Environ. Sci. Technol. **2002**, *36,* 1202−1211

Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999−2000: A National Reconnaissance

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To provide the first nationwide reconnaissance of the occurrence of pharmaceuticals, hormones, and other organic wastewater contaminants (OWCs) in water resources, the U.S. Geological Survey used five newly developed analytical methods to measure concentrations of 95 OWCs in water samples from a network of 139 streams across 30 states during 1999 and 2000. The selection of sampling sites was biased toward streams susceptible to contamination (i.e. downstream of intense urbanization and livestock production). OWCs were prevalent during this study, being found in 80% of the streams sampled. The compounds detected represent a wide range of residential, industrial, and agricultural origins and uses with 82 of the 95 OWCs being found during this study. The most frequently detected compounds were coprostanol (fecal steroid), cholesterol (plant and animal steroid), *N,N*-diethyltoluamide (insect repellant), caffeine (stimulant), triclosan (antimicrobial disinfectant), tri(2-chloroethyl)phosphate (fire retardant), and 4-nonylphenol (nonionic detergent metabolite). Measured concentrations for this study were generally low and rarely exceeded drinking-water guidelines, drinking-water health advisories, or aquatic-life criteria. Many compounds, however, do not have such guidelines established. The detection of multiple OWCs was common for this study, with a median of seven and as many as 38 OWCs being found in a given water sample. Little is known about the potential interactive effects (such as synergistic or antagonistic toxicity) that may occur from complex mixtures of OWCs in the environment. In addition, results of this study demonstrate the importance of obtaining data on metabolites to fully understand not only the fate and transport of OWCs in the hydrologic system but also their ultimate overall effect on human health and the environment.

Introduction

The continued exponential growth in human population has created a corresponding increase in the demand for the Earth's limited supply of freshwater. Thus, protecting the integrity of our water resources is one of the most essential environmental issues of the 21st century. Recent decades have brought increasing concerns for potential adverse human and ecological health effects resulting from the production, use, and disposal of numerous chemicals that offer improvements in industry, agriculture, medical treatment, and even common household conveniences (*1*). Research has shown that many such compounds can enter the environment, disperse, and persist to a greater extent than first anticipated. Some compounds, such as pesticides, are intentionally released in measured applications. Others, such as industrial byproducts, are released through regulated and unregulated industrial discharges to water and air resources. Household chemicals, pharmaceuticals, and other consumables as well as biogenic hormones are released directly to the environment after passing through wastewater treatment processes (via wastewater treatment plants, or domestic septic systems), which often are not designed to remove them from the effluent (*2*). Veterinary pharmaceuticals used in animal feeding operations may be released to the environment with animal wastes through overflow or leakage from storage structures or land application (*3*). As a result, there are a wide variety of transport pathways for many different chemicals to enter and persist in environmental waters.

Surprisingly, little is known about the extent of environmental occurrence, transport, and ultimate fate of many synthetic organic chemicals after their intended use, particularly hormonally active chemicals (*4*), personal care products, and pharmaceuticals that are designed to stimulate a physiological response in humans, plants, and animals (*1, 5*). One reason for this general lack of data is that, until recently, there have been few analytical methods capable of detecting these compounds at low concentrations which might be expected in the environment (*6*). Potential concerns from the environmental presence of these compounds include abnormal physiological processes and reproductive impairment (*7*−*12*), increased incidences of cancer (*13*), the development of antibiotic-resistant bacteria (*14*−*17*), and the potential increased toxicity of chemical mixtures (*18*). For many substances, the potential effects on humans and aquatic ecosystems are not clearly understood (*1, 2, 19*).

The primary objective of this study is to provide the first nationwide reconnaissance of the occurrence of a broad suite of 95 organic wastewater contaminants (OWCs), including

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Published on Web 03/13/2002

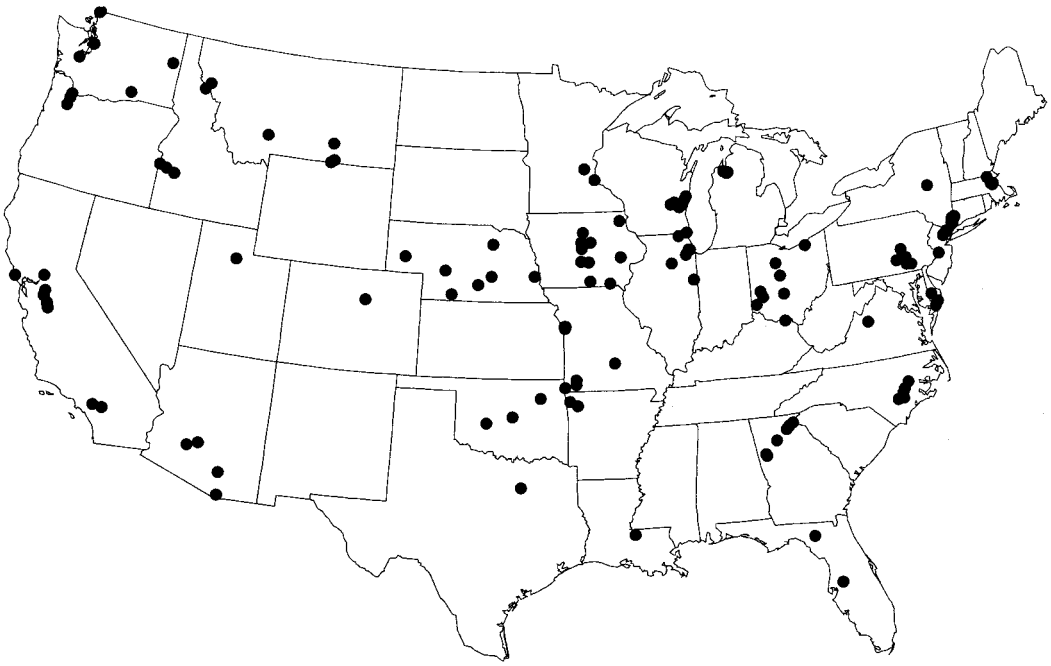

FIGURE 1. Location of 139 stream sampling sites.

many compounds of emerging environmental concern, in streams across the United States. These OWCs are potentially associated with human, industrial, and agricultural wastewaters and include antibiotics, other prescription drugs, nonprescription drugs, steroids, reproductive hormones, personal care products, products of oil use and combustion, and other extensively used chemicals. The target OWCs were selected because they are expected to enter the environment through common wastewater pathways, are used in significant quantities, may have human or environmental health implications, are representative or potential indicators of certain classes of compounds or sources, and/or can be accurately measured in environmental samples using available technologies. Although these 95 OWCs are just a small subset of compounds being used by society, they represent a starting point for this investigation examining the transport of OWCs to water resources of the United States.

This paper describes the analytical results available from 139 streams sampled during 1999−2000 (Figure 1). The results are intended to determine if OWCs are entering U.S. streams and to estimate the extent of their co-occurrence in susceptible waters. In addition, this study provides a focal point for the development and testing of new laboratory methods for measuring OWCs in environmental samples at trace levels, an interpretive context for future assessments of OWCs, and a means for establishing research priorities and future monitoring strategies. More complete interpretations, including an evaluation of the role of potential sources of contamination, will follow in subsequent papers.

Site Selection and Sampling

Little data were available on the occurrence of most of the targeted OWCs in U.S. streams at the onset of this investigation. Therefore, the selection of sampling sites primarily focused on areas considered susceptible to contamination from human, industrial, and agricultural wastewater. The 139 stream sites sampled during 1999−2000 (Figure 1) represent a wide range of geography, hydrogeology, land use, climate, and basin size. Specific information on the individual sampling sites is provided elsewhere (*20*).

All samples were collected by U.S. Geological Survey personnel using consistent protocols and procedures designed to obtain a sample representative of the streamwaters using standard depth and width integrating techniques (*21*). At each site, a composite water sample was collected from about 4−6 vertical profiles which was split into appropriate containers for shipment to the participating laboratories. For those bottles requiring filtration, water was passed through a 0.7 μm, baked, glass-fiber filter in the field where possible, or else filtration was conducted in the laboratory. Water samples for each chemical analysis were stored in precleaned-amber, glass bottles and collected in duplicate. The duplicate samples were used for backup purposes (in case of breakage of the primary sample) and for laboratory replicates. Following collection, samples were immediately chilled and sent to the laboratory. To minimize contamination of samples, use of personal care items (i.e. insect repellents, colognes, perfumes), caffeinated products, and tobacco were discouraged during sample collection and processing.

Each stream site was sampled once during the 1999−2000 study period. Samples collected in 1999 were analyzed for a subset of the OWCs based on the watershed land-use characteristics. Samples collected in 2000 were analyzed for the complete suite of OWCs. The analytical results for each stream sample are available elsewhere (*20*).

Analytical Methods

To determine the environmental extent of 95 OWCs (Table 1) in susceptible streams, five separate analytical methods were used. Each method was developed independently in different laboratories, with somewhat different data objectives, such as identifying hormones versus identifying antibiotics. As a result of these differing objectives, varying approaches were used in the development of the five analytical methods. For example, select methods (Methods 1−3 below) used filtered water for solid-phase extraction (SPE) with liquid chromatography/mass spectrometry positive-ion electrospray (LC/MS-ESI(+)) analysis, while others (Methods 4 and 5 below) used whole-water continuous liquid−liquid extraction (CLLE) with capillary gas chromatography/mass spectrometry (GC/MS) analysis.

All methods use selected ion monitoring (SIM) for improved sensitivity, thus, only the target compounds were reported with no attempt to report data for nontarget

TABLE 1. Summary of Analytical Results of Streams Sampled for 95 Organic Wastewater Contaminants[i]

chemical (method)	CASRN	*N*	RL (μg/L)	freq (%)	max (μg/L)	med (μg/L)	use	MCL or *HAL* (*23*) (μg/L)	lowest LC_{50} for the most sensitive indicator species (μg/L)/no. of aquatic studies identified (*24*)
Veterinary and Human Antibiotics									
carbodox (1)	6804-07-5	104	0.10	0	ND	ND	antibiotic	–	–/1
chlortetracycline (1)	57-62-5	115	0.05	0	ND	ND	antibiotic	–	88000[a]/3
chlortetracycline (2)	57-62-5	84	0.10	2.4	0.69	0.42	antibiotic	–	88000[a]/3
ciprofloxacin (1)	85721-33-1	115	0.02	2.6	0.03	0.02	antibiotic	–	–/0
doxycycline (1)	564-25-0	115	0.1	0	ND	ND	antibiotic	–	–/0
enrofloxacin (1)	93106-60-6	115	0.02	0	ND	ND	antibiotic	–	40[b]/29
erythromycin-H_2O (1)	114-07-8	104	0.05	21.5	1.7	0.1	erythromycin metabolite	–	665000[b]/35
lincomycin (1)	154-21-2	104	0.05	19.2	0.73	0.06	antibiotic	–	–/0
norfloxacin (1)	70458-96-7	115	0.02	0.9	0.12	0.12	antibiotic	–	–/6
oxytetracycline (1)	79-57-2	115	0.1	0	ND	ND	antibiotic	–	102000[a]/46
oxytetracycline (2)	79-57-2	84	0.10	1.2	0.34	0.34	antibiotic	–	102000[a]/46
roxithromycin (1)	80214-83-1	104	0.03	4.8	0.18	0.05	antibiotic	–	–/0
sarafloxacin (1)	98105-99-8	115	0.02	0	ND	ND	antibiotic	–	–/0
sulfachloropyridazine (2)	80-32-0	84	0.05	0	ND	ND	antibiotic	–	–/0
sulfadimethoxine (1)	122-11-2	104	0.05	0	ND	ND	antibiotic	–	–/5
sulfadimethoxine (2)	122-11-2	84	0.05	1.2	0.06	0.06	antibiotic	–	–/5
sulfamerazine (1)	127-79-7	104	0.05	0	ND	ND	antibiotic	–	100000[c]/17
sulfamerazine (2)	127-79-7	84	0.05	0	ND	ND	antibiotic	–	100000[c]/17
sulfamethazine (1)	57-68-1	104	0.05	4.8	0.12	0.02	antibiotic	–	100000[c] 17
sulfamethazine (2)	57-68-1	84	0.05	1.2	0.22	0.22	antibiotic	–	100000[c]/17
sulfamethizole (1)	144-82-1	104	0.05	1.0	0.13	0.13	antibiotic	–	–/0
sulfamethoxazole (1)	723-46-6	104	0.05	12.5	1.9	0.15	antibiotic	–	–/0
sulfamethoxazole (3)	723-46-6	84	0.023	19.0	0.52	0.066	antibiotic	–	–/0
sulfathiazole (1)	72-14-0	104	0.10	0	ND	ND	antibiotic	–	–/0
sulfathiazole (2)	72-14-0	84	0.05	0	ND	ND	antibiotic	–	–/0
tetracycline (1)	60-54-8	115	0.05	0	ND	ND	antibiotic	–	550000[b]/3
tetracycline (2)	60-54-8	84	0.10	1.2	0.11	0.11	antibiotic	–	550000[b]/3
trimethoprim (1)	738-70-5	104	0.03	12.5	0.71	0.15	antibiotic	–	3000[c]/4
trimethoprim (3)	738-70-5	84	0.014	27.4	0.30	0.013	antibiotic	–	3000[c]/4
tylosin (1)	1401-69-0	104	0.05	13.5	0.28	0.04	antibiotic	–	–/0
virginiamycin (1)	21411-53-0	104	0.10	0	ND	ND	antibiotic	–	–/0
Prescription Drugs									
albuterol (salbutamol) (3)	18559-94-9	84	0.029	0	ND	ND	antiasthmatic	–	–/0
cimetidine (3)	51481-61-9	84	0.007	9.5	0.58[d]	0.074[d]	antacid	–	–/0
codeine (3)	76-57-3	46	0.24	6.5	0.019	0.012	analgesic	–	–/0
codeine (4)	76-57-3	85	0.1	10.6	1.0[d]	0.2[d]	analgesic	–	–/0
dehydronifedipine (3)	67035-22-7	84	0.01	14.3	0.03	0.012	antianginal	–	–/0
digoxin (3)	20830-75-5	46	0.26	0	ND[d]	ND[d]	cardiac stimulant	–	10000000[a]/24
digoxigenin (3)	1672-46-4	84	0.008	0	ND	ND	digoxin metabolite	–	–/0
diltiazem (3)	42399-41-7	84	0.012	13.1	0.049	0.021	antihypertensive	–	–/0
enalaprilat (3)	76420-72-9	84	0.15	1.2	0.046[d]	0.046[d]	enalapril maleate (antihypertensive) metabolite	–	–/0
fluoxetine (3)	54910-89-3	84	0.018	1.2	0.012[d]	0.012[d]	antidepressant	–	–/0
gemfibrozil (3)	25812-30-0	84	0.015	3.6	0.79	0.048	antihyperlipidemic	–	–/0
metformin (3)	657-24-9	84	0.003	4.8	0.15[d]	0.11[d]	antidiabetic	–	–/0
paroxetine metabolite (3)	–	84	0.26	0	ND[d]	ND[d]	paroxetine (antidepressant) metabolite	–	–/0
ranitidine (3)	66357-35-5	84	0.01	1.2	0.01[d]	0.01[d]	antacid	–	–/0
warfarin (3)	81-81-2	84	0.001	0	ND	ND	anticoagulant	–	16000[c]/ 33
Nonprescription Drugs									
acetaminophen (3)	103-90-2	84	0.009	23.8	10	0.11	antipyretic	–	6000[a]/ 14
caffeine (3)	58-08-2	84	0.014	61.9	6.0	0.081	stimulant	–	40000[e]/ 77
caffeine (4)	58-08-2	85	0.08	70.6	5.7	0.1	stimulant	–	40000[e]/ 77
cotinine (3)	486-56-6	84	0.023	38.1	0.90	0.024	nicotine metabolite	–	–/0
cotinine (4)	486-56-6	54	0.04	31.5	0.57	0.05	nicotine metabolite	–	–/0
1,7-dimethylxanthine (3)	611-59-6	84	0.018	28.6	3.1[d]	0.11[d]	caffeine metabolite	–	–/0
ibuprofen (3)	15687-27-1	84	0.018	9.5	1.0	0.20	antiinflammatory	–	–/0
Other Wastewater-Related Compounds									
1,4-dichlorobenzene (4)	106-46-7	85	0.03	25.9	4.3	0.09	deodorizer	*75*	1100[c]/190
2,6-di-*tert*-butylphenol (4)	128-39-2	85	0.08	3.5	0.11[d]	0.06[d]	antioxidant	–	–/2
2,6-di-*tert*-butyl-1,4-benzoquinone (4)	719-22-2	85	0.10	9.4	0.46	0.13	antioxidant	–	–/0
5-methyl-1H-benzotriazole (4)	136-85-6	54	0.10	31.5	2.4	0.39	antiocorrosive	–	–/0
acetophenone (4)	98-86-2	85	0.15	9.4	0.41	0.15	fragrance	–	155000[e]/21
anthracene (4)	120-12-7	85	0.05	4.7	0.11	0.07	PAH	–	5.4[e]/188
benzo[*a*]pyrene (4)	50-32-8	85	0.05	9.4	0.24	0.04	PAH	0.2	1.5[a]/428
3-*tert*-butyl-4-hydroxy anisole (4)	25013-16-5	85	0.12	2.4	0.2[d]	0.1[d]	antioxidant	–	870[c]/14
butylated hydroxy toluene (4)	128-37-0	85	0.08	2.4	0.1[d]	0.1[d]	antioxidant	–	1440[a]/15
bis(2-ethylhexyl) adipate (4)	103-23-1	85	2.0	3.5	10[f]	3[f]	plasticizer	400	480[a]/9
bis(2-ethylhexyl) phthalate (4)	117-81-7	85	2.5	10.6	20[f]	7[f]	plasticizer	6	7500[a]/309

TABLE 1. (Continued)

chemical (method)	CASRN	*N*	RL (μg/L)	freq (%)	max (μg/L)	med (μg/L)	use	MCL or *HAL* (*23*) (μg/L)	lowest LC_{50} for the most sensitive indicator species (μg/L)/no. of aquatic studies identified (*24*)
Other Wastewater-Related Compounds									
bisphenol A (4)	80-05-7	85	0.09	41.2	12	0.14	plasticizer	–	3600[e]/26
carbaryl (4)	63-25-2	85	0.06	16.5	0.1[d]	0.04[d]	insecticide	*700*	0.4[a]/1541
***cis*-chlordane** (4)	5103-71-9	85	0.04	4.7	0.1	0.02	insecticide	2	7.4[b]/28
chlorpyrifos (4)	2921-88-2	85	0.02	15.3	0.31	0.06	insecticide	*20*	0.1[a]/1794
diazinon (4)	333-41-5	85	0.03	25.9	0.35	0.07	insecticide	*0.6*	0.56[a]/1040
dieldrin (4)	60-57-1	85	0.08	4.7	0.21	0.18	insecticide	0.2	2.6[c]/1540
diethylphthalate (4)	84-66-2	54	0.25	11.1	0.42	0.2	plasticizer	–	12000[c]/129
ethanol,2-butoxy-phosphate (4)	78-51-3	85	0.2	45.9	6.7	0.51	plasticizer	–	10400[e]/7
fluoranthene (4)	206-44-0	85	0.03	29.4	1.2	0.04	PAH	–	74[e]/216
lindane (4)	58-89-9	85	0.05	5.9	0.11	0.02	insecticide	0.2	30[c]/1979
methyl parathion (4)	298-00-0	85	0.06	1.2	0.01	0.01	insecticide	*2*	12[a]/888
4-methyl phenol (4)	106-44-5	85	0.04	24.7	0.54	0.05	disinfectant	–	1400[a]/74
naphthalene (4)	91-20-3	85	0.02	16.5	0.08	0.02	PAH	*20*	910[c]/519
N,*N*-diethyltoluamide (4)	134-62-3	54	0.04	74.1	1.1	0.06	insect repellant	–	71250[c]/9
4-nonylphenol (4)	251-545-23	85	0.50	50.6	40[g]	0.8[g]	nonionic detergent metabolite	–	130[e]/135
4-nonylphenol monoethoxylate (4)	–	85	1.0	45.9	20[g]	1[g]	nonionic detergent metabolite		14450[a]/4
4-nonylphenol diethoxylate (4)	–	85	1.1	36.5	9[g]	1[g]	nonionic detergent metabolite	–	5500[a]/6
4-octylphenol monoethoxylate (4)	–	85	0.1	43.5	2[g]	0.2[g]	nonionic detergent metabolite	–	–/0
4-octylphenol diethoxylate (4)	–	85	0.2	23.5	1[g]	0.1[g]	nonionic detergent metabolite	–	–/0
phenanthrene (4)	85-01-8	85	0.06	11.8	0.53	0.04	PAH	–	590[a]/192
phenol (4)	108-95-2	85	0.25	8.2	1.3[f]	0.7[f]	disinfectant	*400*	4000[c]/2085
phthalic anhydride (4)	85-44-9	85	0.25	17.6	1[f]	0.7[f]	plastic manufacturing	–	40400[c]/5
pyrene (4)	129-00-0	85	0.03	28.2	0.84	0.05	PAH	–	90.9[a]/112
tetrachloroethylene (4)	127-18-4	85	0.03	23.5	0.70[d]	0.07[d]	solvent, degreaser	5	4680[c]/147
triclosan (4)	3380-34-5	85	0.05	57.6	2.3	0.14	antimicrobial disinfectant	–	180[e]/3
tri(2-chloroethyl) phosphate (4)	115-96-8	85	0.04	57.6	0.54	0.1	fire retardant	–	66000[b]/8
tri(dichlorisopropyl) phosphate (4)	13674-87-8	85	0.1	12.9	0.16	0.1	fire retardant	–	3600[b]/9
triphenyl phosphate (4)	115-86-6	85	0.1	14.1	0.22	0.04	plasticizer	–	280[c]/66
Steroids and Hormones									
***cis*-androsterone** (5)	53-41-8	70	0.005	14.3	0.214	0.017	urinary steroid	–	–/0
cholesterol (4)	57-88-5	85	1.5	55.3	10[d]	1[d]	plant/animal steroid	–	–/0
cholesterol (5)	57-88-5	70	0.005	84.3	60[h]	0.83	plant/animal steroid	–	–/0
coprostanol (4)	360-68-9	85	0.6	35.3	9.8[d]	0.70[d]	fecal steroid	–	–/0
coprostanol (5)	360-68-9	70	0.005	85.7	150[h]	0.088	fecal steroid	–	–/0
equilenin (5)	517-09-9	70	0.005	2.8	0.278	0.14	estrogen replacement	–	–/0
equilin (5)	474-86-2	70	0.005	1.4	0.147	0.147	estrogen replacement	–	–/0
17α-ethynyl estradiol (5)	57-63-6	70	0.005	15.7	0.831	0.073	ovulation inhibitor	–	–/22
17α-estradiol (5)	57-91-0	70	0.005	5.7	0.074	0.03	reproductive hormone	–	–/0
17β-estradiol (4)	50-28-2	85	0.5	10.6	0.2[d]	0.16[d]	reproductive hormone	–	–/0
17β-estradiol (5)	50-28-2	70	0.005	10.0	0.093	0.009	reproductive hormone	–	–/0
estriol (5)	50-27-1	70	0.005	21.4	0.051	0.019	reproductive hormone	–	–/0
estrone (5)	53-16-7	70	0.005	7.1	0.112	0.027	reproductive hormone	–	–/11
mestranol (5)	72-33-3	70	0.005	10.0	0.407	0.074	ovulation inhibitor	–	–/0
19-norethisterone (5)	68-22-4	70	0.005	12.8	0.872	0.048	ovulation inhibitor	–	–/0
progesterone (5)	57-83-0	70	0.005	4.3	0.199	0.11	reproductive hormone	–	–/0
stigmastanol (4)	19466-47-8	54	2.0	5.6	4[d]	2[d]	plant steroid	–	–/0
testosterone (5)	58-22-0	70	0.005	2.8	0.214	0.116	reproductive hormone	–	–/4

[a] *Daphnia magna* (water flea) – 48 h exposure LC_{50}. [b] Other species and variable conditions. [c] *Oncorhynchus mykiss* (rainbow trout) – 96 h exposure LC_{50}. [d] Concentration estimated – average recovery <60%. [e] *Pimephales promelas* (fathead minnow) – 96 h exposure LC_{50}. [f] Concentration estimated – compound routinely detected in laboratory blanks. [g] Concentration estimated – reference standard prepared from a technical mixture. [h] Concentration estimated – value greater than highest point on calibration curve. [i] Compounds suspected of being hormonally active are in bold (*4, 22*). CASRN, Chemical Abstracts Service Registry Number; *N*, number of samples; RL, reporting level; freq, frequency of detection; max, maximum concentration; med, median detectable concentration; MCL, maximum contaminant level; HAL, health advisory level; LC_{50}, lethal concentration with 50% mortality; ND, not detected; –, not available; PAH, polycyclic aromatic hydrocarbon.

compounds. Target compounds within each method were selected from the large number of chemical possibilities based upon usage, toxicity, potential hormonal activity, and persistence in the environment. Some compounds that fit the above criteria, however, could not be included (such as amoxicillin, roxarsone, polybrominated diphenyl ethers) because they were either incompatible with the corresponding method or reference standards were not available. Positive identification of a compound required elution within the expected retention time window. In addition, the sample spectra and ion abundance ratios were required to match that of the reference standard compounds. The base-peak ion was used for quantitation, and, if possible, two qualifier ions were used for confirmation. After qualitative criteria were met, compound concentrations were calculated from 5 to 8 point calibration curves (generally from 0.01 to 10.0 μg/L) using internal standard quantitation. Methods 1 and 2 process calibration standards through the extraction procedure, which generally corrects concentrations for method losses but not matrix effects. Methods 3–5 do not

extract calibration standards, thus the reported concentrations are not corrected for method losses. Reporting levels (RLs) were determined for each method by either an evaluation of instrument response, calculation of limit of detection, or from a previously published procedure (*25*). RLs were adjusted based on experience with the compounds in each method, known interferences, or known recovery problems.

The following descriptions are intended to provide a brief overview of the five analytical methods used for this study. More comprehensive method descriptions are provided elsewhere (*26*−*28*) or will be available in subsequent publications.

Method 1. This method targets 21 antibiotic compounds (Table 1) in 500-mL filtered water samples using modifications from previously described methods (*26, 29*). The antibiotics were extracted and analyzed by tandem SPE and single quadrapole, LC/MS-ESI(+) using SIM. To prevent the tetracycline antibiotics from complexing with Ca^{2+} and Mg^{2+} ions and residual metals on the SPE cartridges, 0.5 mg of disodium ethylenediaminetetraacetate (Na_2EDTA; $C_{10}H_{14}O_8$-Na_2N_2−H_2O) was added to each water sample. Sample pH was adjusted to 3 using concentrated H_2SO_4. The tandem SPE included an Oasis Hydrophilic−Lipophilic-Balance (HLB) cartridge (60 mg) followed by a mixed mode, HLB-cation exchange (MCX) cartridge (60 mg) (Waters Inc., Milford, MA). The HLB and MCX cartridges were conditioned with ultrapure H_2O, CH_3OH, and CH_3OH with 5% NH_4OH. The HLB cartridge was attached to the top of the MCX cartridge, and the sample was passed through the SPE cartridges using a vacuum extraction manifold. The cartridges were eluted with CH_3OH, and the MCX cartridge was eluted separately using CH_3OH with 5% NH_4OH. The eluate was spiked with 500 ng of $^{13}C_6$-sulfamethazine (internal standard), vortexed, and evaporated to 20 μL using N_2 and a water bath of 55° C. Three hundred μL of 20 mM of $NH_4C_2H_3OO$ (pH 5.7) was added to sample eluate, vortexed, transferred to a glass chromatography vial, and frozen until analysis. Samples were extracted as a set of 11 environmental samples, one duplicate sample, two fortified ultrapure water spikes (check standards), and two ultrapure water blanks.

Method 2. This method targets eight antibiotic compounds (Table 1) in filtered water samples. Complete details of this method have been described previously (*26*). The antibiotics were extracted and analyzed using SPE and SIM LC/MS-ESI(+). Samples were prepared for extraction by adding $^{13}C_6$-sulfamethazine and meclocycline as surrogate standards, Na_2EDTA, and H_2SO_4. Target compounds were extracted using 60-mg HLB cartridges preconditioned with CH_3OH, NHCl, and distilled H_2O. Target compounds were eluted with CH_3OH into a test tube containing the internal standard, simatone. The extracts were then concentrated under N_2 to approximately 50 μL, and mobile phase A (10 mM $NH_4H_2O_2$ in 90/10 water/CH_3OH with 0.3% CH_2O_2) was added. The resulting solutions were transferred to amber autosampler vials to prevent photodegradation of tetracyclines (*30*). Mobile phase conditions are described in detail elsewhere (*26*).

For each compound, the proton adduct of the molecular ion (M + H)+ and at least one confirming ion were acquired using LC/MS-ESI(+). All mass spectral conditions are described in detail elsewhere (*26*). Quantitation was based on the ratio of the base peak ion (M + H)+ of the analyte to the base peak of the internal standard. Standard addition was used for quantitation where each sample was analyzed with and without the addition of a 0.5 μg/L spike to correct for suppression of the electrospray signal.

Method 3. This method targets 21 human prescription and nonprescription drugs and their select metabolites (Table 1) in filtered water samples. Compounds were extracted from 1 L water samples using SPE cartridges that contain 0.5 g of HLB (flow rate of 15 mL/min). After extraction, the adsorbed compounds were eluted with CH_3OH followed by CH_3OH acidified with $C_2HCl_3O_2$. The two fractions were reduced under N_2 to near dryness and then combined and brought to a final volume of 1 mL in 10% C_2H_3N:90% H_2O buffered with $NH_4H_2O_2/CH_2O_2$.

Compounds were separated and measured by high-performance liquid chromatography (HPLC) using a polar (neutral silanol) reverse-phase octylsilane (C8) HPLC column (Metasil Basic 3 μm, 150 × 2.0 mm; Metachem Technologies). The compounds were eluted with a binary gradient of mobile phase A (aqueous $NH_4H_2O_2/CH_2O_2$ buffer; 10 mM, pH 3.7) and mobile phase B (100% C_2H_3N).

Method 4. This method (*27, 28*) targets 46 OWCs (Table 1) in unfiltered water. One-liter whole-water samples were extracted using CLLE with CH_2Cl_2. Distilled solvent was recycled through a microdroplet dispersing frit to improve extraction efficiency. Samples were extracted for 3 h at ambient pH and for an additional 3 h at pH 2. The extract was concentrated under N_2 to 1 mL and analyzed by capillary-column GC/MS. Available standards for the 4-nonylphenol compounds were composed of multiple isomers, and thus, laboratory standards for these compounds as well as octylphenol ethoxylates were prepared from technical mixtures.

Method 5. This method (*28*) targets 14 steroid compounds including several biogenic and synthetic reproductive hormones (Table 1). The CLLE extracts from the previously analyzed samples of Method 4 were derivatized and reanalyzed. Analysis of steroid and hormone compounds by GC/MS is enhanced by derivatization to deactivate the hydroxyl and keto functional groups. The technique used in this study is the formation of trimethylsilyl (TMS) ethers of the hydroxyl groups and oximes of the keto groups. Samples were stored in a silanizing reagent to prevent hydrolysis of the derivatives back to the free compound. Surrogate standards ($d_4$estradiol and $d_7$cholesterol) were added to the samples prior to derivatization to evaluate method performance. After derivatization, the samples were analyzed by GC/MS.

Quality Assurance Protocol. At least one fortified laboratory spike and one laboratory blank was analyzed with each set of 10−16 environmental samples. Most methods had surrogate compounds added to samples prior to extraction to monitor method performance. A summary of recoveries for target compounds and surrogate compounds in environmental samples (Table 2) indicates the general proficiency of the methods. The RL (Table 1) is equivalent to the lowest concentration standard that could be reliably quantitated. The compound concentrations reported below the RL or the lowest calibration standard were estimated as indicated in Figure 2. The concentration of compounds with <60% recovery, routinely detected in laboratory blanks, or prepared with technical grade mixtures, was also considered estimated (Table 1).

The laboratory blanks were used to assess potential sample contamination. Blank contamination was not subtracted from environmental results. However, environmental concentrations within twice the values observed in the set blank were reported as less than the RL.

A field quality assurance protocol was used to determine the effect, if any, of field equipment and procedures on the concentrations of OWCs in water samples. Field blanks, made from laboratory-grade organic free water, were submitted for about 5% of the sites and analyzed for all of the 95 OWCs. Field blanks were subject to the same sample processing, handling, and equipment as the stream samples. To date, one field blank had a detection of coprostanol and testosterone, one field blank had a detection of naphthalene and tri(dichlorisopropyl)phosphate, and one field blank had

TABLE 2. Summary of Quality Assurance/Quality Control Results for Target and Surrogate Compounds[b]

compound	spike concn (μg/L)	mean % recovery	% RSD
	Method 1		
target compounds	1.0	99.0	12.1
	Method 2		
target compounds	1.0	97.5	12.2
$^{13}C_6$-sulfamethazine	1.0	80.0	20.0
meclocycline	1.0	80.0	20.0
	Method 3		
target compounds	0.5	85.1	11.6
C_{13}-phenacetin	1.0	96.8	14.0
	Method 4		
target compounds	1.0	81.0	11.0
d_{21}-BHT	2.0	63.0	25.0
n-nonylphenol	2.0	83.0	20.0
	Method 5		
target compounds	NA	NA	NA
d_4-estradiol[a]	0.047	128.8	42.0
d_3-testosterone[a]	0.051	148.5	47.3
d_7-cholesterol[a]	0.053	116.9	55.9

[a] Surrogate standard added after CCLE extraction but prior to derivitization. [b] RSD, relative standard deviation; NA, not currently available.

a detection of naphthalene, 4-nonylphenol, phenol, 4-*tert*-octylphenol monoethoxylate, and ethanol,2-butoxy-phosphate. Most of these detections were near their respective RLs verifying the general effectiveness of the sampling protocols used for this study. In addition all field blanks had low level concentrations of cholesterol being measured using Method 5 (median concentration = 0.09 μg/L) documenting its ubiquitous nature in the environment. Cholesterol concentrations from 0.005 to 0.18 μg/L obtained through Method 5 were set to less than the RL.

Compounds that were measured by more than one analytical method (Table 1; Figure 3) also were used to evaluate the results for this study. The presence or absence of these compounds were confirmed in 100% of the determinations for sulfamerazine, and sulfathiazole; 98.8% for oxytetracycline, sulfadimethoxine, sulfamethazine, and tetracycline; 98.6% for cholesterol and coprostanol; 97.6% for chlortetracyline; 95.7% for 17β-estradiol; 94.4% for cotinine; 94.0% for trimethoprim; 89.1% for sulfamethoxazole; 86.4% for codeine; and 83.3% for caffeine. The comparisons for codeine, caffeine, and cotinine may have been affected by the differing extractions (SPE versus CLLE) as well as differing types of sample (filtered versus whole water).

An interlaboratory comparison of Methods 1 and 3 was conducted using two reagent water blanks and 24 reagent water spikes prepared at concentrations ranging from 0.5 to 1.1 μg/L for two frequently detected antibiotics (sulfamethoxazole and trimethoprim). The results demonstrated that both methods are accurately confirming the presence of sulfamethoxazole and trimethoprim in water, with the measured concentrations being within a factor of 3 or better of the actual concentrations for these compounds. No false positives or false negatives occurred for this experiment.

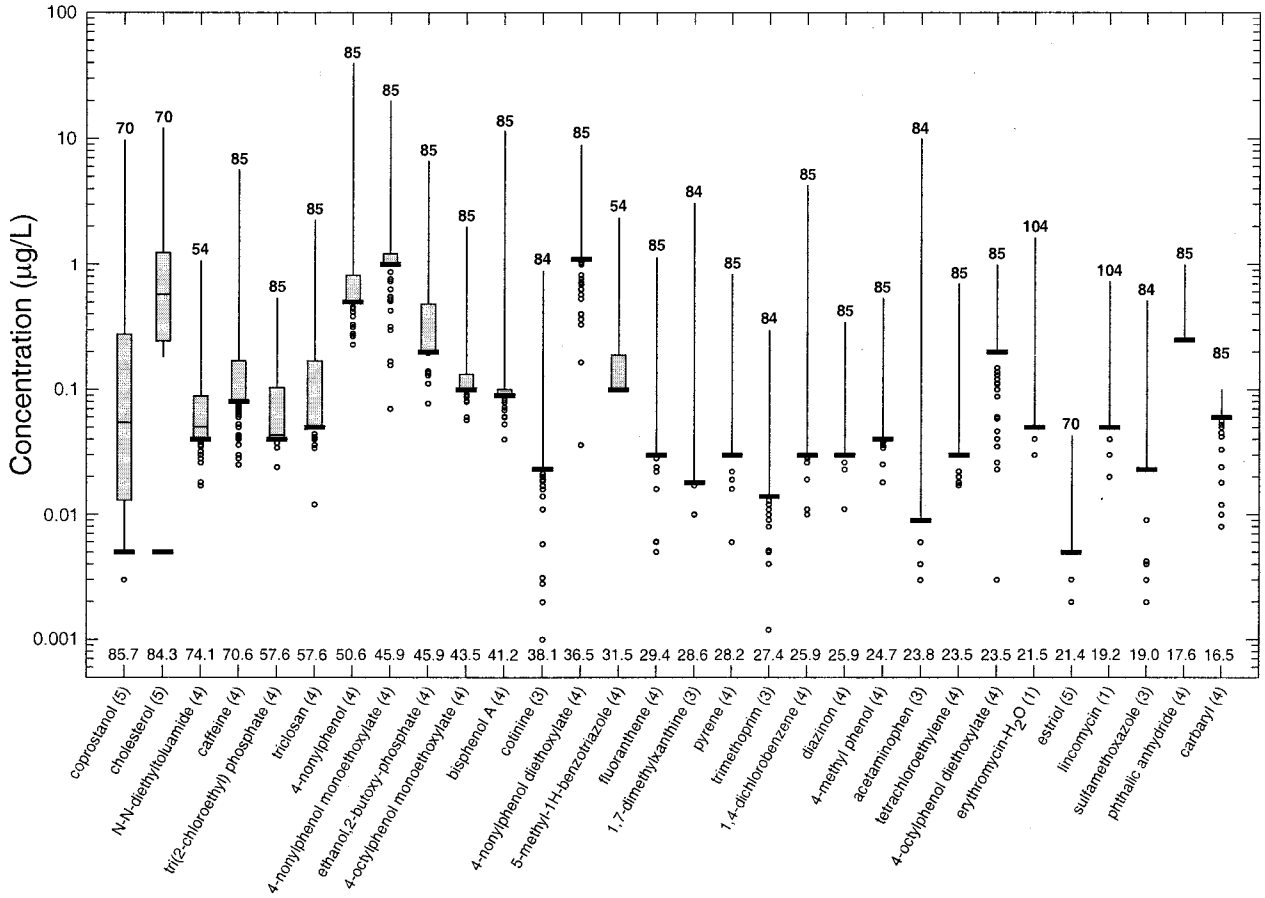

FIGURE 2. Measured concentrations for the 30 most frequently detected organic wastewater contaminants. Boxplots show concentration distribution truncated at the reporting level. Estimated values below the reporting level are shown. Estimated maximum values for coprostanol and cholesterol obtained from Method 5 (Table 1) are not shown. The analytical method number is provided (in parentheses) at the end of each compound name. An explanation of a boxplot is provided in Figure 3.

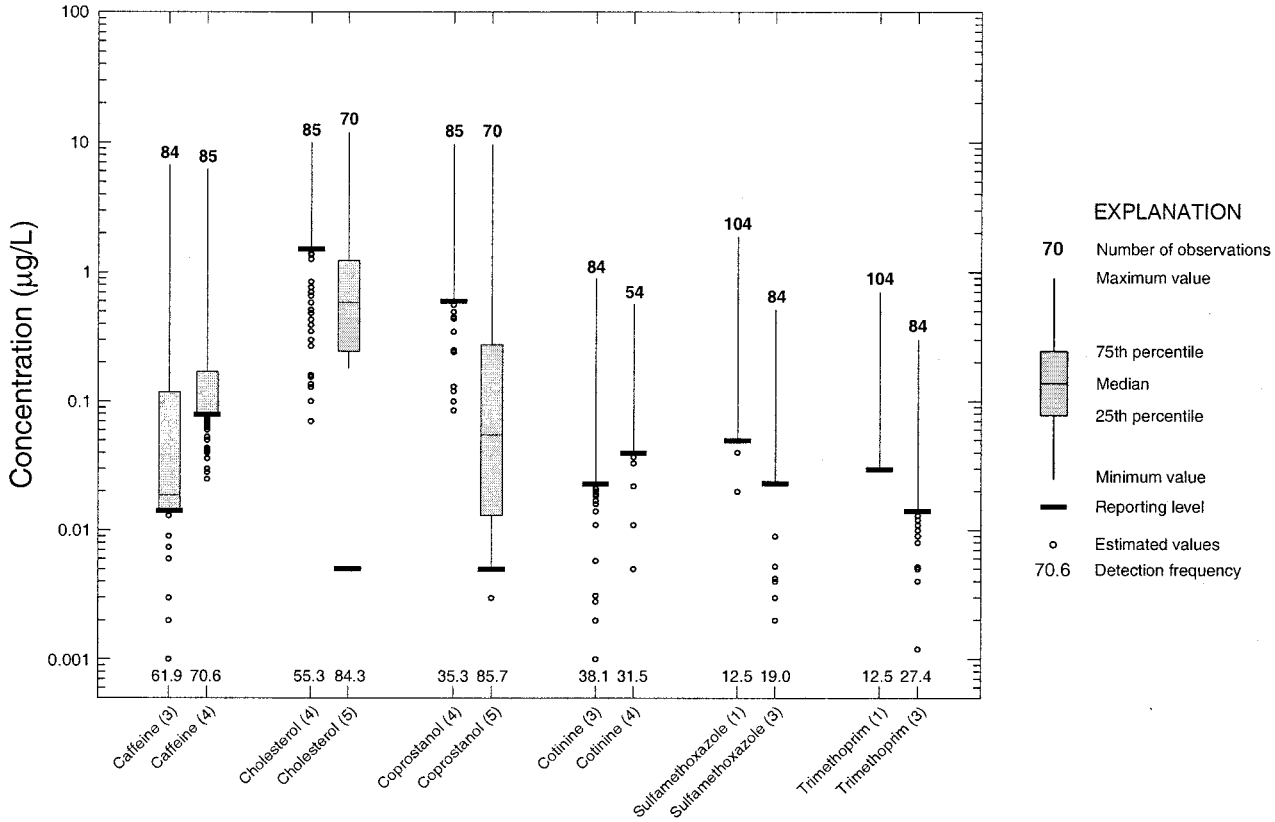

FIGURE 3. Comparison of concentrations of select compounds that were measured using two different methods with significantly different reporting levels. Boxplots show concentration distribution truncated at the reporting level. Estimated values below the reporting level are shown. Estimated maximum values for chloesterol and coprostanol obtained from Method 5 (Table 1) are not shown. The analytical method number is provided (in parentheses) at the end of each compound name.

Results and Discussion

One or more OWCs were found in 80% of the 139 streams sampled for this study. The high overall frequency of detection for the OWCs is likely influenced by the design of this study, which placed a focus on stream sites that were generally considered susceptible to contamination (i.e. downstream of intense urbanization and livestock production). In addition, select OWCs (such as cholesterol) can also be derived from nonanthropogenic sources. Furthermore, some of the OWCs were selected because previous research (*28*) identified them as prevalent in the environment. Thus, the results of this study should not be considered representative of all streams in the United States. A previous investigation of streams downstream of German municipal sewage treatment plants also found a high occurrence of OWCs (*31*).

A large number of OWCs (82 out of 95) were detected at least once during this study (Table 1). Only eight antibiotics and five other prescription drugs were not detected in the samples analyzed (Table 1). Measured concentrations were generally low (median detectable concentrations generally $<1\ \mu$g/L, Table 1), with few compounds exceeding drinking-water guidelines, health advisories, or aquatic-life criteria (Table 1). The concentration of benzo[*a*]pyrene exceeded its maximum contaminant level (MCL) of 0.2 μg/L at one site and bis(2-ethylhexyl)phthalate concentrations exceeded its MCL of 6.0 μg/L at five sites. In addition, aquatic-life criteria were exceeded for chlorpyrifos (Table 1) at a single site. However, many of the 95 OWCs do not have such guidelines or criteria determined (Table 1). In fact, much is yet to be known about the potential toxicological effects of many of the OWCs under investigation (*1*). For many OWCs, acute effects to aquatic biota appear limited because of the low concentrations generally occurring in the environment (*24, 32−34*). More subtle, chronic effects from low-level environmental exposure to select OWCs appear to be of much greater concern (*1*). Such chronic effects have been documented in the literature (*34−38*). In addition, because antibiotics are specifically designed to reduce bacterial populations in animals, even low-level concentrations in the environment could increase the rate at which pathogenic bacteria develop resistance to these compounds (*15−17, 39*).

The 30 most frequently detected compounds represent a wide variety of uses and origins including residential, industrial, and agricultural sources (Figure 2, Table 1). Only about 5% of the concentrations for these compounds exceeded 1 μg/L. Over 60% of these higher concentrations were derived from cholesterol and three detergent metabolites (4-nonyphenol, 4-nonylphenol monoethoxylate, and 4-nonylphenol diethoxylate). The frequent detection of cotinine, 1,7-dimethylxanthine, erythromycin-H_2O, and other OWC metabolites demonstrate the importance of obtaining data on degradates to fully understand the fate and transport of OWCs in the hydrologic system. In addition, their presence suggests that to accurately determine the overall effect on human and environmental health (such as pathogen resistance and genotoxicity) from OWCs, their degradates should also be considered. The presence of the parent compound and/or their select metabolites in water resources has previously been documented for OWCs (*40, 41*) as well as other classes of chemicals such as pesticides (*42, 43*).

Many of the most frequently detected compounds (Figure 2) were measured in unfiltered samples using Method 4. Thus, their frequencies of detection may be somewhat higher because concentrations being measured include both the dissolved and particulate phases, whereas concentrations measured by Methods 1−3 include just the dissolved phase. For example, about 90% of the coprostanol discharged from

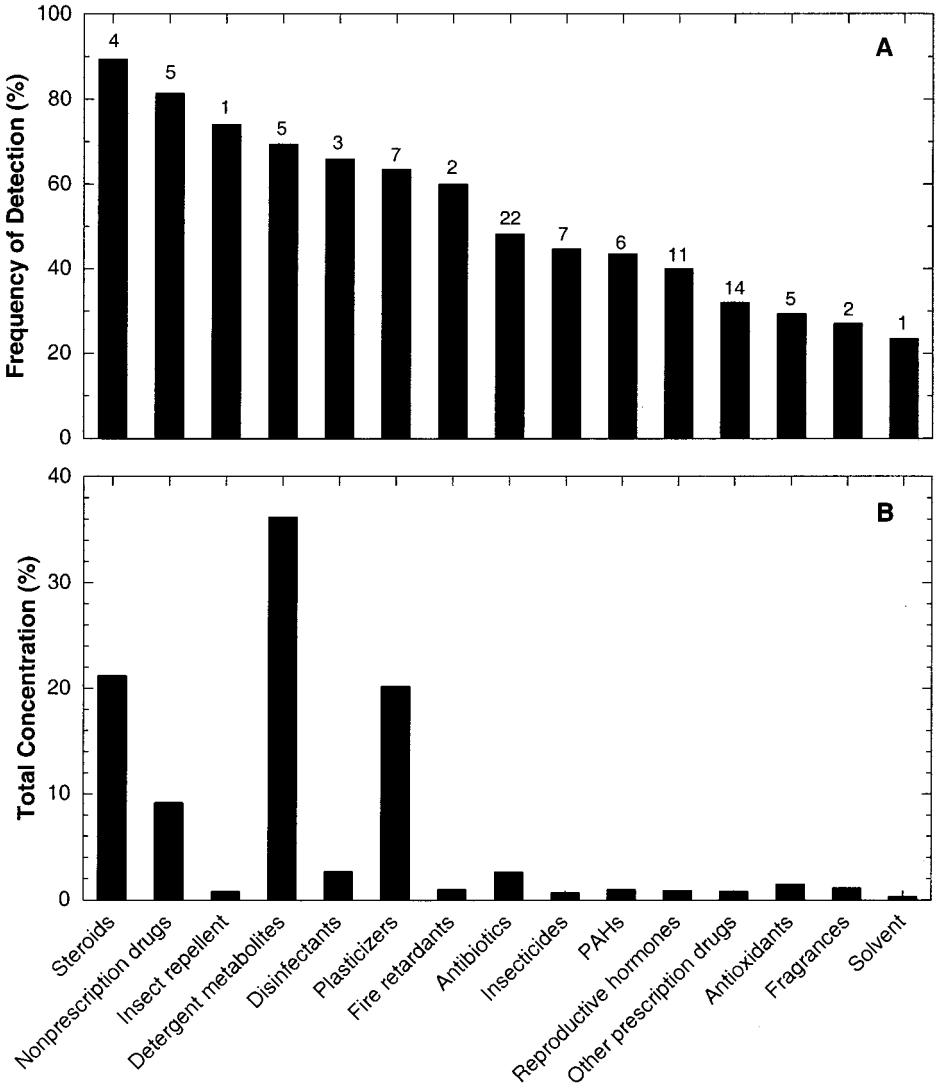

FIGURE 4. Frequency of detection of organic wastewater contaminants by general use category (4A), and percent of total measured concentration of organic wastewater contaminants by general use category (4B). Number of compounds in each category shown above bar.

sewage effluents has been shown to be associated with particulate matter (*44*). Thus, the concentration and frequency of detection for select compounds would likely have been reduced if sample filtration had taken place.

Variations in RL also influence the frequency of OWC detection (Figure 2). For example, the detection of 4-nonylphenol would likely have been much greater if an order of magnitude lower RL (similar to other OWCs) could have been achieved. The effect of RL on frequencies of detection is more clearly demonstrated by comparison of concentrations of select compounds that were measured using multiple analytical methods (Figure 3). As expected, the frequency of detection for a given compound was higher with the lower RL. The only exception being caffeine, where filtration of Method 3 may have reduced caffeine concentrations compared to that of the unfiltered Method 4. Figures 2 and 3 also demonstrate the importance of estimated values (*45*) below the RL. Clearly the numerous estimated concentrations illustrate that the current RLs are not low enough to accurately characterize the total range of OWC concentrations in the stream samples and that the frequencies of detection for this study are conservative.

To obtain a broader view of the results for this study, the 95 OWCs were divided into 15 groups based on their general uses and/or origins. The data show two environmental determinations: frequency of detection (Figure 4A) and percent of total measured concentration (Figure 4B) for each group of compounds. These two views show a vastly different representation of the data. In relation to frequency of detection, there were a number of groups that were frequently detected, with seven of the 15 groups being found in over 60% of the stream samples (Figure 4A). However, three groups (detergent metabolites, plasticizers, and steroids) contributed almost 80% of the total measured concentration (Figure 4B).

For those groups of compounds that have received recent public attention—namely antibiotics, nonprescription drugs, other prescription drugs, and reproductive hormones (*1, 2, 10*)—nonprescription drugs were found with greatest frequency (Figure 4A). Antibiotics, other prescription drugs, and reproductive hormones were found at relatively similar frequencies of detection. The greater frequency of detection for nonprescription drugs may be at least partially derived from their suspected greater annual use compared to these other groups of compounds. When toxicity is considered, measured concentrations of reproductive hormones may have greater implications for health of aquatic organisms than measured concentrations of nonprescription drugs. Previous research has shown that even low-level exposure ($<0.001\,\mu g/L$) to select hormones can illicit deleterious effects in aquatic species (*7, 46, 47*).

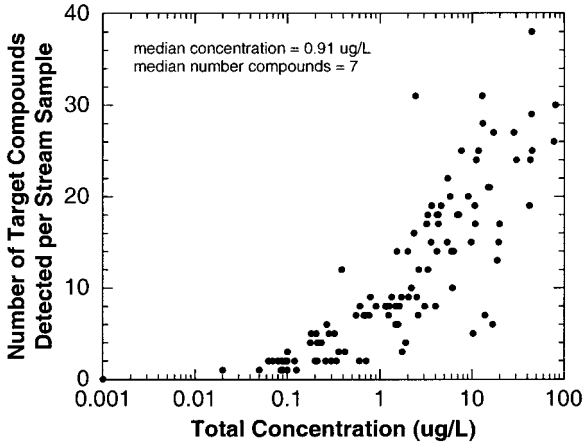

FIGURE 5. Relation between total concentration (summation from all detections) and number of organic wastewater contaminants found per water sample (Spearman's rank correlation coefficient = 0.94, $P < 0.001$).

Mixtures of various OWCs were prevalent during this study, with most (75%) of the streams sampled having more than one OWC identified. In fact, a median of seven OWCs were detected in these streams, with as many as 38 compounds found in a given streamwater sample (Figure 5). Because only a subset of the 95 OWCs were measured at most sites collected during the first year of study, it is suspected that the median number of OWCs for this study is likely underestimated. Although individual compounds were generally detected at low-levels, total concentrations of the OWCs commonly exceeded 1 μg/L (Figure 5). In addition, 33 of the 95 target OWCs are known or suspected to exhibit at least weak hormonal activity with the potential to disrupt normal endocrine function (*4, 7, 8, 10, 12, 22, 36, 37, 48−50*), all of which were detected in at least one stream sample during this study (Table 1). The maximum total concentration of hormonally active compounds was 57.3 μg/L. Aquatic species exposed to estrogenic compounds have been shown to alter normal hormonal levels (*7, 48, 51*). Thus, the results of this study suggest that additional research on the toxicity of the target compounds should include not only the individual OWCs but also mixtures of these compounds. The prevalence of multiple compounds in water resources has been previously documented for other contaminants (*52, 53*). In addition, research has shown that select chemical combinations can exhibit additive or synergistic toxic effects (*54−56*), with even compounds of different modes of action having interactive toxicological effects (*57*).

The results of this study document that detectable quantities of OWCs occur in U.S. streams at the national scale. This implies that many such compounds survive wastewater treatment (*1, 6, 58*) and biodegradation (*59*). Future research will be needed to identify those factors (i.e. high use and chemical persistence) that are most important in determining the occurrence and concentration of OWCs in water resources.

Although previous research has also shown that antibiotics (*60*), other prescription drugs (*1, 2, 19, 61−63*), and non-prescription drugs (*1, 40, 62, 64*) can be present in streams, this study is the first to examine their occurrence in a wide variety of hydrogeologic, climatic, and land-use settings across the United States. Much is yet to be learned pertaining to the effects (particularly those chronic in nature) on humans, plants, and animals exposed to low-level concentrations of pharmaceuticals and other OWCs. Furthermore, little is known about the potential interactive effects (synergistic or antagonistic toxicity) that may occur from complex mixtures of these compounds in the environment. Finally, additional research also needs to be focused on those OWCs not frequently detected in this stream sampling. Select OWCs may be hydrophobic and thus may be more likely to be present in stream sediments than in streamwater (*65, 66*). For example, the low frequency of detection for the tetracycline (chlortetracycline, doxycycline, oxytetracycline, tetracycline) and quinolone (ciprofloxacin, enrofloxacin, norfloxacin, sarafloxacin) antibiotics is not unexpected given their apparent affinity for sorption to sediment (*66*). In addition, select OWCs may be degrading into new, more persistent compounds that could be transported into the environment instead of (or in addition to) their associated parent compound.

Acknowledgments

The authors wish to acknowledge the USGS scientists and field technicians who provided essential assistance to this project by identifying candidate stream sites across the United States and in collecting and processing stream samples. In addition, the authors thank Michele Lindsey, Jeff Cahill, and Greg Brown for their important contributions to developing the analytical methods being used. The authors also acknowledge Steffanie Keefe for her efforts in compiling the existing ecotoxicological data, Jessica Hopple for her assistance in generating select figures for this paper, and Kymm Barnes for her assistance in compiling the water-quality data for this study. This project was supported by the U.S. Geological Survey, Toxic Substances Hydrology Program. The use of trade, firm, or brand names in this paper is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

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Received for review June 12, 2001. Revised manuscript received November 26, 2001. Accepted December 12, 2001.

ES011055J