persistence, the initial phase in determining if these compounds are useful chemical indicators of human fecal contamination. Because organic compounds can be at least partially removed or transformed during wastewater treatment procedures (*68*−*70*), including chlorination (*71*), the effluents of WWTPs, rather than their raw influents, were targeted in this study because we were interested in behavior following discharge, rather than reductions during treatment. It should be noted that this study was designed to explore the correlation between the presence of the chemicals and known human waste sources, and not the relation of the chemicals directly to the pathogens that are presumably present within the waste. Traditional microbial indicator data were collected as part of this study to compare the behavior of microbial and chemical indicators and to determine if there is added interpretive power provided by using these two complementary data types together.

Experimental Design

Site Selection and Sampling. This study focused on 10 WWTPs across the United States (Figure 1). Site selection was primarily based on the results of previous research activities (*42*, *52*). Most of the sample sets consisted of one upstream, one effluent, and two downstream samples (DS1 = sites proximal to WWTP discharge and DS2 = sites further

downstream from WWTP discharge). The network consisted of 40 sampling sites: 9 upstream samples (one site had no upstream sampling point), 11 WWTP effluent samples (one site had two WWTP discharge points), and 20 downstream samples. The 10 locations represent a variety of climatic conditions, populations served, stream sizes, and treatment practices (Table 1). The distances from the treatment plants to the upstream and downstream locations vary because of sampling accessibility. The discharge from the WWTPs contributed between 10 and 95% of the streamflow at the DS1 site (Table 1). The samples from the Arizona location are unique in that the stream is composed entirely of wastewater; thus, the "upstream" sample is actually a sample from the channel immediately downstream from a wastewater-treatment plant. For all of the data interpretation, the Arizona location was considered to have two WWTP samples and no upstream sample.

In addition to the 10 WWTP-influenced locations, samples were collected from 2 remote sites in Michigan and Montana (Figure 1) in areas having minimal direct impact from human wastewater. These samples were not included when calculating frequencies of detection (Table 2) or any other statistical analysis.

All of the samples were collected by USGS personnel. For the stream samples, standard width- and depth-integrating techniques were used to ensure a representative water sample (*72*). More details on the integrated sampling protocols can be found in a paper published previously (*42*). The effluent sample was collected as a grab sample from the discharge.

Chemical Analysis. The collected water samples were placed in baked amber glass bottles and shipped on ice for analysis to the USGS National Water Quality Laboratory in Lakewood, Colorado and the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas for analysis. Three different analytical methods were used because of differences in the physicochemical properties of the compounds. For the majority of the pharmaceuticals, the method consisted of passing 500−1000 mL of filtered water though solid-phase extraction (SPE) cartridges, which were eluted, concentrating the eluent, and analyzing the final extract using liquid chromatography/mass spectrometry positive-ion electrospray [LC/MS-ESI(+)] (*73*). Throughout this paper, this method will be referred to as the "pharmaceutical method". For the wastewater compounds, a whole-water sample was extracted using continuous liquid−liquid extraction and then analyzed using gas chromatography/mass spectrometry (GC/MS) (*74*). This will be identified as the "wastewater method", although other classes of compounds (such as pharmaceuticals) are included as analytes. Twenty-five antibiotic compounds were extracted and analyzed by the third method, SPE using tandem cartridges, and LC/MS-ESI(+) on a single quadrapole mass spectrometer (the "antibiotic method"). Additional details on this method can be found in Kolpin et al. (*42*). The target compounds, their methods of analysis, and their respective reporting levels are listed in Table 2. Qualitatively identified compound detections for which the calculated concentrations were less than the reporting level were reported as concentration estimates and were included in the statistical analysis. As in our previous study (*42*), estimated concentrations were included in statistical analyses so that a more comprehensive and complete data set could be used to determine the range of potential concentrations in ambient water samples impacted by wastewater, maximizing the scientific value of our results (*75*)

Microbial Analysis. Samples were collected in presterilized Teflon bottles and shipped overnight on ice for analysis to the USEPA, Office of Research and Development's National Exposure Research Laboratory in Cincinnati, Ohio for analysis. Three different aliquots (1, 10, and 100 mL) were analyzed in triplicate by two different USEPA methods: the modified *E. coli* method (modified from method 1103.1; mTEC), and modified enterococci method (method 1600; MEI). The experimental details for each method were published previously (*76*). The median time between collection and microbial analyses was 26 h, which exceeded the 8-hour ideal time between collection and analysis because of the shipping considerations. However, because all of the samples associated with a WWTP were subject to the same approximate delay, the results show within-site changes in microbial populations, and concentrations reported herein are considered estimates.

Quality Control. Compound concentrations were blank corrected to zero if the concentrations in the environmental samples were less than 10 times that measured in the associated laboratory blanks. In addition, two replicate field blanks were collected and processed after collection of a surface water sample comprised wholly of treated wastewater effluent at a site in Arizona. The purpose of these replicate field blanks was to evaluate the potential for cross-contamination resulting from sample collection and equipment cleaning procedures.

No wastewater compounds were detected in either blank. No antibiotics were detected in the blanks using the antibiotic method. A total of 15 detections out of a possible 60 detections were identified in the 2 blanks analyzed by the pharmaceutical method. The mean and median concentrations of these detections were 0.0069 and 0.007 μg/L, respectively. Five compounds, diltiazem, diphenhydramine, caffeine, metformin, and trimethoprim, were detected in both blank samples. Carbamazepine, codeine, cotinine, fluoxetine, and sulfamethoxazole were detected in one of the two replicate blank samples. These field blank results suggest that under "worst-case" conditions, field sample collection protocols and equipment cleaning procedures were sufficient to minimize cross contamination. Field blank samples were also used to blank correct the associated field samples, using the same correction level of 10 times the detected concentration.

Thirty-three of the samples collected for this project were analyzed in duplicate for the 21 compounds in the pharmaceutical method (*73*), for a total of 693 (33×21) replicate pair measurements. Of these replicate pairs, 408 had non-detections in both samples, 55 had a detection in only one of the samples, and 230 had detections in both samples. In about half of the 55 replicate pairs that had only one detection, the detected compound was near or less than the reporting level. For the 230 pairs having measurable detections in both samples, the relative percent difference (RPD) was calculated. The median RPDs for the pharmaceuticals ranged from 6.07% for acetaminophen to 16.6% for fluoxetine. The overall median RPD for all samples and all compounds was 10.2%. This result indicates that the precision of ambient concentrations in environmental samples was acceptable for making comparisons between sites.

Two compounds, caffeine and cotinine, were determined by both the pharmaceutical (*73*) and the wastewater methods (*74*). The reporting levels for these compounds were lower in the pharmaceutical method, and thus, concentrations determined by the pharmaceutical method were the values used in the environmental data analysis. In 22 of the 40 samples, caffeine was detected by both methods. The median RPD for caffeine between these two methods was 41.6%. Cotinine was detected by both methods in 13 samples; the median RPD was 83.3%. The presence or absence of caffeine was confirmed by these two methods (that is, either detected by both methods, or not detected by both methods) in 70% of the samples. The presence or absence of cotinine was confirmed in 37.5% of the samples. The considerable RPDs between the pharmaceutical and wastewater methods likely reflect differences between sample type (filtered water versus

TABLE 1. Selected Ancillary Information on the Wastewater Treatment Plants Investigated

location	population served	land use[a] (%)	number hospitals/ pharm. manuf. served	treatment level	biological treatment used	disin-fectant	nitrogen removal	distance from Up[b] to WWTP[c] (m)	distance from WWTP to DS1[d] (m)	distance from WWTP to DS2[e] (m)	stream flow at Up (m^3/ s)	flow of WWTP effluent (m^3/ s)	stream flow at DS1 (m^3/ s)	stream flow at DS2 (m^3/ s)	stream flow at DS1 relative to base-flow conditions
Arizona	419 000	U = 33 A = 0	5/0	secondary	trickling filter	chlorine	no	N/A[f]	N/A	N/A	N/A	1.40	N/A	N/A	N/A
	320 278	ND = 67	2/0	secondary	activated sludge	chlorine	no	N/A	2330	13 780	N/A	1.42	2.32	1.21	high
Colorado	1 500 000	U = 8 A = 4 ND = 88	40/0	secondary	activated sludge	chlorine	yes	7242	14 484	96 561	5.97	4.81	15.0	2.07	low
Georgia	800 000	U = 7 A = 16 ND = 77	15/0	secondary[g]	activated sludge	UV	no	48 753	2736	64 372	26.0	4.50	36.2	32.0	normal
Iowa	29 700	U = 5 A = 85 ND = 10	0/0	secondary	activated sludge	UV	ammonia only	6.1	393	8441	0.0028	0.13[h]	0.14	0.10	low
Kansas	115 000	U = 85 A = 5 ND = 10	1/0	secondary	activated sludge	UV	no	457	1067	1372	0.025	0.40	0.42	0.45	normal
Minnesota	90 000	U = 5 A = 84 ND = 11	4/0	secondary	N/A	chlorine	yes	91.4	305	1067	3.96	0.61	4.58	4.58	normal
Nevada	625 000	N/A	4/0	tertiary	activated sludge	chlorine and UV	yes	3219	1609	9656	0.32	3.72[h]	6.42	4.53	normal
New Jersey	65 000	U = 70 A = 20 ND = 10	0/0	secondary	activated sludge	chlorine	no	1175	96	3584	0.17	0.44[h]	0.48	0.62	low
New York	10 000	U = 47 A = 5 ND = 48	0/0	tertiary	trickling filter	chlorine	no	100	100	805	0.39	0.044[h]	0.42	0.71	normal
South Dakota	134 000	U = 3 A = 84 ND = 13	6/0	tertiary	trickling filter and activated sludge	chlorine	no	11 265	1609	6437	2.83	0.82	2.55	2.09	low

[a] Land use classifications: U = urban; A = agricultural; ND = not developed (includes forest, wetland, and water). [b] Up= upstream sample. [c] WWTP = wastewater treatment plants. [d] DS1= first downstream sample. [e] DS2= second downstream sample. [f] N/A = not available. [g] With biological phosphorus removal. [h] Flows estimated based on volume treated on sample day (MGD × 0.0438 = m^3/s).

TABLE 2. Analytical Results of 110 Chemicals Investigated in This Study

name	CAS number	reporting level (μg/L)	median concn (μg/L)	maximum concn (μg/L)	frequency of detection (%)	primary use[a]
E. coli[b]			202	27 330	92.5	microbial indicator
enterococci[b]			315	22 670	97.5	microbial indicator
1,4-dichlorobenzene	106-46-7	0.5	<RL[c]	0.91	27.5	6-moth repellant
1,7-dimethylxanthine	611-59-6	0.14	0	8.55	35	2-caffeine metabolite
1-methylnaphthalene	90-12-0	0.5	0	0.1	5	9-gasoline and diesel fuel component
2,6-dimethylnaphthalene	581-42-0	0.5	ND	ND	0	9-diesel fuel component
2-methylnaphthalene	91-57-6	0.5	0	0.061	5	9-gasoline and diesel fuel component
3,4-dichlorophenyl isocyanate	102-36-3	0.5	0.077	0.32	72.5	8-intermediate in the production of dyes
4-cumylphenol	599-64-4	1.0	ND[d]	ND	0	4-detergent metabolite
4-*n*-octylphenol	1806-26-4	1.0	<RL	0.36	2.5	4-detergent metabolite
4-nonylphenol diethoxylate	NA	5.0	1.19	38	62.5	4-detergent metabolite
4-nonylphenol monoethoxylate	NA	5.0	0.485	18	62.5	4-detergent metabolite
4-octylphenol diethoxylate	NA	1.0	<RL	0.36	32.5	4-detergent metabolite
4-octylphenol monoethoxylate	NA	1.0	<RL	1.9	2.5	4-detergent metabolite
4-tert-octylphenol	140-66-9	1.0	<RL	1.1	22.5	4-detergent metabolite
5-methyl-1H-benzotriazle	136-85-6	2.0	<RL	1.7	45	8-antioxident
acetaminophen	103-90-2	0.036	0.001	1.78	50	2-antipyretic
acetophenone	98-86-2	0.5	<RL	0.78	7.5	6-fragrance
albuterol	18559-94-9	0.023	<RL	0.034	32.5	1-antiasthmatic (H,V)
anthracene	120-12-7	0.5	ND	ND	0	9-wood preservative
anthraquinone	84-65-1	0.5	<RL	0.58	30	10-pesticide
atrazine	1912-24-9	0.5	<RL	0.46	45	10-herbicide
benzo[*a*]pyrene	50-32-8	0.5	<RL	0.084	7.5	9-used in cancer research
benzophenone	119-61-9	0.5	0.1	0.61	67.5	7-fixative in perfumes and soaps
bisphenol A	80-05-7	1.0	<RL	0.31	30	8-used in manuf of polycarbanate resins
bromacil	314-40-9	0.5	<RL	0.69	17.5	10-herbicide
bromoform	75-25-2	0.5	<RL	0.62	10	8-wastewater ozonation byproduct
butylated hydroxyanisole (BHA)	25013-16-5	5.0	<RL	0.32	5	6-antioxidant
caffeine	58-08-2	0.016	0.046	7.99	70	2-stimulant (H)
camphor	76-22-2	0.5	<RL	0.13	5	7-flavor, odorant
carbadox	6804-07-5	0.05	ND	ND	0	1A-antibiotic (V)
carbamazepine	298-46-4	0.011	0.074	0.27	82.5	1-antiepileptic (H)
carbaryl	63-25-2	1.0	<RL	0.22	5	10-pesticide
carbazole	86-74-8	0.5	<RL	0.2	12.5	8-used in manuf of dyes and expolosives
chlorpyrifos	2921-88-2	0.5	<RL	0.032	10	10-domestic pest/ termite control
chlortetracycline	64-72-2	0.02	<RL	2.8	2.5	1A-antibiotic (V)
cholesterol	57-88-5	1.5	1.05	8.7	90	3-plant/animal sterol
cimetidine	51481-61-9	0.012	<RL	0.426	42.5	2-antacid (H)
ciprofloxacin	85721-33-1	0.01	ND	ND	0	1A-antibiotic (H)
codeine	76-57-3	0.015	0.027	0.73	72.5	1-analgesic (H)
coprostanol	360-68-9	2.0	0.355	5.9	60	3-fecal sterol
cotinine	486-56-6	0.023	0.021	1.03	92.5	2-nicotine metabolite (H)
cumene	98-82-8	0.5	ND	ND	0	8-intermediate in the production of plastics
dehydronifedipine	67035-22-7	0.015	0.004	0.022	57.5	1-antianginal (H)
demeclocycline	127-33-3	0.02	ND	ND	0	1A-antibiotic (H)
diazinon	333-41-5	0.5	<RL	0.15	47.5	10-insecticide
dichlorvos	62-73-7	1.0	<RL	0.049	5	10-insectide
diethyl phthalate	84-66-2	0.5	<RL	0.71	5	8-plasticizer
diethylhexyl phthalate	117-81-7	0.5	<RL	27	22.5	8-plasticizer
diltiazem	42399-41-7	0.016	0.012	0.146	67.5	1-antihypertensive (H)
diphenhydramine	58-73-1	0.015	0.005	0.387	55	2-antihistamine (H)
d-limonene	5989-27-5	0.5	ND	ND	0	6-antimicrobial, fragrance
doxycycline	564-25-0	0.05	ND	ND	0	1A-antibiotic (H, V)
enrofloxacin	93106-60-6	0.01	ND	ND	0	1A-antibiotic (V)
erythromycin-H2O	114-07-8	0.02	0.035	0.61	52.5	1A-antibiotic (H,V)
ethanol,2-butoxy-,phosphate	78-51-3	0.5	0.23	12	70	8-plasticizer
ethyl citrate	77-93-0	0.5	0.094	0.52	72.5	7-fragrance, tobacco addative
fluoranthene	206-44-0	0.5	<RL	0.19	10	9-coal tar and asphalt component
fluoxetine	54910-89-3	0.014	<RL	0.021	2.5	1-antidepressant (H)
galaxolide (HHCB)	1222-05-5	0.5	0.093	0.53	57.5	7-fragrance, musk
gemfibrozil	25812-30-0	0.013	ND	ND	0	1-antihyperlipidemic (H)
ibuprofen	15687-27-1	0.042	ND	ND	0	2-antiinflammatory (H)
indole	120-72-9	0.5	<RL	0.2	7.5	7-fragrance, pesticide inert
isoborneol	124-76-5	0.5	<RL	0.12	2.5	7-fragrance
isophorone	78-59-1	0.5	ND	ND	0	8-solvent
isoquinoline	119-65-3	0.5	<RL	0.095	2.5	7-flavor, fragrance
lincomycin	154-21-2	0.01	ND	ND	0	1A-antibiotic (H, V)
menthol	1490-04-6	0.5	<RL	1.3	20	7-cigarette and household item flavorant
metalaxyl	57837-19-1	0.5	ND	ND	0	8-soil pathogen, mildew
metformin	657-24-9	0.003	<RL	0.698	17.5	1-antidiabetic (H)
methotrexate	59-05-2	0.02	ND	ND	0	1A-antibiotic (H)
methyl salicylate	119-36-8	0.5	<RL	0.099	5	7-liniment, lotions
metolachlor	51218-45-2	0.5	<RL	0.097	37.5	10-herbicide
minocycline	10118-90-8	0.02	ND	ND	0	1A-antibiotic (H)
N,*N*-diethyl-*m*-toluamide (DEET)	134-62-3	0.5	0.097	2.1	70	6-insect repellant

TABLE 2 (Continued)

name	CAS number	reporting level (μg/L)	median concn (μg/L)	maximum concn (μg/L)	frequency of detection (%)	primary use[a]
naphthalene	91-20-3	0.5	<RL	0.16	5	9-fumagant, moth repellant
naproxen	22204-53-1		ND	ND	0	2-antiinflammatory (H, V)
norfloxacin	70458-96-7	0.01	ND	ND	0	1A-antibiotic (H)
oxytetracycline	79-57-2	0.05	ND	ND	0	1A-antibiotic (H, V)
para-cresol	106-44-5	1.0	<RL	0.4	15	8-wood preservative
para-nonylphenol-total	84852-15-3	5.0	<RL	22	17.5	4-detergent metabolite
pentachlorophenol	87-86-5	2.0	<RL	0.086	35	8-wood preservative
phenanthrene	85-01-8	0.5	<RL	0.066	5	9-manufacture of explosives
phenol	108-95-2	0.5	<RL	1.8	40	8-disinfectant
prometon	1610-18-0	0.5	<RL	0.27	25	10-herbicide
pyrene	129-00-0	0.5	<RL	0.13	7.5	9-coal tar and asphalt component
ranitidine	66357-35-5	0.01	<RL	0.295	27.5	2-antacid (H)
roxithromycin	80214-83-1	0.01	ND	ND	0	1A-antibiotic
sarafloxacin	98105-99-8	0.01	ND	ND	0	1A-antibiotic (V)
sitosterol	83-46-5	2.0	0.835	2.9	72.5	3-plant sterol
skatol	83-34-1	1.0	<RL	0.09	30	7-fragrance
stigmastanol	19466-47-8	2.0	<RL	1.2	22.5	3-plant sterol
sulfachloropyridazine	80-32-0	0.05	ND	ND	0	1A-antibiotic
sulfadimethoxine	122-11-2	0.01	ND	ND	0	1A-antibiotic (V)
sulfamerazine	127-79-7	0.02	ND	ND	0	1A-antibiotic (V)
sulfamethazine	57-68-1	0.01	ND	ND	0	1A-antibiotic (V)
sulfamethizole	72-14-0	0.05	ND	ND	0	1A-antibiotic (V)
sulfamethoxazole	723-46-6	0.064	0.068	0.763	72.5	1-antibiotic (H, V)
sulfathiazole	72-14-0	0.05	ND	ND	0	1A-antibiotic (V)
tetrachloroethylene	127-18-4	0.5	<RL	0.2	12.5	8-solvent, degreaser
tetracycline	60-54-6	0.02	ND	ND	0	1A-antibiotic (H, V)
thiabendazole	148-79-8	0.011	<RL	0.515	7.5	1-antifungal agent and antihelmintic (H, V)
tonalide (AHTN)	1506-02-1	0.5	0.56	2.6	80	7-fragrance, musk
tri(2-chloroethyl)phosphate	115-96-8	0.5	0.18	0.48	75	5-fire retardant
tri(dichlorisopropyl)phosphate	13674-87-8	0.5	0.2	0.48	77.5	5-fire retardant
tributyl phosphate	126-73-8	0.5	0.1	0.47	70	5-antifoaming agent and flame retardant
triclosan	3380-34-5	1.0	0.12	1.6	62.5	6-disinfectant, antimicrobial
trimethoprim	738-70-5	0.013	0.011	0.414	60	1-antibiotic (H, V)
triphenyl phosphate	115-86-6	0.1	<RL	0.18	37.5	8-plasticizer
tylosin	1401-69-0	0.02	ND	ND	0	1A-antibiotic (V)
virginiamycin	21411-53-0	0.1	ND	ND	0	1A-antibiotic (V)
warfarin	81-81-2	0.012	ND	ND	0	1-anticoagulant (H)

[a] Use classifications: 1, prescription pharmaceutical (registered for H= human, V= veterinary uses in the United States); 2, nonprescription pharmaceutical (registered for H= human, V= veterinary uses in the United States); 3, plant or animal sterol; 4, detergents and their degradates; 5, flame retardants; 6, household wastewater compounds; 7, flavors and fragrances; 8, industrial wastewater compounds; 9, polycyclic aromatic hydrocarbons; and 10, pesticides. Chemicals in use classes 1 and 2 were analyzed using LC/MS (1A= antibiotic method); the remaining compounds were analyzed by GC/MS. [b] The concentration unit for the microorganisms is colony forming unit/100 mL (cfu/100 mL). [c] RL = reporting level.[d] ND = not detected.

whole water, respectively), extraction mechanism (SPE versus continuous liquid−liquid extraction), and analysis technique (LC/MS versus GC/MS). When only the measurements that were greater than the reporting level of the wastewater method (0.5 μg/L caffeine, 1.0 μg/L cotinine) were considered, the correspondence between the methods improved. Caffeine was detected in seven instances by both methods, and the median RPD decreased to 37.5%. Cotinine was never simultaneously detected; only one measurement of cotinine by the pharmaceutical method surpassed the reporting level of the wastewater method. Confirmation of presence or absence for both caffeine and cotinine was determined in 97.5% of the samples.

There were also two compounds that were included in both the pharmaceutical method (*73*) and the antibiotic method (*42*), sulfamethoxazole and trimethoprim. The agreement between the measured concentration and the rate of detection by both methods was good. In 20 of the 40 samples, sulfamethoxazole was detected by both methods and had a median RPD of 17.6%. Twenty-two samples had detections of trimethoprim by both methods and had a median RPD of 18.8%. The presence or absence of sulfamethoxazole was confirmed by these two methods in 75% of the 40 samples, whereas the presence or absence of trimethoprim was confirmed in 92.5% of the 40 samples. The small RPDs between the pharmaceutical and antibiotic methods are likely a result of identical sample type, extraction mechanism, and instrumental analyses, with minor differences in SPE elution solvents and instrumental analysis conditions. When only the measurements that were greater than the reporting level of the antibiotic method (0.02 μg/L sulfamethoxazole, 0.01 μg/L trimethoprim) were considered, the correspondence between the methods showed little to no improvement. Sulfamethoxazole again was detected in 20 instances by both methods; the median RPD remained 17.6%. The number of simultaneous detections of trimethoprim decreased to 19, but the median RPD also decreased slightly to 18.1%. With this more stringent reporting level, the presence or absence for sulfamethoxazole was confirmed by both methods in 75% of the samples; trimethoprim was confirmed in 85% of samples.

Results and Discussion

Summary Results for Chemical Samples. Of the 110 chemicals investigated in this study, 78 were found in at least one sample (Table 2). Not surprisingly, many of these same chemicals were also detected in a previous national stream-reconnaissance study of surface-water sites susceptible to wastewater discharge (*42*). The median number of compounds detected at each sample location was: upstream (11), WWTP effluent (35), 1st downstream (33), 2nd downstream (24), and reference (1.5) (Tables 3 and 4). Among the

TABLE 3. Number of Compounds and Total Concentration of Analytes Found at Each Location, Classified by Sample Site

	number of compounds detected				total concentration at each site[a] (μg/L)			
location	upstream	WWTP effluent	DS1	DS2	upstream	WWTP effluent	DS1	DS2
Arizona 1		46				97.7		
Arizona 2		41	40	34		91.7	72.9	26.0
Colorado	33	37	46	12	11.4	46.7	30.0	1.26
Georgia	4	32	19	23	0.325	9.5	2.72	2.96
Iowa	11	50	47	35	2.75	27.5	29.9	10.6
Kansas	18	29	31	32	4.18	27.3	6.28	8.14
Minnesota	23	30	29	25	14.6	11.2	8.94	6.60
Nevada	9	28	35	23	2.41	21.6	8.66	4.07
New Jersey	10	35	34	30	2.74	12.3	12.2	7.21
New York	11	43	30	20	2.48	91.1	23.5	9.07
South Dakota	6	31	22	20	2.23	39.2	10.9	2.92

[a] Sum of all detections.

TABLE 4. Compounds Detected at the Reference Locations

compound	location[a]	concentration (μg/L)
acetaminophen	Michigan	0.012
caffeine	Michigan	0.0056
methyl salicylate	Michigan	0.015

[a] No compounds detected at the Montana location.

detected compounds, the median concentration detected was less than 1 μg/L for most compounds; however, seven compounds had at least one detection greater than 5 μg/L, and one concentration of 38 μg/L was detected (Table 5). Not surprisingly, these high concentrations were all derived from the WWTP effluent samples (Table 5).

When the compounds are divided into categories based on their type of use, fire retardants and the fecal and plant sterols were the two classes most commonly detected (Figure 2). In general, there were substantial differences in the frequency of detection within the chemical groups. For example, of the nonprescription drugs, cotinine was detected in 92.5% of the environmental samples, but ibuprofen was never detected (Table 2).

For the two reference locations, three compounds were detected at low concentrations at the Michigan reference site, whereas none were detected at the Montana reference location (Table 4). The relative absence of detected compounds at the reference sites indicates that the target compounds are not ubiquitous in all streams, and therefore could potentially serve as chemical indicators of human wastewater.

Several compounds in this study, ibuprofen, trimethoprim, sulfamethoxazole, cabamazepine, cholesterol, coprostanol, galaxolide (HHCB), tonalide (AHTN), caffeine, *N*,*N*-diethyl-*m*-toluamide (DEET), fluoxetine, and triclosan, have been monitored in WWTP effluents and/or waters directly impacted by human wastewater (*15*, *17*−*22*, *26*, *36*, *43*). It should be noted that none of these other studies included more than 5 of the compounds in the above list, and the maximum number of compounds included in any one study was 15. In general, the concentrations in this study are lower than those measured in the other studies, all of which were conducted outside of the United States. This can be attributed to several factors, such as distinct usage patterns, discrepancies in household water consumption, differences in treatment regulations and efficiencies, and variations in analytical methodology.

Several studies (*20*, *24*, *25*, *31*, *32*) have examined both cholesterol and coprostanol, so it is possible to calculate coprostanol-to-cholesterol ratios as an indicator of human fecal contribution. In this study, the median coprostanol-to-cholesterol ratios in the upstream, WWTP effluent, DS1, and DS2 samples were 0, 0.66, 0.55, and 0.48, respectively. The effluent and downstream coprostanol-to-cholesterol ratios are similar to that found in human fecal material (*39*), making the human contribution of coprostanol from the WWTP to the streams apparent. In other studies, the range of upstream ratios was 0.003−0.017 (*24*, 25), the range of effluent ratios was 0.50−1.79 (*31*, *32*), and the range of downstream ratios was 0.061−0.38 (*24*, *25*), all of which agree well with our study.

Summary Results for Microbial Samples. Overall, microorganisms were the most commonly detected constituents in this study, with the two microbial indicators, *E.* coli and enterococci, detected in greater than 90% of the samples (Table 2). Given the multiple possible sources (e.g., humans, livestock, wildlife), this is not an unexpected result. Not only were these two indicators detected frequently, they were also found in high densities, with 75% of all of the samples exceeding the levels recommended for recreational waters (*6*, *7*) for either *E. coli* or enterococci (235 and 61 colony forming units/100 mL (cfu/100 mL), respectively). In 25% of the samples, high densities (>5000 cfu/100 mL) of *E. coli* or enterococci were determined.

In contrast to the chemical analytes, *E. coli* and enterococci were found in both collected reference samples (Michigan: *E. coli*, 51.3 cfu/100 mL, enterococci, 40.3 cfu/100 mL; Montana: *E. coli,* 56.7 cfu/100 mL, enterococci, 373 cfu/100 mL). This illustrates the lack of specificity of microbial indicators for identifying solely human fecal contamination. The two locations were minimally impacted by human waste, but still showed bacterial counts, presumably caused by fecal material from other sources (e.g., livestock, wildlife). Most of the pathogens that cause illness in humans come from human hosts; thus, it is useful to know if drinking or recreational water is contaminated with human or animal waste, and therefore use indicators are needed that are specific for human or animal waste. These data suggest that the chemical indicators of fecal contamination might be more useful for discriminating human from animal sources of fecal contamination.

Instream Analysis. More specific trends in the number of detections and concentrations can be determined if the data from each WWTP site are compared with the upstream and downstream samples from within the same stream reach (Table 3). The results clearly show the contributions of WWTPs to water quality, with both the overall frequency of detection and, in most cases, the total concentration (that is, the sum of the concentrations of all detected compounds) being greater in the samples collected downstream than those collected upstream from the WWTPs. Previous studies (*15*, *24*, *25*) with similar sampling design and target compounds (e.g., pharmaceuticals and fecal sterols) also documented the contributions of WWTP effluents to the stream concentrations of pharmaceuticals and other wastewater derived compounds.

The percent change between the different sample types was calculated for both the number of compounds and the total concentration (Table 6) and evaluated using the Mann−Whitney U test. Significant increases ($P < 0.050$) in the total number of compounds were found between the upstream samples and the WWTP, DS1, and DS2 samples (Table 6), reflecting the contribution of chemical input from the WWTPs. No statistical differences were identified between the WWTP and the DS1 sample types, but statistical decreases were found between the WWTP and the DS2 sample types (Table 6). As with the number of compounds, the total concentration of the upstream sample was significantly different from the WWTP and DS1 but not significantly different from the DS2 sample (Table 6). The total concen-

TABLE 5. Patterns of Median Concentration and Frequency of Detection for the 35 Most Commonly Detected Chemicals

	type[a]	median concn (μg/L)				maximum concn (μg/L)				detection freq (%)				Kruskal–Wallis χ^2 *P* value[b]		
		Up[c]	WWTP	DS1	DS2	Up	WWTP	DS1	DS2	Up	WWTP	DS1	DS2	Up to WWTP	WWTP to DS1	WWTP to DS2
E. coli (cfu/100 mL)		287	78	483.5	174.2	27 330	9300	8170	11 130	89	91	90	100	0.403	0.218	0.503
enterococci (cfu/100 mL)		1277	105.3	713.5	211.5	14 900	1277	22 670	3770	100	91	100	100	**0.040**	0.078	0.291
1,4-dichlorobenzene	6	<RL	0.110	<RL	<RL	<RL	0.910	0.280	<RL	0	64	40	0	**0.005**	0.264	0.003
1,7-dimethylxanthine	2	<RL	<RL	<RL	0.017	0.488	8.550	1.760	0.504	11	36	40	50	0.178	0.809	0.969
3,4-dichlorophenyl isocyanate	8	<RL	0.150	0.064	0.046	0.110	0.320	0.230	0.280	33	100	80	70	**0.000**	0.062	**0.002**
4-nonylphenol diethoxylate	4	<RL	2.200	2.100	0.770	2.100	38.0	15.0	3.100	22	91	70	60	**0.002**	0.377	**0.028**
4-nonylphenol monoethoxylate	4	<RL	0.880	0.760	0.405	1.300	18.0	12.0	2.300	22	91	70	60	**0.006**	0.274	**0.033**
4-octylphenol diethoxylate	4	<RL	0.120	<RL	<RL	0.340	0.360	0.220	0.120	11	55	40	20	0.082	0.648	**0.048**
5-methyl-1H-benzotriazle	8	<RL	0.820	<RL	<RL	0.270	1.700	1.100	0.880	11	82	40	40	**0.001**	0.088	**0.050**
acetaminophen	2	<RL	0.006	<RL	<RL	1.780	1.060	0.684	1.720	44	73	40	40	0.583	0.509	0.509
benzophenone	7	<RL	0.200	0.130	0.072	0.076	0.610	0.420	0.510	11	100	80	70	**0.000**	0.089	**0.021**
bisphenol A	8	<RL	0.120	<RL	<RL	0.190	0.310	0.300	0.120	11	55	40	10	**0.040**	0.361	**0.023**
caffeine	2	0.040	0.053	0.041	0.050	0.807	7.990	2.600	0.807	67	73	80	60	0.488	0.943	0.473
carbamazepine	1	<RL	0.080	0.079	0.075	0.158	0.270	0.172	0.186	33	91	100	100	**0.006**	0.398	0.324
cholesterol	3	0.840	2.000	1.200	0.785	4.700	8.700	8.000	5.100	89	91	90	90	**0.013**	0.275	**0.012**
codeine	1	<RL	0.139	0.039	0.018	0.217	0.730	0.211	0.195	33	82	90	80	**0.021**	0.438	0.120
coprostanol	3	<RL	1.300	0.720	0.175	4.000	5.900	5.600	3.300	22	82	80	50	**0.010**	0.224	**0.026**
cotinine	2	0.012	0.024	0.022	0.024	0.215	1.030	0.481	0.072	100	91	90	90	0.057	0.379	0.647
dehydronifedipine	1	<RL	0.011	0.004	0.005	<RL	0.021	0.022	0.017	0	73	70	80	**0.002**	0.336	0.338
diazinon	9	<RL	0.037	0.011	<RL	0.046	0.150	0.092	0.090	33	73	50	30	0.051	0.192	**0.044**
diltiazem	1	<RL	0.049	0.016	0.010	0.074	0.146	0.067	0.057	22	91	80	70	**0.006**	0.121	**0.022**
diphenhydramine	2	<RL	0.078	0.009	<RL	0.273	0.387	0.244	0.154	22	91	80	20	**0.006**	0.091	**0.004**
ethanol,2-butoxy-,phosphate	8	0.230	0.180	0.310	0.170	1.800	12.0	5.500	1.700	67	64	80	70	0.846	0.859	0.747
ethyl citrate	7	<RL	0.270	0.105	0.082	0.074	0.520	0.400	0.260	11	100	100	70	**0.000**	**0.031**	**0.002**
galaxolide (HHCB)	7	<RL	0.280	0.140	0.038	0.057	0.530	0.350	0.160	11	100	60	50	**0.000**	**0.031**	**0.000**
N,N-diethyl-*m*-toluamide(DEET)	6	<RL	0.180	0.145	0.117	0.160	2.100	0.640	0.420	33	82	80	80	**0.017**	0.646	0.416
pentachlorophenol	8	<RL	0.024	0.004	<RL	0.033	0.072	0.086	0.033	22	55	50	10	0.086	0.970	**0.026**
phenol	8	<RL	0.270	<RL	<RL	0.650	1.800	0.680	0.610	33	55	40	30	0.298	0.594	0.292
sitosterol	3	0.710	1.100	1.020	0.570	2.000	2.900	2.900	2.100	78	82	70	60	0.062	0.570	**0.021**
sulfamethoxazole	1	<RL	0.150	0.081	0.057	0.292	0.589	0.763	0.321	22	91	80	90	**0.003**	0.139	0.062
tonalide (AHTN)	7	<RL	1.000	0.710	0.240	0.240	2.600	2.100	0.880	22	100	100	90	**0.000**	**0.037**	**0.001**
tri(2-chloroethyl)phosphate	5	<RL	0.330	0.170	0.180	0.200	0.430	0.480	0.320	33	100	90	70	**0.000**	0.090	**0.013**
tri(dichlorisopropyl)phosphate	5	<RL	0.300	0.210	0.140	0.150	0.480	0.390	0.350	22	100	100	80	**0.000**	0.052	**0.015**
tributyl phosphate	5	<RL	0.180	0.120	0.106	0.100	0.470	0.340	0.270	11	100	90	70	**0.000**	0.180	0.062
triclosan	6	<RL	0.250	0.200	0.110	0.100	1.600	1.000	0.640	11	100	70	60	**0.000**	0.245	**0.015**
trimethoprim	1	<RL	0.038	0.014	0.012	0.054	0.353	0.414	0.093	22	73	70	70	**0.019**	0.336	0.144
triphenyl phosphate	8	<RL	0.072	0.027	<RL	0.057	0.180	0.096	0.070	11	64	50	20	**0.011**	0.287	**0.021**

[a] The number following the compound name indicates the compound class: 1, prescription pharmaceutical; 2, nonprescription pharmaceutical; 3, plant or animal sterol; 4, detergents and their degradates; 5, flame retardants; 6, household wastewater compounds; 7, flavors and fragrances; 8, industrial wastewater compounds; 9, pesticides. [b] *P* values in bold indicate significant difference at the 95% confidence level. [c] Up = upstream sample.

trations in the WWTP and DS2 were significantly different, but the total concentrations in the WWTP and DS1 as well as the total concentration in the two downstream samples were not statistically different (Table 6). The trends in both the number of compounds and the total concentration suggest that with additional distances from a WWTP processes (e.g., dilution, degradation, sorption, etc.) act to decrease chemical concentrations with transport downstream.

To examine instream trends in more detail, we considered only those compounds found in greater than 50% of the WWTP effluent samples. This reduced the number of compounds from 110 to 35 (Table 5). Because the reference samples consisted of a single sample, they were not included in this spatial analysis. Individual compounds exhibited different incidence patterns and persistence. Of the 35 compounds, 22 were found in greater than 80% of the WWTP effluent samples. In contrast, only two compounds, cotinine (a nicotine metabolite) and cholesterol, were found at similar frequencies in the upstream and WWTP effluent samples, emphasizing the importance of effluent-point discharges as the predominant source of the potential chemical indicators of fecal contamination. Among DS2 samples, however, 4 of these 22 chemical indicators were found in <50% of the samples, 10 were found between 60 and 70% of the samples, and 8 were found more than 80% of the samples.

A Kruskal–Wallis test, the nonparametric equivalent of the analysis of variance (ANOVA), was performed to determine the variation between the upstream and WWTP effluent samples, the WWTP effluent and DS1 samples, and the WWTP effluent and the DS2 samples, based on the concentration of each compound at each of the 10 locations (Table 5). A change is considered statistically significant if $P < 0.050$. Concentrations of 25 of the compounds were found to have significant increases between the upstream and WWTP effluent samples; enterococci were shown to decrease significantly between the upstream and WWTP samples. The increases in concentrations were expected and demonstrates that many compounds present in WWTP effluent are not found upstream of this source. In comparison, when the WWTP effluent and the DS1 samples are compared, only ethyl citrate, galaxolide, and tonalide were found to be statistically different. The similarity in chemical concentrations between WWTP effluent and proximal downstream sampling points clearly shows the effect of WWTP effluent on stream water quality. However, a comparison between the WWTP effluent and the DS2 sampling sites found 21 compounds to be statistically different. Thus, with further distances from WWTP discharge, instream processes (e.g., dilution, degradation, sorption, etc) are causing decreases in chemical concentrations. Because the sampling design for this study did not take into account stream travel times,

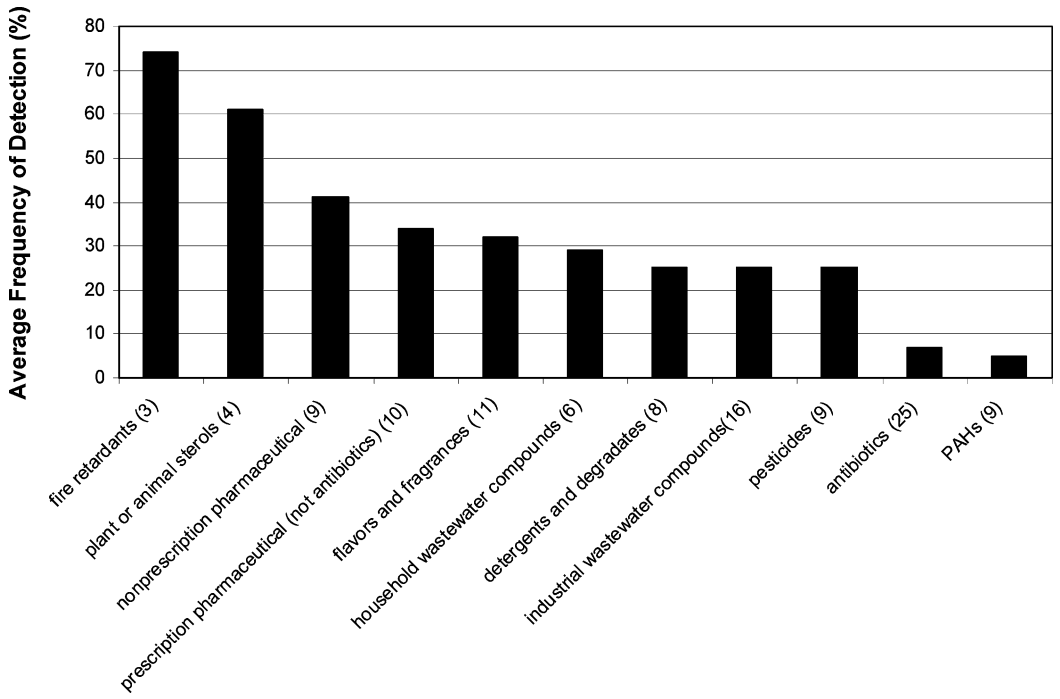

FIGURE 2. Average frequency of detection by compound class. Numbers in parentheses indicate the number of compounds included in each class. Because of the infrequent detection of antibiotics, they were separated from the rest of the prescription pharmaceuticals.

TABLE 6. Percent Change between the Sample Sites for Both the Number of Compounds and Total Concentration (μg/L), as Well as the Mann−Whitney U-test *P* Values

	number of compounds		total concentration	
sample comparisons	median % change	*P* value[a]	median % change	*P* value
upstream − WWTP effluent	250	**0.000**	796	**0.000**
upstream − DS1	240	**0.001**	345	**0.010**
upstream − DS2	156	**0.008**	94.7	0.156
WWTP effluent − DS1	−3.10	0.512	−47.8	0.051
WWTP effluent − DS2	−23.0	**0.006**	−70.9	**0.000**
DS1 − DS2	−14.4	0.123	−52.2	0.052

[a] *P* values in bold indicate significant difference at the 95% confidence level.

TABLE 7. Median Ratios between the Concentrations of Ephemeral (Galaxolide and Tonalide), Intermediate (Coprostanol and Triclosan) and Recalcitrant (Carbamazepine and DEET) Chemicals in the WWTP Effluent, First, and Second Downstream Samples

compounds evaluated	WWTP	DS1	DS2
galaxolide: tonalide	0.22	0.17	0.11
triclosan: coprostanol	0.21	0.18	0.19
DEET: carbamazepine	0.20	0.41	0.35
tonalide: triclosan	4.33	2.32	1.41
tonalide: coprostanol	0.58	0.47	0.28
galaxolide: triclosan	0.88	0.43	0.32
galaxolide: coprostanol	0.13	0.06	0.03
tonalide: DEET	9.20	2.34	1.69
tonalide: carbamazepine	13.14	9.62	4.26
galaxolide: DEET	1.64	0.46	0.32
galaxolide: carbamazepine	2.47	1.53	0.20
triclosan: DEET	1.78	0.47	0.98
triclosan: carbamazepine	3.27	2.17	0.67
coprostanol: DEET	7.38	3.51	0.46
coprostanol: carbamazepine	15.03	9.29	1.20

it was not possible to determine elimination rates for the various chemicals.

Note that two compounds that are consumed daily by many people, caffeine and cotinine, were found at similar frequencies in the upstream, WWTP effluent, and downstream samples. Their ubiquitous presence may be linked to either their abundant use, inefficient removal in WWTPs that may be present further upstream from the sampling sites, contributions from diffuse sources, such as individual septic systems, other point sources, such as stormwater discharges, or the inherent stability of the compounds.

Five compounds [benzophenone, ethyl citrate, galaxolide (HHCB), tributyl phosphate, and triclosan] were found in only one upstream sample but in all of the WWTP effluent samples. The DS2 concentrations of these five compounds declined at different rates; with frequencies of detection ranging from 50 to 70%. Thus, these compounds may make them candidates for chemical indicators of human fecal contamination.

As noted by Buser et al. (*19*), the ratio between the concentrations of an ephemeral compound and a recalcitrant compound should decrease during wastewater treatment, as the less permanent compound is preferentially decreased. In addition, they noted that the ratio should decrease with increased residence time in a water body. To explore this trend, the ratio of six compounds was investigated. The six consisted of two compounds, galaxolide and tonalide, that were ephemeral (as indicated by significant Kruskal−Wallis χ^2 *P* values between the WWTP and DS1 and the WWTP and DS2; Table 5), two intermediate persistence compounds, coprostanol and triclosan (as indicated by significant Kruskal−Wallis χ^2 *P* values between the WWTP and DS2), and two compounds that were recalcitrant, cabamazepine and DEET (no significant Kruskal−Wallis χ^2 *P* values downstream of the WWTP). The ratios between the concentrations of the compounds were calculated in the WWTP effluent and the downstream samples (Table 7).

When the compounds with similar persistence were compared to each other, their ratios remained fairly consistent in the WWTP, DS1, and DS2 samples. The ephemeral and intermediate compounds showed slight downward trends, suggesting that the compounds in the numerator (galaxolide and triclosan) were slightly less persistent that

those in the denominator (tonalide and coprostanol, respectively). The recalcitrant compounds show a slight increasing trend, suggesting that DEET may be slightly more persistent than carbamazepine. But, overall, the compounds in each class behaved similarly, and there was no extremely apparent preferential elimination of one compound relative to another. Conversely, when the concentrations of the ephemeral compounds are compared to the intermediate and persistent compounds, as well as when the intermediates are compared to the persistent compounds, there is a dramatic decrease in the ratios when the WWTP, DS1, and DS2 ratios are compared. The decrease in the ratios reflects the preferential removal of the less persistent compound in the numerator. These ratios may not only be a useful tool in evaluating the composition of compounds in a lake as compared to the lake's hydraulic residence time, as Buser et al. found (*19*), but also the temporal and spatial distance from a known source in a stream or riverine system.

The median and maximum concentrations and frequency of detection for the microbial indicators are listed in Table 5. A trend seen with the microbial indicators was generally lower densities in the WWTP effluent samples compared to both the upstream and DS1 and DS2 samples. The recreational water guideline for *E. coli* of 235 cfu/100 mL (*6*, *7*) was exceeded in 60% of the upstream samples, 36% of the WWTP effluent samples, 50% of the first downstream samples, and 40% of the second downstream samples. Similarly, the enterococci guideline of 61 cfu/100 mL (*6*, *7*) was exceeded in 78% of the upstream samples, 64% of the WWTP effluent samples, 80% of the first downstream samples, and 70% of the second downstream samples. The most probable explanation of this phenomenon is that disinfection performed at the WWTP reduced the concentrations of bacteria in the WWTP outflow, but the rapid regrowth of the bacteria downstream, as residual disinfection was consumed or dispersed, resulted in increased concentrations. Even when these lowered WWTP effluent concentrations are taken into account, the microbial indicators do not follow the same general pattern of the chemicals, that is, low concentrations upstream, high concentrations at the WWTP, and gradually decreasing concentrations downstream. The concentration of the bacteria upstream from the WWTP was often close to, or greater than, the concentration in the second downstream sample. If blind samples were sent to a laboratory to determine where a WWTP effluent plume was located (and thus, where there would be a higher probability of contamination by human pathogens), these results suggest that the source of fecal contamination could be misidentified. As with the detections in the locations minimally impacted by humans in Michigan and Montana, the high densities in the upstream samples illustrate the limitations of the microbial indicators.

Correlation Analysis. To further identify possible correlative relations between the 35 compounds in Table 5, the data were examined using two different statistical analyses. The first was a standard parametric correlation analysis using an Excel (Microsoft Corporation, Redmond, WA) data analysis package. The data were examined by sample site type. A relation was determined to be significantly correlated at the 99% confidence level (two-tailed) if its correlation coefficient was greater than the critical value of 0.750 for the upstream sample (degrees of freedom (df) =7), 0.685 for the WWTP effluent samples (df = 9), and 0.716 for both of the downstream samples (df = 8). Standard water-chemistry measurements and other physical properties measured at the time of sample collection (pH, conductivity, water temperature, turbidity, dissolved oxygen, and streamflow) were also included in this analysis. Of these, only two physical properties, water temperature and turbidity, correlated to the concentration of the compounds; these relations were primarily negative correlations in the WWTP effluent samples.

In examining the correlations between chemicals, most of the correlations were between those compounds that shared use classifications. For example, the pharmaceuticals trimethoprim, diltiazem, sulfamethoxazole, dehydronifedipine, codeine, diphenhydramine, and carbamazepine were all correlated to one another in the upstream and downstream samples. Cholesterol, coprostanol, and sitosterol (the fecal sterols) were positively correlated with each other in all four sample-site types. The wastewater (nonpharmaceutical) compounds were correlated to each other, but chiefly in the upstream samples. Surprisingly, caffeine was only correlated to its metabolite, 1,7-dimethylxanthine, in the WWTP effluent and downstream samples and several of the other wastewater compounds in the upstream samples but not to any other pharmaceuticals, which would be ingested like caffeine. Cotinine was similarly correlated to some of the wastewater compounds in the WWTP effluent and downstream samples, but also to the fecal sterols in those samples.

The second type of analysis was a clustering analysis performed using an algorithm in statistiXL (Kalamunda, Western Australia), a companion statistical software package for Excel. For this analysis on a quantitative data set, the Pearson Correlation, a parametric analysis, was used as the similarity measure, and the group average was used as the cluster method. As the dendrogram in Figure 3 shows, compounds with similar use classifications were frequently grouped together. For example, the pharmaceuticals trimethoprim, sulfamethoxazole, dehydronifedipine, diphenhydramine, diltiazem, and carbamazepine were all grouped together. The fact that trimethoprim and sulfamethoxazole were grouped together was particularly interesting because these two antibiotics often are prescribed in tandem. Other notable groupings were fecal sterols, cholesterol, coprostanol, and sitosterol; caffeine and its metabolite; and the musks tonalide and galaxolide. Perhaps the most unexpected result was that acetaminophen grouped with the two microorganisms, *E. coli* and enterococci, and not the other pharmaceuticals. The use of clustering analysis and other parametric approaches, particularly with a ranked measure, such as the Pearson correlation, removes the effect of large differences in concentration range on statistical inference, and may reflect relations between constituents more accurately.

Utility as Indicators of Human Fecal Contamination. The results of this work indicate that chemicals, particularly the 35 listed in Table 5, may be useful as indicators of human fecal contamination. For most of these chemicals, there is an increase in the frequency of detection and concentration in the WWTP effluent sample as compared to the water sample collected upstream. In addition, the chemical concentrations and occurrences decrease downstream with distance from the WWTPs. Specifically, the distinct changes that the concentrations of the wastewater compounds ethyl citrate, galaxolide, and tonalide undergo between the upstream, WWTP effluent, and two downstream sites suggest that they may be good indicator candidates. Compounds that are typically only used by humans, such as the pharmaceuticals carbamazepine and diphenhydramine, and even caffeine, would also be potential indicator candidates. These compounds are slightly more desirable than the wastewater compounds as indicators because they are ingested and would be excreted from the human body. Of the fecal sterols, coprostanol, because it has a human source and exhibits the most changes in concentration between the sample sites, has the best potential for use as an indicator of human fecal material. However, no compound should be ruled in or out until its presence or concentration has been compared to the incidence of illness (such as gastroenteritis) caused by contact with the water. This correlation requires an epidemiological study.

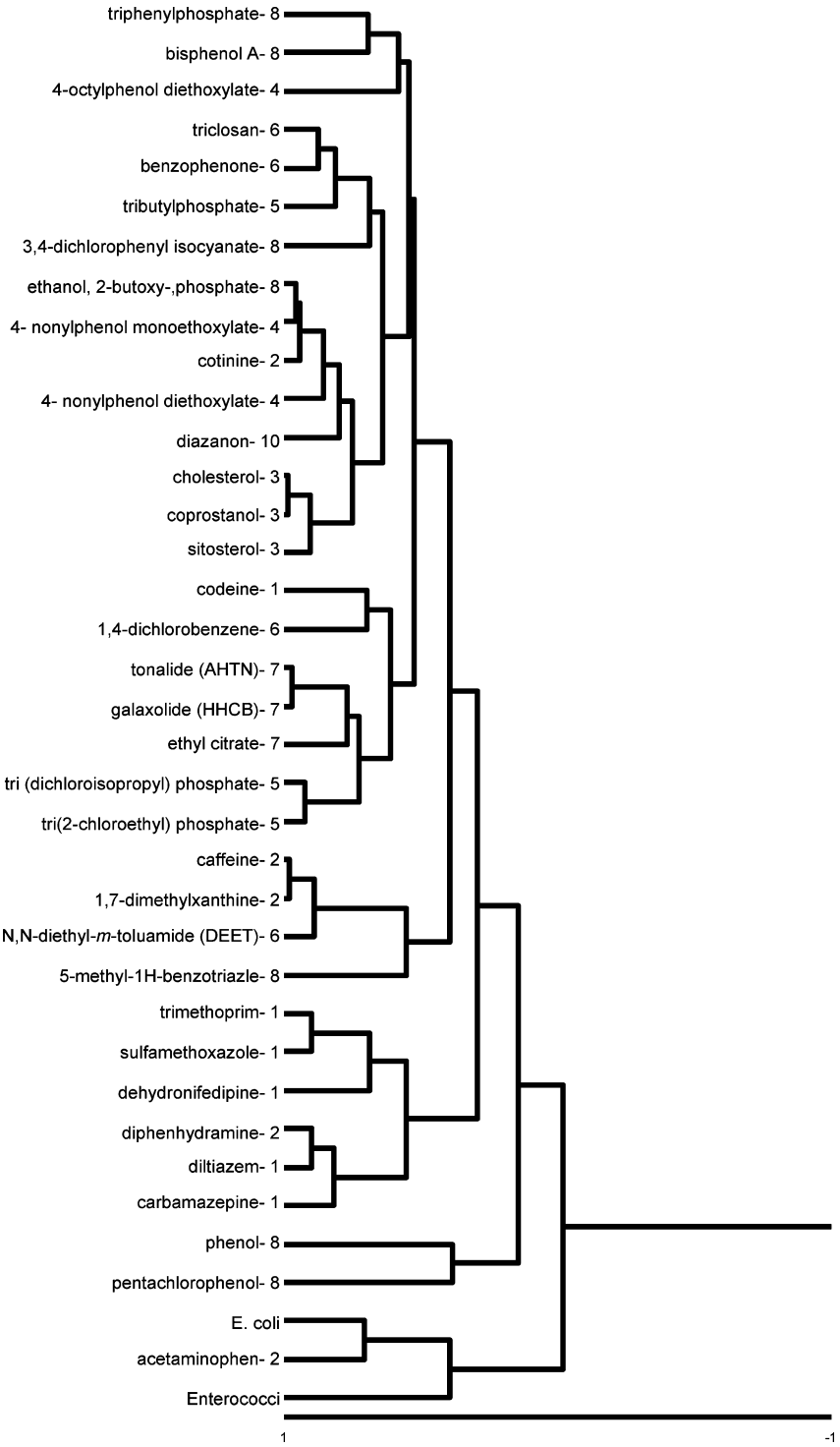

FIGURE 3. Dendrogram of indicator bacteria and 35 of the most commonly detected chemicals showing clustering relations. The number after the name indicates the use classification described in Table 2.

Acknowledgments

This work was assisted by U.S. Geological Survey field personnel who led local sample collection efforts: Gail Cordy, Arizona; Ken Covay, Nevada; Lori Sprague, Colorado; John Lambing, Montana; Steve Sando, South Dakota; Doug Schnoebelen, Iowa; Kathy Lee, Minnesota; Sheridan Haack, Michigan; David Mau, Kansas; Betsy Frick, Georgia; Pat Phillips, New York; and Paul Stackelberg, New Jersey. The U.S. Environmental Protection Agency, through its Office of Research and Development, supported and collaborated in the research described here under an Interagency Agreement (DW-14-93940201) with the U.S. Geological Survey. This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for publication. The use of trade, product, or firm names in this paper is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited

(1) Bonde, G. J. Bacteriological methods for estimation of water pollution. *Health Lab. Sci.* **1966**, *3*, 124−128.

(2) Elmund, G. K.; Allen, M. J.; Rice, E. W. Comparison of *Escherichia coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency. *Water Environ. Res.* **1999**, *71*, 332−339.
(3) Federal Register. National Primary Drinking Water Regulation: Total coliforms (including fecal coliforms and *E. coli*). *Fed. Regst.* **1989**, *54*, 27544−27568.
(4) USEPA. Bacterial Water Quality Standards for Recreational Waters (Freshwater and marine water). USEPA 2003, EPA-823-R-03-008.
(5) Cabelli, V. J. Health effects criteria for marine recreational waters. USEPA 1983, EPA-600/1-80-031.
(6) Dufour, A. P. Health effects criteria for fresh recreational waters. USEPA 1984, EPA-600/1-84-004.
(7) USEPA. Ambient water quality criteria for bacteria−1986. USEPA 1986, EPA-440/5-84-002.
(8) Federal Register. Guidelines establishing test procedures for the analysis of pollutants, analytical methods for biological pollutants in ambient water; final rule. *Fed. Regst*. **2003**, *68*, 43272−43283.
(9) USEPA. Draft implementation guidance for ambient water quality criteria for bacteria. USEPA 2002, EPA-823-B-02-003.
(10) Toze, S. PCR and the detection of microbial pathogens in water and wastewater. *Water Res.* **1999**, *33*, 3545−3556.
(11) Seidler, R. J.; Evans, T. M.; Kaufman, J. R.; Warvick, C. E.; LeCheveiler, M. W. Limitations of standard coliform enumeration techniques. *J.—Am. Water Works Assoc.* **1981**, *73*, 538−542.
(12) Oshiro, R.; Fujioka, R. Sand, soil and pigeon droppings: Sources of indicator bacteria in the waters of Hanauma Bay, Oahu, Hawaii. *Water Sci. Technol.* **1995**, *31*, 251−254.
(13) Pontius, F. W. Reconsidering the total colifrom rule. *J.—Am. Water Works Assoc.* **2000**, *92*, 14−26.
(14) Solo-Gabriele, H. M.; Wolfert, M. A.; Desmarais, T. R.; Palmer, C. J. Sources of *Escherichia coli* in a coastal subtropical environment. *Appl. Environ. Microbiol.* **2000**, *66*, 230−237.
(15) Ashton, D.; Hilton, M.; Thomas, K. V. Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. *Sci. Total Environ.* **2004**, *333*, 167−184.
(16) Atherholt, T.; Feerst, E.; Hovendon, B.; Kwak, J.; Rosen, J. D. Evaluation of indicators of fecal contamination in groundwater. *J.—Am. Water Works Assoc.* **2003**, *95*, 119−131.
(17) Buerge, I. J.; Buser, H. R.; Müller, M. D.; Poiger, T. Behavior of the polycyclic musks HHCB and AHTN in lakes, two potential anthropogenic markers for domestic wastewater in surface waters. *Environ. Sci. Technol.* **2003**, *37*, 5636−5644.
(18) Buerge, I. J.; Poiger, T.; Müller, M. D.; Buser, H.-D. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environ. Sci. Technol.* **2003**, *37*, 691−700.
(19) Buser, H. R.; Poiger, T.; Müller, M. D. Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and wastewater. *Environ. Sci. Technol.* **1999**, *33*, 2529−2535.
(20) Chaler, R.; Simoneit, B. R. T.; Grimalt, J. O. Bile acids and sterols in urban sewage treatment plants. *J. Chromatogr., A.* **2001**, *927*, 155−160.
(21) Chen, Z.; Pavelic, P.; Dillon, P.; Naidu, R. Determination of caffeine as a tracer of sewage effluent in natural waters by on-line solid-phase extraction and liquid chromatography with diode-array detection. *Water Res.* **2002**, *36*, 4830−4838.
(22) Clara, M.; Strenn, B.; Kreuzinger, N. Carbamazepine as a possible anthropogenic marker in the aquatic environment: Investigations on the behavior of Carbamazepine in wastewater treatment and during groundwater infiltration. *Water Res.* **2004**, *38*, 947−954.
(23) Close, M. E.; Hodgson, L. R.; Tod, G. Field evaluation of fluorescent whitening agents and sodium tripolyphosphate as indicators of septic tank contamination in domestic wells. *N. Z. J. Mar. Freshwater Res.* **1989**, *23*, 563−568.
(24) Gilpin, B. J.; Gregor, J. E.; Savill, M. G. Identification of the source of fecal pollution in contaminated rivers. *Water Sci. Technol.* **2002**, *46*, 9−15.
(25) Gilpin, B.; James, T.; Nourozi, F.; Saunders, D.; Scholes, P.; Savill, M. The use of chemical and molecular microbial indicators for fecal source identification. *Water Sci. Technol.* **2003**, *47*, 39−43.
(26) Gregor, J.; Garrett, N.; Gilpin, B.; Randall, C.; Saunders, D. Use of classification and regression tree (CART) analysis with chemical fecal indicators to determine sources of contamination. *N. Z. J. Mar. Freshwater Res.* **2002**, *36*, 387−398.
(27) Isobe, K. O.; Tarao, M.; Zakaria, M. P.; Chiem, N. H.; Minh, L. Y.; Takada, H. Quantitative application of fecal sterols using gas chromatography−mass spectrometry to investigate fecal pollution in tropical waters: Western Malaysia and Mekong Delta, Vietnam. *Environ. Sci. Technol.* **2002**, *36*, 4497−4507.
(28) Maldonado, C.; Dachs, J.; Bayona, J. M. Trialkylamines and coprostanol as tracers of urban pollution in waters from enclosed seas: The Mediterranean and Black Sea. *Environ. Sci. Technol.* **1999**, *33*, 3290−3296.
(29) McCarty, P. L.; Aieta, E. M. Chemical indicators and surrogate parameters in water treatment. *J.—Am. Water Works Assoc.* **1984**, *76*, 98−106.
(30) Metcalfe, C. D.; Miao, X.; Koenig, B. G.; Struger, J. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada. *Environ. Toxicol. Chem.* **2003**, *22*, 2881−2889.
(31) Nichols, P. D.; Leeming, R.; Rayner, M. S.; Latham, V. Use of capillary gas chromatography for measuring fecal-derived sterols: Application to stormwater, the sea-surface microlayer, beach greases, regional studies, and distinguishing algal blooms and human and nonhuman sources of sewage pollution. *J. Chromatogr., A.* **1996**, *733*, 497−509.
(32) Ottoson J.; Stenström, T. A. Fecal contamination of graywater and associated microbial risks. *Water Res.* **2003**, *37*, 645−655.
(33) Roser, D.; Ashbolt, N.; Storey, M.; Leeming, R.; Scott, P.; Bennetts, R.; Ryken-Rapp, D.; Toze, S. Application of physicochemical parameters and particle-bound biomarkers to indicate microbial contamination of aquifers. *Water Sci. Technol.: Water Supply* **2002**, *2*, 419−426.
(34) Sinton, L. W.; Finlay, R. K.; Hannah, D. J. Distinguishing human from animal fecal contamination in water: A review. *N. Z. J. Mar. Freshwater Res.* **1998**, *32*, 323−348.
(35) Standley, L. J.; Kaplan, L. A.; Smith, D. Molecular tracers of organic matter sources to surface water resources. *Environ. Sci. Technol.* **2000**, *34*, 3124−3130.
(36) Weigel, S.; Berger, U.; Jensen, E.; Kallenborn, R.; Thoresen, H.; Hühnerfuss, H. Determination of selected pharamceuticals and caffeine in seawage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites. *Chemosphere* **2004**, *56*, 583−592.
(37) Murtaugh, J. J.; Bunch, R. L. Sterols as a measure of fecal pollution. *J.—Water Pollut. Control Fed.* **1967**, *39*, 404−409.
(38) Leeming, R.; Nichols, P. D. Concentration of coprostanol that correspond to existing bacterial indicator guideline limits. *Water Res.* **1996**, *30*, 2997−3006.
(39) Veiga, P.; Juste, C.; Lepercq, P.; Saunier, K.; Béguet, F.; Gérard, P. Correlation between faecal microbial community structure and cholesterol-to-coprostanol conversion in the human gut. *FEMS Microbiol. Lett.* **2005**, *242*, 81−86.
(40) Leeming, R.; Ball, A.; Ashbolt, N.; Nichols, P. Using fecal sterols from humans and animals to distinguish fecal pollution in receiving waters. *Water Res.* **1996**, *30*, 2893−2900.
(41) Leeming, R.; Bate, N.; Hewlett, R.; Nichols, P. D. Distinguishing fecal pollution: A case study of stormwater entering Port Phillip Bay, Australia. *Water Sci. Technol.* **1998**, *38*, 15−22.
(42) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U. S. Streams, 1999−2000: A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.
(43) Elhmmali, M. M.; Roberts, D. J.; Evershed, R. P. Combined analysis of bile acids and sterols/stanols from riverine particulates to assess sewage discharges and other fecal sources. *Environ. Sci. Technol.* **2000**, *34*, 39−46.
(44) Writer, J. H.; Leenheer, J. A.; Barber, L. B.; Amy, G. L.; Chapra, S. C. Sewage contamination in the upper Mississippi River as measured by the fecal sterol, coprostanol. *Water Res.* **1995**, *29*, 1427−1436.
(45) Piocos, E. A.; de la Cruz, A. A. Solid-phase extraction and high performance liquid chromatography with photodiode array detection of chemical indicators of human fecal contamination in water. *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 1281−1291.
(46) Seiler, R. L.; Zaugg, S. D.; Thomas, J. M.; Harcroft, D. L. Caffeine and Pharmaceuticals as indicators of wastewaster contamination in wells. *Ground Water* **1999**, *37*, 405−410.
(47) Gardinali, P. R.; Zhao, X. Trace determination of caffeine in surface water samples by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometery. *Environ. Int.* **2002**, *28*, 521−528.
(48) Drewes, J. E.; Heberer, T.; Rauch, T.; Reddersen, K. Fate of pharmaceuticals during ground water recharge. *Ground Water Monit. Rem.* **2003**, *23*, 64−72.
(49) Stan, H. J.; Heberer, T. Pharmaceuticals in the aquatic environment. *Analusis Magn.* **1997**, *25*, M20-M23.

(50) Ternes, T. A. Occurrence of drugs in German sewage treatment plants and rivers. *Water Res.* **1998**, *32*, 3245−3260.
(51) Hirsch, R.; Ternes, T.; Haberer, K.; Kratz, K. Occurrence of antibiotics in the aquatic environment. *Sci. Total Environ.* **1999**, *225*, 109−118.
(52) Kolpin, D. W.; Skopec, M.; Meyer, M. T.; Furlong, E. T.; Zaugg, S. D. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. *Sci. Total Environ.* **2004**, *328*, 119−130.
(53) Snyder, S. A.; Westerhoff, P.; Yoon, Y.; Sedlak, D. L. Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry. *Environ. Eng. Sci.* **2003**, *20*, 449−469.
(54) Giger, W.; Alder, A. C.; Golet, E. M.; Kohler, H. E.; McArdell, C. S.; Molnar, E.; Siegrist, H.; Suter, M. J. Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. *Chimia* **2003**, *57*, 485−491.
(55) Ferrari, B.; Paxeus N.; Lo Giudice, R.; Pollio, A.; Garric, J. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: Study of carbamazepine, clofibric acid, and diclofenac. *Ecotoxicol. Environ. Saf.* **2003**, *55*, 359−370.
(56) Bruchet, A.; Prompsy, C.; Filippi, G.; Souali, A. A broad spectrum analytical scheme for the screening of endocrine disruptors (EDs), pharmaceuticals and personal care products in wastewaters and natural waters. *Water Sci. Technol.* **2002**, *46*, 97−104.
(57) Anderson, P. D.; D'Aco, V. J.; Shanahan, P.; Chapra. S. C.; Buzby., M. E.; Cunningham, V. L.; Duplessie, B. M.; Hayes, E. P.; Mastrocco, F. J.; Parke, N. J.; Rader, J. C.; Samuelian, J. H.; Schwab, B. W. Screening analysis of human pharmaceutical compounds in U.S. surface waters. *Environ. Sci. Technol.* **2004**, *38*, 838−849.
(58) Almeida, M. C.; Butler, D.; Friedler, E. At-source domestic wastewater quality. *Urban Water* **1999**, *1*, 49−55.
(59) Eganhouse, R. P.; Blumfield, D. L.; Kaplan, I. R. Long-chain alkylbenzenes as molecular tracers of domestic wastes in the marine environment. *Environ. Sci. Technol.* **1983**, *17*, 523−530.
(60) Krueger, C. J.; Radakovich, K. M.; Sawyer, T. E.; Barber, L. B.; Smith, R. L.; Field, J. A. Biodegradation of the surfactant linear alkylbenzenesulfonate in sewage-contaminated groundwater: A comparison of column experiments and field tracer tests. *Environ. Sci. Technol.* **1998**, *32*, 3954−3961.
(61) Poiger, T.; Field, J. A.; Field, T. M.; Giger, W. Occurrence of fluorescent whitening agents in sewage and river water determined by solid-phase extraction and high-performance liquid chromatography. *Environ. Sci. Technol.* **1996**, *30*, 2220−2226.
(62) Poiger, T.; Kari, F. G.; Giger, W. Fate of fluorescent whitening agents in the River Glatt. *Environ. Sci. Technol.* **1999**, *33*, 533−539.
(63) Eriksson, E.; Auffarth, K.; Eilersen, A.; Henze, M.; Ledin, A. Household chemicals and personal care products as sources for xenobiotic organic compounds in grey wastewater. *Water SA* **2003**, *29*, 135−146.
(64) Kallenborn, R.; Gatermann, R.; Rimkus, G. G. Synthetic musks in environmental samples: Indicator compounds with relevant properties for environmental monitoring. *J. Environ. Monit.* **1999**, *1*, 70N−74N.
(65) Rimkus, G. G. Polycyclic musk fragrances in the aquatic environment. *Toxicol. Lett.* **1999**, *111*, 37−56.
(66) Rimkus, G. G.; Gatermann, R.; Hühnerfuss, H. Musk xylene and musk ketone amino metabolites in the aquatic environment. *Toxicol. Lett.* **1999**, *111*, 5−15.
(67) Stackelberg, P. E.; Furlong, E. T.; Meyer, M. T.; Zaugg, S. D.; Henderson, A. K.; Reissman, D. B. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Sci. Total Environ.* **2004**, *329*, 99−113.
(68) Carballa, M.; Omil, F.; Lema, J. M.; Llompart, M.; García-Jares, C.; Rodríguez, I.; Gómez, M.; Ternes, T. Behavior of pharmaceuticals, cosmetics, and hormones in a sewage treatment plant. *Water Res.* **2004**, *38*, 2918−2926.
(69) Ternes, T. A.; Stüber, J.; Herrmann, N.; McDowell, D.; Reid, A.; Kampmann, M.; Teiser, B. Ozonation: A tool for removal of pharmaceuticals, contrast media, and musk fragrances from wastewater? *Water Res.* **2003**. *37*, 1976−1982.
(70) Zweiner, C.; Frimmel, F. H. Oxidative treatment of pharmaceuticals in water. *Water Res.* **2000**, *34*, 1881−1885.
(71) Glassmeyer, S. T.; Shoemaker, J. A. Effects of chlorination on the persistence of pharmaceuticals in the environment. *B. Environ. Contam. Tox.* **2005**, *74*, 24−31.
(72) Shelton, L. R. Field guide for collecting and processing stream-water samples for the National Water-Quality Assessment Program. U.S. Geol. Survey Open-File Report 1994, no. 94-455, p 42.
(73) Cahill, J. D.; Furlong, E. T.; Burkhardt, M. R.; Kolpin, D. W.; Anderson, L. G. Determination of pharmaceutical compounds in surface- and ground-water samples by solid-phase extraction and high-performance liquid chromatography-electrospray ionization mass spectrometry. *J. Chromatogr., A* **2004**, *1041*, 171−180.
(74) Zaugg, S. D.; Smith, S. G.; Schroeder, M. P.; Barber, L. B.; Burkhardt, M. R. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of wastewater compounds by polystyrene-divinylbenzene solid-phase extraction and capillary-column gas chromatography/mass spectrometry: U.S. Geological Survey Water-Resources Investigations Report, 2002, 01-4186, p 37.
(75) Stackelberg, P. E.; Furlong, E. T.; Meyer, M. T.; Zaugg, S. D.; Henderson, A. K.; Reissman, D. B. Response to comment on "Stackelberg, P. E.; Furlong, E. T.; Meyer, M. T.; Zaugg, S. D.; Henderson, A. K.; Reissman, D. B. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Sci. Total Environ.* **2004**, *329*, 99−113", *Sci. Total Environ.*, in press, 2005.
(76) USEPA. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. USEPA 2000, EPA-821-R-97-004.

Received for review November 29, 2004. Revised manuscript received April 19, 2005. Accepted May 3, 2005.

ES048120K