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EVALUATING THE EXTENT OF PHARMACEUTICALS IN SURFACE WATERS OF THE UNITED STATES USING A NATIONAL-SCALE RIVERS AND STREAMS ASSESSMENT SURVEY

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Abstract: To assess the potential exposure of aquatic ecosystems to active pharmaceutical ingredients, the authors conducted a national-scale, probability-based statistical survey of the occurrence of these compounds in surface waters of the United States. The survey included 182 sampling sites and targeted rivers with close proximity to urban areas. The 46 analytes reported represent many classes of active pharmaceutical ingredients (APIs), including antibiotics, diuretics, antihypertensives, anticonvulsants, and antidepressants. Of the 46 analytes, 37 were detected in at least 1 sampling location. Sulfamethoxazole (an antibiotic) was the most frequently detected compound, being measured in 141 of the 182 surface waters surveyed at concentrations ranging up to 570 ng/L. Ten of the compounds were detected in 20% or more of the sampling sites. Weighted means of the analytical measurements are used with the statistical survey design and analysis to provide national estimates of the extent of contamination for these APIs in the nation's urban rivers. *Environ Toxicol Chem* 2015;9999:1–8. Published 2015 Wiley Periodicals, Inc. on behalf of SETAC. This article is a US government work and is in the public domain in the United States of America.

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INTRODUCTION

The reported presence of many active pharmaceutical ingredients (APIs) in the environment has become of increasing concern because of their potential to cause undesirable ecological effects [1–9]. Pharmaceuticals used in human medicine can enter the environment either by excretion or by disposal of surplus drugs into sewage systems [10,11]. Presently, wastewater treatment plants (WWTPs) are not specifically designed to remove many classes of trace-level contaminants, such as APIs, and many of these compounds are consequently released into surface waters [12,13]. More than 1000 human APIs are approved for use in the United States today, with widely varying chemical and physical properties, uses, modes of action, and potencies. These compounds are expected to have different environmental fates, potencies, and effects, and studying the full range of approved APIs would be impractical.

To efficiently explore potential risks from the broad range of human prescription pharmaceuticals in use today, we have previously prioritized a list of the most prescribed APIs in the United States based on the potential of their wastewater residues to cause biological effects [14,15]. An analytical method using selective solid-phase extraction and liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS) was developed for the analysis of the top rated APIs from that process, which includes 48 APIs and 6 selected metabolites, some of which had not been previously included in an environmental monitoring method [16]. Our most recent study [17] reported the concentrations of these APIs in effluent samples from 50 very large WWTPs located across the United States. The results of this previous wastewater effluent study were used, in combination with the previously mentioned theoretical work [14] and measurements, to draw tentative conclusions about risks to aquatic life from water-column exposure to all human prescription pharmaceuticals [17].

To more completely assess the potential exposure of aquatic ecosystems to these prioritized APIs, we conducted a national-scale, probability-based statistical survey of the occurrence of these compounds in surface waters of the United States. The surface water survey included 182 sampling sites and targeted rivers with close proximity to urban areas. The prioritized analytes measured for the present study represent many classes of APIs, including antibiotics, diuretics, antihypertensives, anticonvulsants, and antidepressants. Weighted means of the analytical measurements are used with the statistical survey design and analysis to provide national estimates of the extent of contamination by these pharmaceuticals in the nation's urban rivers. The present study reports the results from the national surface water survey and, when compared with the results from our recent risk assessment in wastewater effluent study [17], will provide further information needed to assess the risks to aquatic life from pharmaceuticals in the environment.

METHODS

Site selection and sample collection

Samples were collected from navigable US streams that were fifth order and greater [18] as part of the 2008 to 2009 US Environmental Protection Agency's (USEPA's) National Rivers and Streams Assessment. The larger National Rivers and Streams Assessment study was intended to provide a national assessment of the condition of rivers and streams across the United States and included several chemical indicators that were analyzed as part of the assessment in fish tissue from more than 500 sampling sites with locational subgroups, as described in detail elsewhere [19,20]. The sampling structure [21] was derived using the National Hydrography Dataset [22] and included Strahler stream order attributes [18]. Sampling sites

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were selected using a probability-based approach [23–25], generally applying the spatial methodology that has been used previously for lakes [26].

One of the subgroups in the larger National Rivers and Streams Assessment study design included a subset of rivers and streams identified as urban rivers. This portion of the study focused on river and stream segments that were within urban tracts, with sampling sites included from 42 of the 48 states. Because the main pathway of human prescription pharmaceuticals into the environment is through wastewater treatment discharge, surface water at urban sites was chosen as the focus for API analysis. National Hydrography Dataset-Plus and the US Census Bureau's national urban boundary geographic information system coverage layers [27] were used to identify urban sampling areas, which were defined as densely settled census block groups (minimum population density of 50 000 people). Surface water samples were collected for pharmaceutical analysis from 182 urban rivers and streams within the conterminous United States. A map of the sites, which also designates which sampling locations are downstream of a WWTP discharge, is shown as Figure 1. The 182 sampling sites selected in the present study represent 30 169 km of fifth order and higher urban streams in the United States. Each sampling location is weighted, and from the measured surface water concentrations of each analyte at these locations the survey can provide a national estimate of urban river miles that are expected to contain these APIs.

Samples were collected between June 2008 and January 2010. For nonwadeable sites, a composite sample of surface water was obtained by collecting and combining 2-L grab samples from 5 equally spaced locations. For wadeable sites, a single sample was collected at the midpoint of the site. A sample aliquot was transferred to clean 500-mL amber glass bottles, shipped overnight on wet ice to the laboratory in Cincinnati (OH, USA), and stored at 4 °C until extraction. All samples were extracted within 4 d of collection. Because of the large number of sampling sites and chemical analytes, it was logistically too difficult and expensive to collect and analyze field blanks.

Sample analysis

Surface water samples were extracted and analyzed using a previously reported method [16]. Immediately after arrival at the laboratory, 2 mL of a solution containing 5.0 g/L of Na_2 ethylenediamine tetraacetic acid and 25 mg/L of ascorbic acid was added to each sample. A laboratory reagent blank consisting of distilled water, a laboratory fortified blank distilled water sample, and a laboratory fortified matrix spike sample were also included in each extraction batch. To avoid blockages of the solid-phase extraction columns, 500 mL of each sample was filtered through a 0.7-μm filter; therefore, the reported results represent the dissolved portion of these analytes. All samples were then spiked with respective isotopically labeled procedural internal standards (at a concentration of 500 ng/L) prior to extraction. The quality control samples (laboratory fortified blank and laboratory fortified matrix) were also spiked with a mixture of the target analytes at a concentration of 500 ng/L.

Samples were extracted with 150 mg Oasis HLB MCX (Waters) cartridges at an unadjusted pH. Acidic and neutral analytes were eluted by acetonitrile, and basic analytes were eluted by 95% acetonitrile and 5% ammonium hydroxide into separate silanized glass tubes. Extracts were stored in silanized glass vials at –10 °C until analysis. Immediately before analysis, stored extracts were concentrated to dryness under a constant flow of nitrogen at 40 °C prior to reconstitution in either 20% acetonitrile (acidic and neutral analytes) or 20% methanol (basic analytes). Reconstituted extracts were transferred to polypropylene vials for LC-MS/MS analysis. Extracts were analyzed for 54 APIs using a Waters Aquity ultra performance liquid chromatograph coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer with an electrospray ionization source operated using multiple reaction monitoring. Analytes were separated on a BEH C18 column (1.0 × 100 mm, 1.7 μm) equipped with 0.2-μm inline filter (Waters). Four separate

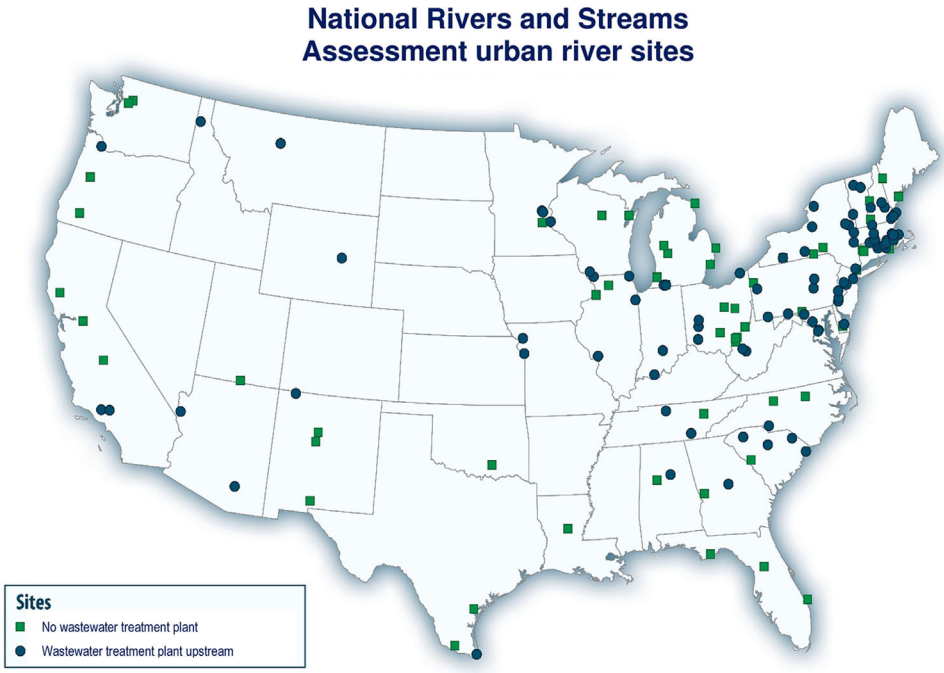

Figure 1. A map of the urban river sites included in the present study.

injections were used to cover the range of analytes, in accordance with LC-MS/MS conditions described previously [16].

Percent recovery for each analyte was calculated in the laboratory fortified blank and laboratory fortified matrix control samples, which were included with each extraction batch for a total of 67 distilled water and matrix spike samples over the 2-yr sample collection period. Because of the complexity of the sample matrix and the long study period, the acceptable target recoveries were set between 50% and 150% for all compounds. Any compound that did not meet the predetermined quality criteria (more specifically, low recoveries <50%) in 75% of the laboratory fortified matrix samples was excluded from further analysis; such compounds included cimetidine, prednisolone, methylprednisone, betamethasone, theophylline, 2-hydroxyibuprofen, glipizide, and glyburide. Any analyte detected in a laboratory reagent blank was reported as a nondetect if its concentration in the sample was less than 10 times the blank concentration. The method detection limit (MDL; Table 1), which is defined as the minimum concentration of an analyte that can be identified and detected with 99% confidence that the analyte concentration is greater than 0, was determined for each analyte using the procedure described by the USEPA [28]. The quantitation limit was defined as 3 times the MDL (Supplemental Data, Table S1). Concentrations were reported to the MDL; however, any concentrations greater than the MDL but less than the quantitation limit were flagged as estimated (Supplemental Data, Table S1). Targets for MDLs were previously chosen to encourage quantification of concentrations with similar potential for eliciting biological effect [16]. Although the majority of the MDLs were well below the target, it was found for a few analytes (testosterone, progesterone) that the MDLs were above the target.

Data analysis

The National Rivers and Streams Assessment survey design and analysis provide national and regional estimates of contaminant concentrations. The statistical estimates are based on weighted means of the analytical results from sites. The weights are based on the survey design and are the inverse of the probability of selecting a sampling site. The probability of selecting a site depends on the stratification and unequal probability of selection associated with the site. The weights are the total stream length represented by the sample site. Percentiles and mean estimates of pharmaceutical concentrations were calculated from the weighted data using routines developed by the USEPA in the statistical calculation package spsurvey R. For the purpose of calculating percentile and mean estimates, the MDL was used in place of nondetected values. Additional information on the statistical algorithms employed in site selection and data analysis in the National Rivers and Streams Assessment survey are available in Stevens et al. [23–25,29] and from the USEPA [30].

Effect-level parameters of minimum daily dose, maximum plasma concentration after a minimum dose (C_{max}), fraction bound to plasma proteins, lowest minimum inhibitory concentration, and antibiotic breakpoint, as well as modes of action and predicted environmental concentrations, were taken from Kostich et al. [14,17].

RESULTS AND DISCUSSION

Measured concentrations

Of the 46 analytes with reported results included in the present study, 37 were detected in at least 1 sampling location. Table 1 provides a summary of the surface water survey findings, including the percentage of the 30 169 km of urban stream length where APIs were estimated to be present above the MDL. Ten analytes were estimated to be present in >10% of the urban stream length represented, with their stream length detection ranging from 12.1% to 82.7%. The most widely detected compound was sulfamethoxazole (an antibiotic), which was estimated to be present in 82.7% of urban stream kilometers represented in the study. To provide an evaluation of the maximum possible exposure for aquatic organisms, nondetects were replaced with the MDL for the calculation of the reported means (Table 1) and percentile estimates (Table 1; Supplemental Data, Table S3), because a nondetect does not definitively indicate that the concentration of an analyte is 0. Percentile estimates for the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles are reported in Supplemental Data, Table S3 for all analytes that were detected in at least 1 sample. The calculated means, 95th percentile estimates, and maximum concentrations detected are presented in Table 1 to provide an overview of the range of concentrations detected for each analyte. Means for detected analytes were all in the low ng/L range. The majority of the maximum concentrations detected were also in the low ng/L range; however, 11 compounds were detected at concentrations greater than 100 ng/L, and the maximum concentration detected for any analyte was 620 ng/L (hydrochlorothiazide).

The cumulative frequency distribution plot for the most frequently detected compound, sulfamethoxazole, is shown in Figure 2. This provides a national estimate of the distribution of concentrations of sulfamethoxazole across the 30 169 km of urban rivers, as indicated by the measured concentrations of sulfamethoxazole (in nanograms per liter, x axis) against the corresponding percentage of US urban river length (y axis). The remaining analytes displayed detected concentrations that were near the detection limit, as shown by the percentile estimates in Supplemental Data, Table S3.

We have previously reported the concentrations of these APIs in effluent samples from 50 very large WWTPs located across the United States [17]. These 50 plants sampled represented 17% of all wastewater produced by wastewater-treatment plants in the United States. Our initial assessment of risk posed by these APIs compared maximum measured effluent concentrations with national average predicted environmental concentrations (PECs). Predicted environmental concentrations were determined by dividing minimum daily dose equivalents prescribed per year by the estimated annual wastewater volume, as described in detail [14], and maximum effluent concentrations were measured using the same analytical methodology [16] applied to the present surface water study. The previously reported PECs and measured effluent concentrations [17] are both listed in Table 1 for comparison purposes. The only compounds detected in surface water that exceeded PECs are fluticasone and warfarin, although both APIs were only detected in 2 samples and exceed the PEC by less than a factor of 5. For most APIs, maximum surface water concentrations were similar to or lower than maximum effluent concentrations (Table 1). When comparing these effluent and surface water maximum concentrations, it should be noted that effluent samples were collected during winter months, the majority of surface water samples were collected during summer months, and temporal trends were not within the scope of either study.

Table 1. An overall summary of the concentrations detected for each pharmaceutical[a]

CAS no.	API	MDL (ng/L)	No. of detections ($n = 182$)	Frequency of detection (%)	Percentage of stream length detected	Mean estimate (ng/L)	95th percentile estimate (ng/L)	Surface water maximum (ng/L)	PEC (ng/L)	Effluent maximum (ng/L)	1% $C_{max\text{-}free}$ (ng/L)	Yr/dose
64520-05-4	10-Hydroxy-amitriptyline[b]	0.2	0	0	0.0	<MDL	<MDL	0	5029	<RL	68.2	N/A
103-90-2	Acetaminophen	1.5	11	6.0	4.3	3.3	<MDL	111.3	306 955	1500 (4500)	80 000.0	14 769
18559-94-9	Albuterol	3.1	2	1.1	0.9	3.1	<MDL	9.8	471	35	46.0	839
28981-97-7	Alprazolam	2.9	3	1.6	3.4	3.3	<MDL	25	103	31	23.0	41
50-48-6	Amitriptyline	0.2	1	0.5	0.2	0.2	<MDL	0.8	5029	110	22.5	68 493
111470-99-6	Amlodipine	0.4	0	0	0.0	<MDL	<MDL	N/A	94	18	520.0	N/A
300-62-9	Amphetamine	0.5	15	8.2	7.5	0.6	1.4	7.1	387	40	142.8	965
51706-40-2	Atenolol	1.9	88	48.4	28.2	9.2	35.4	186.4	4137	3000	5820.0	367
134523-00-5	Atorvastatin	12.0	0	0	0.0	<MDL	<MDL	N/A	2906	<RL	0.6	N/A
132-17-2	Benztropine	0.5	1	0.5	0.3	0.5	<MDL	3.7	33	ND	1.5	185
298-46-4	Carbamazepine	1.4	74	40.7	28.7	12.0	35.6	249.3	5607	240 (460)	3000.0	1099
4205-91-8	Clonidine	11.0	0	0	0.0	<MDL	<MDL	N/A	43	ND	2.2	N/A
87857-41-8	Desmethylsertraline[b]	3.0	1	0.5	0.2	3.0	<MDL	7	3343	100	10.0	48 924
33286-22-5	Diltiazem	0.9	60	33.0	17.9	1.6	3.9	56.8	615	24	435.0	2894
85100-17-0	Desmethyldiltiazem[b]	0.5	18	9.9	5.0	0.6	0.5	16.8	3343	340	1450.0	32 616
76095-16-4	Enalipril	0.3	2	1.1	1.8	0.3	<MDL	1.9	369	38	275.0	1802
356-12-7	Fluocinonide	2.8	0	0	0.0	<MDL	<MDL	N/A	12	ND	5.0	N/A
54910-89-3	Fluoxetine	0.9	10	5.5	3.6	1.6	<MDL	24.8	NA	31	5.0	552
90566-53-3	Fluticasone	6.2	2	1.1	1.3	6.3	<MDL	16.2	4.2	ND	11.5	0.8[c]
106391-48-4	Furosemide	12.0	5	2.7	0.8	12.1	<MDL	37.2	7283	810 (2100)	100.0	736
25812-30-0	Gemfibrozil	1.2	27	14.8	9.8	3.2	16.5	112.5	NA	2300	7500.0	14 612
58-93-5	Hydrochlorothiazide	3.2	41	22.5	13.8	15.5	35.1	619.9	13 947	2800	420.0	28
143-71-5	Hydrocodone	1.2	6	3.3	1.3	1.3	<MDL	16.7	2561	92 (100)	60.5	410
50-23-7	Hydrocortisone	4.6	0	0.0	0.0	<MDL	<MDL	N/A	2368	ND	8.4	N/A
15687-27-1	Ibuprofen	3.8	9	4.9	4.6	4.6	<MDL	43.6	20 257	4200 (4600)	4200.0	6284
37350-58-6	Metoprolol	4.3	73	40.1	25.1	11.8	33.2	217.7	1451	660 (1200)	1364.0	79
68-22-4	Norethindrone	2.2	0	0.0	0.0	<MDL	<MDL	N/A	111	ND	0.9	N/A
83891-03-6	Norfluoxetine[b]	2.3	5	2.7	0.8	2.3	<MDL	15.4	NA	15	9.1	1617
67814-42-4	Norverapamil	1.4	18	9.9	5.7	2.4	2.2	30.4	5328	20	236.4	24 579
124-90-3	Oxycodone	0.8	18	9.9	4.2	1.1	<MDL	93.1	NA	310	55.0	294
61869-08-7	Paroxetine	0.5	1	0.5	0.2	0.5	<MDL	8.3	NA	ND	2.5	1650
53-03-2	Prednisone	9.5	0	0.0	0.0	<MDL	<MDL	N/A	2194	ND	130.0	N/A
57-83-0	Progesterone	60.0	1	0.5	0.8	63.0	<MDL	440.8	NA	<RL	0.01	622
58-33-3	Promethazine	0.4	1	0.5	0.7	0.4	<MDL	1.5	1668	ND	2.6	17 123
469-62-5	Propanolol	1.4	10	5.5	3.2	1.5	<MDL	11.2	991	260	552.0	21 404
525-66-6	Propoxyphene	5.1	3	1.6	1.1	5.1	<MDL	6.4	8300	34 (46)	4.0	3669
66357-35-5	Ranitidine	3.5	7	3.8	2.4	3.5	<MDL	21	NA	1400	3825.0	9785
79559-97-0	Sertraline	0.9	2	1.1	1.1	1.1	<MDL	19	615	71	1.0	1802
79902-63-9	Simvastatin	13.0	0	0.0	0.0	<MDL	<MDL	N/A	548	<RL	0.9	N/A
723-46-6	Sulfamethoxazole	0.5	141	77.5	82.7	28.2	111.1	576.4	NA	2900	120 000.0	1901
58-22-0	Testosterone	2.0	3	1.6	1.6	4.9	<MDL	361.4	NA	ND	0.04	190
396-01-0	Triamterene	0.4	47	25.8	15.6	0.9	2.4	23.6	4504	170	88.0	2177
738-70-5	Trimethoprim	0.8	67	36.8	21.4	1.8	6.6	60.9	NA	370	5600.0	3599
137862-53-4	Valsartan	3.6	71	39.0	28.0	15.3	64.5	319.4	2628	5300 (8200)	300.0	172
52-53-9	Verapamil	0.8	39	21.4	12.1	1.4	2.2	35.8	5328	97	52.0	4592
2610-86-8	Warfarin	3.6	2	1.1	0.6	4.2	<MDL	131.3	28	ND	75.0	21

[a]Frequency of detection was calculated from the number of samples ($n = 182$). Percentage of stream length was calculated as a percentage of the 30 169 stream km represented in the study where active pharmaceutical ingredients were present above the method detection limit. Predicted environmental concentration (PEC) and effluent maximum concentration are taken from Kostich et al. [17]. Effluent maximum concentrations in parentheses are estimated (high or low associated matrix spike recovery).

[b]Metabolite.

[c]The oral bioavailability of fluticasone is negligible. See discussion under *Potential drinking water exposure* for more information.

CAS = Chemical Abstracts Service; API = active pharmaceutical ingredient; MDL = method detection limit; PEC = predicted environmental concentration; $C_{max\text{-}free}$ = free plasma concentrations; ND = not detected; <RL = detected but below method reporting level.

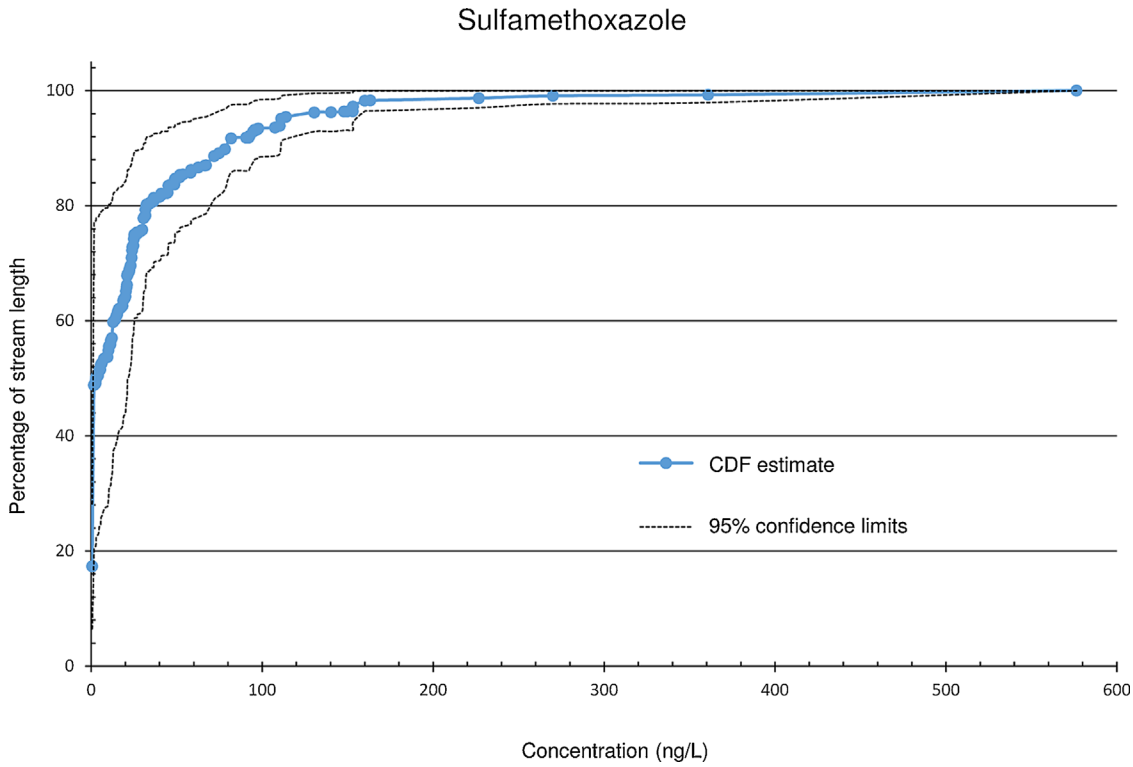

Figure 2. Estimated cumulative distribution plot for the most frequently detected active pharmaceutical ingredient, sulfamethoxazole. The *x* axis represents measured concentration in ng/L, and the *y* axis represents the percentage of the 30 169 km of urban streams represented in the present study. CDF = cumulative distribution frequency.

Potential ecosystem exposure

To investigate the potential risks to aquatic organisms exposed to environmental concentrations of APIs, therapeutic plasma concentrations were used to identify 3 reference levels. The reference levels were then applied to estimate the length of river miles that may be exceeded by detected levels of APIs. Although there have been some reports that indicate effects of pharmaceuticals on aquatic life [5–7], there are still no definitive effect-level benchmarks. Plasma concentrations after therapeutic dosing have been previously proposed as a threshold concentration that may elicit a biological response [31–33], and the application of such was discussed thoroughly in our previous work [17]. Free plasma concentrations ($C_{max\text{-}free}$; Supplemental Data, Table S4) were determined using maximal plasma concentration in human subjects after a minimum therapeutic dose minus the fraction of plasma drug bound to plasma proteins. For the biologically active metabolites included in the present study, the $C_{max\text{-}free}$ was adjusted to account for the reduced biological activity of the metabolites using the potency of the metabolite relative to the parent compound. This approach assumes a similar response at a similar molecular receptor in aquatic organisms as in humans, that the pharmaceutical readily enters the organism, and that the organism does not have the ability to actively rid itself of the pharmaceutical. Since pharmaceuticals most likely exist in the environment as mixtures and the effects of the ambient environment on the interaction of these compounds and aquatic organisms are largely unknown, 2 additional reference levels were included (10% of the $C_{max\text{-}free}$ and 1% of the $C_{max\text{-}free}$; Supplemental Data, Table S4) to represent a conservative estimation.

Table 1 compares the calculated mean, estimated 95th percentile, and maximum concentrations detected to the lowest reference level used (1% of the $C_{max\text{-}free}$). Twelve compounds were found to exceed the 1% $C_{max\text{-}free}$. For 7 of the compounds, the MDL was above the 1% $C_{max\text{-}free}$. Atorvastatin, clonidine, norethindrone, and simvastatin were not detected in any sample, and their MDLs were below the 10% $C_{max\text{-}free}$ (clonidine, norethindrone, simvastatin) or $C_{max\text{-}free}$ (atorvastatin). Propoxyphene (a narcotic pain reliever) had an MDL of 5.1% and a 1% $C_{max\text{-}free}$ of 4.0. Propoxyphene was detected in 3 samples (5.4 ng/L, 5.9 ng/L, and 6.4 ng/L), which were all below the 10% reference level. The MDL for progesterone (60 ng/L) was much higher than any other compound in the method, the result of an isobaric interference that occasionally appears in laboratory samples. Unfortunately, the MDL was far above the $C_{max\text{-}free}$ of 1 ng/L for progesterone; therefore, no reference level analysis could be applied.

The reference-level analysis presents results in terms of the length of urban rivers that are estimated to contain concentrations of pharmaceuticals that exceed any of the 3 reference levels investigated. The results for any API that exceeded any of the 3 reference levels are listed in Table 2 and are expressed both as river length (in kilometers) and as a percentage of the total 30 169 urban river km represented. Although sulfamethoxazole was the most frequently detected compound, with the highest 95th percentile concentration (111 ng/L) and the second highest maximum concentration (576 ng/L), it is also the least potent pharmaceutical included in the present study, with a 1% $C_{max\text{-}free}$ of 120 000 ng/L. Fluoxetine was found to exceed the 1% reference threshold in an estimated 985.1 stream km of the 30 169 km represented in the present study (3.3% of the urban rivers), with a maximum concentration of 28 ng/L.

Table 2. A summary of the stream length estimates (in km) for each active pharmaceutical ingredient that exceeded reference level values based on free plasma concentrations ($C_{max\text{-}free}$)

Active pharmaceutical ingredient	Mode of action	Reference level	Reference level value (ng/L)	No. of samples exceeded	Percentage of urban river length exceeded	Urban river length exceeded (km)
Alprazolam	Neurotransmitter modulator (benzodiazepine)	1%	23	1	0.2	53.0
Benztropine	Neurotransmitter modulator (anticholinergic)	1%	1.5	1	0.3	91.2
Fluoxetine	Antidepressant (serotonin reuptake inhibitor)	1%	5	4	3.3	985.1
Fluticasone	Anti-inflammatory (corticosteroid)	1%	11.5	1	0.4	121.4
Hydrochlorthiazide	Antihypertensive (diuretic)	1%	420	1	0.7	210.1
Norfluoxetine	Antidepressant (serotonin reuptake inhibitor)	1%	9.09	2	0.3	100.9
Oxycodone	Neurotransmitter modulator (opioid)	1%	55	3	0.1	18.6
Paroxetine	Antidepressant (serotonin reuptake inhibitor)	1%	2.5	1	0.2	46.4
Sertraline	Antidepressant (serotonin reuptake inhibitor)	10%	10	1	0.9	281.1
Sertraline	Antidepressant (serotonin reuptake inhibitor)	1%	1	2	1.1	327.4
Testosterone	Reproductive hormone (androgen)	100%	4	2	1.2	358.9
Valsartan	Antihypertensive (angiotensin 1 antagonist)	1%	300	1	0.1	24.0
Warfarin	Anticoagulant (anti–clotting factor)	1%	75	1	0.4	121.4

Norfluoxetine, a metabolite of fluoxetine, was also found to exceed the 1% reference level in an estimated 100.9 stream km, with a maximum concentration of 15.4 ng/L. Sertraline exceeded the 1% reference level in an estimated 327.4 stream km, or 1.1% of the nation's urban rivers. The maximum concentration of sertraline detected (19 ng/L) also exceeded the 10% reference level, which equates to 281.1 stream km (0.9%). Fluoxetine, its metabolite norfluoxetine, sertraline, and paroxetine (also found to exceed the 1% reference level in 46.4 stream km; Table 2) all belong to the same class of selective serotonin reuptake inhibitor antidepressants. The effects of selective serotonin reuptake inhibitor exposure on a number of fish and invertebrates have been investigated, with effects (behavioral and reproductive) being reported typically when water concentrations are in the mid to high μg/L range, which is far above any concentrations detected in the present study, although there are a few reports of effects in the low ng/L range [7,9,34]. The remaining compounds, which included other psychiatric drugs and antihypertensives, were found to only exceed the lowest reference level and represented <1% of the urban rivers in the study, with the exception of testosterone. These are similar findings to what was observed in effluent, where the main classes of APIs found to come close to the $C_{max\text{-}free}$ were antidepressants (sertraline and desmethylsertraline) and antihypertensives (valsartan and propranolol) [17].

Testosterone was detected in 2 samples, at concentrations of 6.5 ng/L and 361.4 ng/L (Table 1; Supplemental Data, Table S2). In the same sample where testosterone was detected at its maximum concentration, progesterone was detected at 440.8 ng/L, the only detection of progesterone in the present study. In addition to being prescription pharmaceuticals, testosterone and progesterone are naturally occurring hormones that are produced by the human body. Unfortunately, because of the large number of samples included, it was not financially or logistically possible to collect field blanks at each location, so contamination in the field by handling glassware cannot be effectively ruled out. However, laboratory blanks and laboratory fortified blank and laboratory fortified matrix samples were processed with each extraction batch, and no contamination was seen in any of these quality assurance/quality control samples in the sample preparation. Furthermore, no other analytes were detected in this sample at this level (Supplemental Data, Table S1), which indicates that these concentrations were not the result of contamination from a standard during analysis. The instrument method utilizes triple-quadrupole MS, collects multiple reaction monitoring transitions for 2 product ions, and calculates a product ion ratio, which is currently accepted as the standard for specificity in environmental analysis. Although these concentrations far exceed anything we have detected in our laboratory with our previous studies [17], testosterone and progesterone surface water concentrations have been reported at up to 0.2 μg/L in another national-scale surface water study [35]. In addition to being prescribed as pharmaceuticals, testosterone and progesterone are naturally occurring hormones produced by animals and have been detected near agricultural practices that house large numbers of animals, such as confined animal feeding operations and dairies [36–39]. Progesterone also has been found to form from the decomposition of cow manure [39] and plant material [40]. The 100% $C_{max\text{-}free}$ for testosterone is 4 ng/L and that for progesterone is 1 ng/L, which were both exceeded in this sample by about 2 orders of magnitude. Because the MDL of progesterone exceeded all reference levels, stream length estimates could not be calculated. Testosterone concentrations exceeded the 100% $C_{max\text{-}free}$ reference level (Table 2) in 358.9 stream km (0.8%). Although the maximum concentrations of testosterone and progesterone were in the mid ng/L range, they clearly are not representative of a typical surface water concentration for these hormones. This may be an example of how APIs near certain locations—such as confined animal feeding operations [36], health-care facilities [41],

and pharmaceutical manufacturing facilities [42]—can exceed typical concentrations.

Potential drinking water exposure

Surface waters can receive discharges from wastewater-treatment plants and can also be used as source water for drinking water treatment. Drinking water treatment may remove more of the pharmaceuticals; but, to put the surface water concentrations in context, the measured concentrations were compared with therapeutic dosages for each pharmaceutical. The minimum daily therapeutic dose rates prescribed for humans are presented in Supplemental Data, Table S5 and are used in the present study because they are well established. Exposures at lower concentrations (below the therapeutic dose), however, may elicit an effect for some compounds for sensitive individuals, such as individuals with allergies or sensitivities to specific compounds or classes of compounds. The number of years it would take to consume a single dose of each pharmaceutical detected, assuming that a person consumes 2 L of drinking water per day at the maximum concentrations detected in this surface water study, is presented in Table 1. The maximum concentration of fluticasone was 16.2 ng/L, and with a minimum daily dose of 0.01 mg, the years per dose would be less than 1 (0.8). However, fluticasone is a corticosteroid often prescribed as a topical cream or nasal spray, and the oral bioavailability of fluticasone propionate is negligible (<1%) [43]. Warfarin had the second lowest years per dose (21 yr), and the remaining pharmaceuticals would all take multiple decades to consume a single dose. Sulfamethoxazole, for example, was again the most frequently detected compound with the second highest maximum concentration; however, at that maximum, it would take almost 2000 yr for an individual to consume a single dose. These results are in agreement with our previous studies [17] and indicate that the potential for exposure to APIs for humans through drinking water is very low.

Potential for antibiotic resistance

Two antibiotics were also analyzed in the present study: sulfamethoxazole and trimethoprim. These 2 APIs are typically used together as 1 pharmaceutical formulation at a ratio of 5 parts sulfamethoxazole to 1 part trimethoprim, because these 2 antibiotics exhibit a synergistic effect by inhibiting 2 different steps of folate synthesis. To investigate the possible contribution of environmental concentrations of antibiotics to antibiotic-resistant bacteria, we previously compared the minimum inhibitory concentration and breakpoint concentration to maximum concentrations of antibiotics detected in wastewater effluent. The minimum inhibitory concentration is the lowest concentration of an antibiotic that causes reliable inhibition of microbial growth. For sulfamethoxazole, the minimum inhibitory concentration is 0.16 μg/mL (1.6E5 ng/L), and it is 0.015 μg/mL (1.5E4 ng/L) for trimethoprim, which are almost 300 times greater than the maximum concentrations measured in surface water (576.4 ng/L for sulfamethoxazole and 60.9 ng/L for trimethoprim; Table 1). The breakpoint is the concentration of an antibiotic that can be expected to be safely maintained in a patient without causing adverse effects. For sulfamethoxazole, the breakpoint is 76 μg/mL (7.6E7 ng/L), and it is 4 μg/mL for trimethoprim (4.0E6 ng/L), which are again several orders of magnitude greater than the maximum concentrations observed. These results indicate that the environmental concentrations observed for these 2 antibiotics are not likely to directly select for antibiotic-resistant bacteria. However, recent studies have suggested that environmentally relevant antibiotic concentrations (including sulfamethoxazole at a water concentration of 500 ng/L) can induce a variety of functional shifts in river bacterial community composition [44,45].

CONCLUSION

The results of the surface water survey are in agreement with our previous findings in wastewater, and concentrations of these APIs, when compared with prescribed doses, indicate that the risk of exposure for humans through drinking water would be very low. However, a few APIs—namely, the selective serotonin reuptake inhibitor antidepressants and antihypertensives (valsartan, for example)—may pose a risk for aquatic organisms. More work is needed to provide reliable sublethal effect–level data at all environmentally relevant concentrations for aquatic life across the full range of trophic levels, although this is complicated by the large range of sensitivities to APIs expected for different species of fish and invertebrates. As future effects-level data become available, however, this data set and the described reference-level methods could be applied to predict national-level contamination with new benchmarks.

SUPPLEMENTAL DATA

Tables S1–S5. (108 KB XLSX).

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Data availability—Data collected for the present study can be accessed in the Supplemental Data.

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