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Selective Uptake and Bioaccumulation of Antidepressants in Fish from Effluent-Impacted Niagara River

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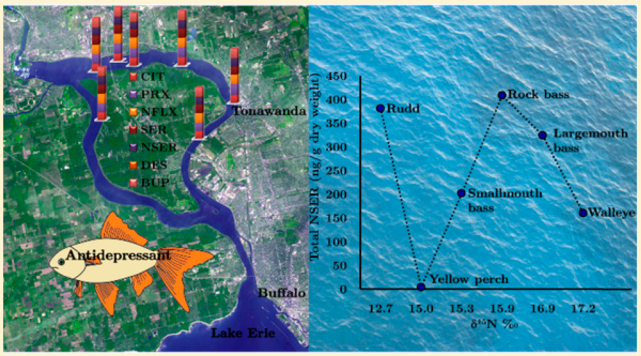
Supporting Information

ABSTRACT: The continuous release of pharmaceuticals and personal care products (PPCPs) into freshwater systems impacts the health of aquatic organisms. This study evaluates the concentrations and bioaccumulation of PPCPs and the selective uptake of antidepressants in fish from the Niagara River, which connects two of the North American Great lakes (Erie and Ontario). The Niagara River receives PPCPs from different wastewater treatment plants (WWTPs) situated along the river and Lake Erie. Of the 22 targeted PPCPs, 11 were found at part-per-billion levels in WWTP effluents and at part-per-trillion levels in river water samples. The major pollutants observed were the antidepressants (citalopram, paroxetine, sertraline, venlafaxine, and bupropion, and their metabolites norfluoxetine and norsertraline) and the antihistamine diphenhydramine. These PPCPs accumulate in various fish organs, with norsertraline exhibiting the highest bioaccumulation factor (up to about 3000) in the liver of rudd (*Scardinius erythrophthalmus*), which is an invasive species to the Great Lakes. The antidepressants were selectively taken up by various fish species at different trophic levels, and were further metabolized once inside the organism. The highest bioaccumulation was found in the brain, followed by liver, muscle, and gonads, and can be attributed to direct exposure to WWTP effluent.



INTRODUCTION

The continuous use of pharmaceuticals and personal care products (PPCPs) ultimately leads to their release into aquatic ecosystems. The presence of PPCPs in the environment is an important ecological concern due to the potential impairment of organism-specific functions, and the food web transmission resulting in biomagnification of contaminants in the environment. The inefficient removal of PPCPs by current municipal wastewater treatment systems results in the occurrence of PPCP residues in different environmental compartments such as sewage, rivers, lakes, soil, sediments, fish, and other aquatic organisms.[1−22]

Antidepressants are among the most prescribed drugs in the United States (U.S.).[23,24] According to the National Center for Health Statistics, there has been a 400% increase in antidepressant use from 1988 to 2008 in the US. Like many PPCPs, antidepressants are not completely eliminated during conventional wastewater treatment process; numerous studies confirmed the ubiquitous occurrence of antidepressants in the effluents of wastewater treatment plants (WWTPs) that are eventually discharged into the aquatic environments.[1,18,19,22,25−30] Antidepressants are classified based on how they affect serotonin and other neurotransmitter levels in the body. Selective serotonin reuptake inhibitors (SSRIs), one of the most widely used classes of antidepressants, prevent the reuptake of the neurotransmitter serotonin. Examples of SSRIs are fluoxetine, fluvoxamine, sertraline, paroxetine, escitalopram, and citalopram.[19,31] The newer types of antidepressants, serotonin−norepinephrine reuptake inhibitors (SNRIs) and norepinephrine−dopamine reuptake inhibitor (NDRI), target other neurotransmitters either alone or in addition to serotonin; and venlafaxine and bupropion are examples of these groups, respectively.[19,31]

The Niagara River, located in western New York, U.S., is an important waterway connecting Lake Erie and Lake Ontario,

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two of the North American Great Lakes. This 56-km river is part of the international boundary between the U.S. and Canada, dropping at the crest of the famous Niagara Falls and constituting an important recreational water resource. Unfortunately, many WWTPs discharge into the Niagara River and its tributaries; due to the impact of legacy industrial wastes in the river, some parts of the Niagara River has been designated as Area of Concern by the US Environmental Protection Agency (EPA). The occurrence of PPCPs in the region has been reported previously, including the pharmaceuticals ketoprofen (anti-inflammatory, NSAID), fenoprofen (NSAID), and carbamazepine (antiseizure) that have been detected in surface waters of the Niagara River.[27] More recently in Lake Michigan, 32 pharmaceuticals including fluoxetine have been reported at concentrations ranging from 0.3 to 9200 ng/L in WWTP effluents, and between 1 to 510 ng/g in sediments.[1] In addition, pharmaceutical concentrations at near shore habitats of Lake Michigan have been reported to be in the range between 0.92 to 46.2 ng/L.[28]

As WWTPs discharge into the aquatic ecosystems, the resident organisms are chronically exposed to the active ingredients of PPCPs and their persistent metabolites. Mixtures of SSRI drugs, even at trace levels, influence the physiology of exposed organisms.[3,6−8,32] For instance, ecotoxicological studies have demonstrated the physiological effects of SSRIs on fish, mollusks, crustaceans, algae, and protozoans.[6,9,32−35] It has been shown that SSRIs alter fish behavior relevant to population survival and community structure.[6,33−35] An exposure study using fathead minnows (*Pimephales promelas*) revealed that the antidepressants fluoxetine and sertraline affect their reproductive system, feeding habit, growth, and survivorship behavior.[6] In other studies using hybrid striped bass (*Morone saxatilis x Morone chrysops*), fluoxetine and venlafaxine caused the decrease of brain serotonin concentrations and affected the ability of the fish to capture its prey.[34,35] Many PPCPs have been measured in different fish tissues such as muscle, liver, brain and blood plasma.[2,4,13,14,36,37] However, PPCPs in several tissues of individual fish are seldom reported.[14,36] In addition to bioaccumulation of these chemicals, it is important to have information on their distribution in various fish organs in order to understand the potential toxicity of the contaminants and the ability of the fish to metabolize these compounds. The following fish organs are of interest: brain, because SSRIs act on the brain; liver, due to its function in xenobiotic metabolism; gonad, to see if the chemicals may have implications on the reproductive system of an aquatic organism; and muscle, in order to determine if the consumption of contaminated fish could potentially impact human consumers.

Bioaccumulation of pollutants in fish not only affect the fish that are directly exposed to the chemicals, but also pose risks to their predators.[10] Our study aims to investigate the occurrence of five parent SSRIs, three SSRI metabolites, and 16 other PPCPs (Supporting Information (SI), Table S1) in various environmental matrices, and determine their bioaccumulation in fish that inhabit the wastewater-impacted aquatic environment. The study includes analysis of SSRIs and other PPCPs in tissues from fish collected from the Niagara River, and the concentrations of these wastewater-derived compounds in surface water samples from different transects in the river. Because PPCPs pose significant risk to fish, and since the risks can be species-specific, ten species of fish were analyzed.[33] To examine the selective bioaccumulation of PPCPs in various fish organs, we analyzed brain, gonad, liver, and muscle samples.

MATERIALS AND METHODS

Study Sites. The study sites for water and fish sample collection, including the locations of the two WWTPs relative to the Niagara River are shown in Figure S1. The upper Niagara River (upstream of Niagara Falls) receives effluents from WWTPs, and untreated sewage from storm drains due to combined sewer overflows from the cities of Buffalo, Tonawanda, North Tonawanda, and Niagara Falls (New York, U.S.A.). This river and its tributaries have a history of industrial use and contamination, and is listed by the US EPA as an Area of Concern.[38] Water samples (1 L) from five sites, distributed along the U.S. East-branch of the river were collected, with two sites on the West-branch (see map in Figure S1). The river pH during the sampling period was 7.99 ± 0.39. These sites are located in the general area where the outflows of WWTPs enter the river (Figure S1 and Table S2). For "control" samples, a site at the mouth of a creek in Grand Island that runs through a State Park (Burnt Ship Creek) was used for collecting samples that are expected to have very low contamination. This site is in the North part of the island that faces the Canadian shoreline, and is less exposed to wastewater than any of the sites in the East-branch of the river. To show evidence that WWTPs that discharge effluents into the tributaries to Niagara River are important sources of PPCPs, effluent samples were also collected from two nearby WWTPs shown in Figure S1 as W1 and W2. The purpose of analyzing effluents from these WWTPs is to demonstrate that the same PPCPs detected in the Niagara River can be traced back from these WWTPs that discharge into the tributaries of the river.

Chemicals and Supplies. Acetaminophen (ACT), acetaminophen-d4 (d4-ACT), acetyl-sulfamethoxazole (ASMX), acetyl-SMX-d4 (d4-ASMX), caffeine (CAF), carbamazepine (CBZ), erythromycin (ERY), ibuprofen (IBU), ibuprofen-d3 (d3-IBU), naproxen (NPX), naproxen-d3 (d3-NPX), sulfamethoxazole (SMX), sulfamethoxazole-d4 (d4-SMX), trimethoprim (TMP), trimethoprim-d9 (d9-TMP), meprobamate (MEP), meprobamate-d7 (d7-MEP), metformin (MET), and iopamidol (IOPA) were purchased from Sigma-Aldrich. 13C-erythromycin-H_2O (13C-ERY), ciprofloxacin (CIP), desvenlafaxine (DES), diclofenac (DIC), diclofenac-d4 (d4-DIC) and dilantin (DIL) were obtained from Cambridge Isotopes (Tewksbury, MA). Carbamazepine-d10 (d10-CBZ), ciprofloxacin-d8 (d8-CIP), and caffeine-d3 (d3-CAF) were purchased from CDN Isotopes (Quebec, Canada). Diphenhydramine HCl (DPH), diphenhydramine-d3 (d3-DPH), bupropion HCl (BUP), bupropion-d9 HCl (d9-BUP), citalopram HBr (CIT), citalopram-d6 HBr (d6-CIT), paroxetine maleate (PRX), paroxetine-d6 maleate (d6-PRX), venlafaxine (VEN), venlafaxine-d6 (d6-VEN), desvenlafaxine (DES), desvenlafaxine-d6 (d6-DES), sertraline HCl (SER), sertraline-d3 HCl (d3-SER), norfluoxetine oxalate (NFLX), norfluoxetine-d6 oxalate (d6-NFLX), norsertraline HCl (NSER), and norsertraline-13C6 HCl (13C6-NSER) were obtained from Cerilliant (Sigma-Aldrich, St Louis, MO). Barnstead NANOpure Diamond (Waltham, MA) purification system was used to obtain 18.2 MΩ water used throughout all experiments. LC−MS grade methanol and acetonitrile (Omnisolv) was obtained from EMD Millipore Corporation (Billerica, MA). Formic acid (88%) was purchased from Fisher Chemical (Pittsburgh, PA).

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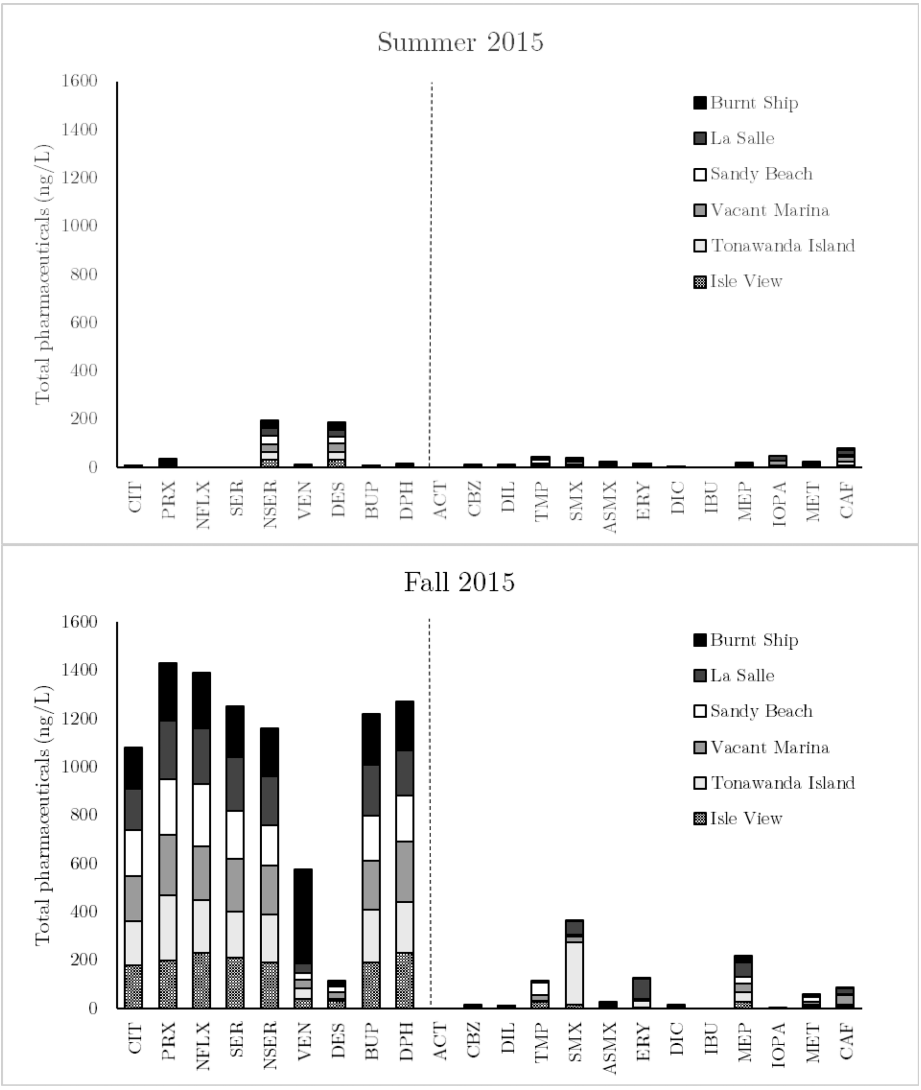

Figure 1. Total concentrations of each pharmaceutical in surface water analyzed in the Niagara River during summer 2015 (July, top) and fall 2015 (October, bottom). The breakdown of concentrations is arranged based on sampling location. The SSRI antidepressants are presented on the left of the dashed line. The control site, Burnt Ship Creek, is the solid black bar at the top of the plot.

Water Sample Collection and Preparation. Grab surface water samples were collected from different sites along the Niagara River using acid-washed amber bottles with polytetrafluoroethylene caps. The samples were collected in July (summer sample) and October (fall sample), 2015. The coordinates of the sampling sites are shown in Table S2. Collected samples were immediately stored in a cooler packed with ice, and transported to the laboratory where the samples were prepared using a slightly modified version of a method published from the same group.[39] The samples were spiked with a surrogate mix, filtered using glass microfiber filters (0.45 μm), and then acidified to pH 3.0 using phosphoric acid. After filtration, 500 mL of each sample was loaded into a 6 mL Waters Oasis HLB cartridge with 500 mg packing (Milford, MA) at a flow rate of 3 to 5 mL/min. The cartridges were vacuum-dried for at least 30 min after sample loading. Elution was done using 8 mL of acetonitrile; then extracts were dried under a nitrogen stream (5−10 psi). Samples were reconstituted to 1 mL using 80% aqueous (0.1%) formic acid solution in water and 20% 50:50 (v/v) acetonitrile: methanol mixture.

Fish Samples and Organ Collection. The fish were obtained from various locations in the upper Niagara River by electrofishing and hook-and-line fishing during the summer and fall of year 2015. General information on fish samples is shown in Table S3, which includes local sportfish that are typically consumed by recreational fishers. The species studied included smallmouth bass (*Micropterus dolomieu*), largemouth bass (*Micropterus salmoides*), the exotic common rudd (*Scardinius erythrophthalmus*), rock bass (*Ambloplites rupestris*), white bass (*Morone chrysops*), white perch (*Morone americana*), walleye (*Sander vitreus*), bowfin (*Amia calva*), steelhead trout (*Oncorhynchus mykiss*), and yellow perch (*Perca flavescens*). Each fish sample was individually extracted for analysis, except for the small yellow perch whose muscle, gonad, liver, and brain samples were combined to obtain enough sample mass for chemical analysis. The yellow perch samples were sorted into three groups based on the location from which they were taken (YPG1 consisted of three yellow perch from Burnt Ship Creek, which was considered the control site; YPG2 consisted of four yellow perch from Gun Creek, Sandy Beach and La Salle, which were grouped as upper river sites; YPG3 consisted of six yellow

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perch from Strawberry Island, Beaver Island, and Motor Island which were grouped as lower river sites).

After collection of the different organs, the tissues were extracted by sonication using an optimized solvent consisting of 5% formic acid in acetonitrile/isopropanol (50/50, v/v) mixture as extractant. Two cleanup steps using the optimized lipid removal sorbents and Waters Oasis HLB solid-phase extraction (SPE) cartridges (Milford, MA) were performed on the crude fish extracts. A detailed discussion on how the fish organs were dissected and prepared for analysis by liquid chromatography with tandem mass spectrometry (LC−MS/MS) is presented in the SI.

Method Development and Validation. Due to the inherent complexity and high lipid content of the various fish tissues a fully optimized and validated analytical method is required for the accurate quantification of PPCPs including SSRIs in these biological matrices. The optimized method consists of extraction and cleanup steps for various fish tissues to determine %extraction recoveries as a function of solvent composition, type of extraction, extraction time, sample size, and sorbent material for lipid removal. A detailed description of the method development and optimization are provided in the SI.

The analytical figures of merit of the optimized method were evaluated for the determination of PPCPs in different fish tissues, and are summarized in Tables S5 and S6; protocols followed for quality assurance/quality control are also provided in the SI. Method detection limits (MDLs) in all tissues were in the range of 0.004−250 ng/g dry weight, and method quantification limits (MQLs) were in the range of 0.014−795 ng/g dry weigh, except for ciprofloxacin. The linear dynamic range for the PPCPs was from the MDL to 40 ng/g dry weight of each compound in muscle, liver, and gonad. For brain tissue the observed linear dynamic range for each compound was from MDL − 500 ng/g dry weight. The linearity, expressed as correlation coefficient (r^2), was greater than 0.998 for most target PPCPs, except for ciprofloxacin. Surrogate recoveries (% R) (Table S6) were evaluated at two levels of spiking concentrations: 10 ng/g dry weight and 100 ng/g dry weight for all analytes, except for iopamidol in brain tissue where 500 ng/g dry weight was used due to higher MDL. Isotope dilution was employed for quantification in order to correct for the low recoveries and matrix effects. Method precision, expressed as the relative standard deviation (%RSD) of surrogate recoveries in the different fish tissues (Table S6), were less than or equal to 12% for all fish samples.

LC−MS/MS Method for Quantifying PPCPs. The LC−MS/MS method was optimized for the determination of all target PPCPs. Separation was carried out using an Agilent 1100 LC system (Palo Alto, CA) with degasser, quaternary pump, and autosampler. The LC−MS/MS was equipped with a Waters XSelect CSH column (2.1 × 150 mm^2, 3.5 μm particle size) from Waters Corporation (Milford, MA). Column temperature was kept at 25 °C. A 20 μL sample was injected into the LC−MS/MS system and separated using a gradient elution program with 0.1% formic acid (A) and 50:50 (methanol: acetonitrile) (B) at a rate of 0.2 mL/min. The gradient started at 80% A and 20% B, and was kept for 2 min before ramping up to 100% B within 12 min; mobile phase was kept constant for 6 min then was brought back to the starting condition. The column was given 12 min to re-equilibrate before injecting the next sample (30 min total run time). An Agilent 6410 triple quadrupole MS detector was used under positive electrospray ionization (+ESI) and multiple-reaction monitoring (MRM) mode. The details of the MRM transitions and the MS parameters used are listed in Table S4. Isotope dilution method was used for quantification of all analytes, except for iopamidol and metformin which were quantified with internal standard method using trimethoprim-d9 as an internal standard.

RESULTS AND DISCUSSION

Impact of WWTP Effluents on Surface Water. Surface water samples were analyzed for SSRIs and other PPCPs to estimate the bioaccumulation factor (BAF) of these chemicals in fish. Figure 1 shows the distribution of the different PPCPs at the different sampling points along the Niagara River during the two sampling events. The concentrations of SSRIs are notably higher relative to the other PPCPs during the fall sampling event. However, a different pharmaceutical profile was found during summer, with norsetraline and desvenlafaxine being the highest. Moreover, there is a pronounced difference in the levels of all target PPCPs between the summer and fall samples. The high concentrations observed during fall may be attributed to the contributions of combined sewer overflows containing untreated or partially treated municipal wastewater that drain into the river due to the higher frequency of rain events in the season, lower pharmaceutical removal efficiency of WWTPs during fall, and difference in pharmaceutical usage.[40] One may expect that the relative concentrations of these pollutants in surface water should follow the same trend as the WWTP effluent, however, it must be taken into account that the system under study is a very dynamic body of water in terms of water flow, temperature, and rainfall. For example, the levels of pollutants in the river may be affected by input from upstream sources, Lake Erie, and their respective tributaries. Despite the statistically significant differences (two-way ANOVA, 95% CL, p-value of 4.55×10^{-85}) in the concentrations of the different PPCPs in the various sampling sites, no further conclusions can be drawn out since the control site was surprisingly as polluted as the other sampling sites.

SSRIs in Surface Water and Bioaccumulation in Fish. SSRIs alter the levels of the fish neurotransmitters associated with the stress response and related behaviors, which may affect survivorship in aquatic organisms inhabiting SSRI-polluted waters.[33,34,41−43] Sertraline, detected in the Niagara river at concentrations as high as 218 ng/L, has been shown to elicit different levels of toxicity in fathead minnow depending on water pH.[44] Fathead minnows in the above-mentioned study experienced a decrease in survivorship, growth, and feeding rates after 7 days of exposure to sertraline at 30−60 μg/L (pH 8.5), 120 μg/L (pH 7.5), and 250−500 μg/L (pH 6.5).[44] Changes in behavior relevant to survival after chronic exposure to other SSRIs were observed in the same fish species. Bupropion, detected at concentrations up to 217 ng/L in surface water from Niagara River, also showed the ability to alter the predator avoidance behavior of minnows exposed to concentrations between 200−2000 ng/L for 12 days.[45]

Similarly, exposure of hybrid striped bass at 200 μg/L concentration of venlafaxine for 3 days caused a decrease in brain serotonin concentration and the ability to capture prey.[34] Note that venlafaxine was detected in the Niagara River at concentrations up to 387 ng/L. Citalopram, detected at concentrations ranging from 168 to 188 ng/L in the Niagara River, is known to decrease cortisol levels in rainbow trout when exposed at a concentration of 5 μg/L for 10 days.[46]

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Table 1. Estimated Bioaccumulation Factor (BAF) of Antidepressants and Antihistamine DPH in Fish Muscle, Liver, Gonad and Brain[a]

species/organ		BAF						
		CIT[b,c]	NFLX[b,c]	SER[b,c]	NSER[b,c]	VEN[b,c]	BUP[b,c]	DPH[b,c]
smallmouth bass								
	brain	NA (0/5)	15−49 (4/5)	24−27 (2/5)	470−910 (4/5)	NA (0/5)	3−8 (4/5)	4−6 (5/5)
	gonad	NA (1/5)[d]	5−39 (4/5)	27 (1/5)	150 (1/5)	1 (1/5)	1−9 (5/5)	1−5 (5/5)
	liver	NA (0/5)	NA (0/5)	NA (0/5)	240−320 (5/5)	7−20 (4/5)	1−4 (4/5)	2−4 (5/5)
	muscle	2 (1/5)	1−7 (4/5)	NA (0/5)	84−170 (5/5)	1 (3/5)	NA (0/5)	1 (5/5)
largemouth bass								
	brain	8 (1/5)	15−97 (3/5)	68 (1/5)	590−1200 (5/5)	NA (0/5)	6 (1/5)	5−15 (5/5)
	gonad	4−5 (2/5)	8 (1/5)	NA (0/5)	130−160 (2/5)	3−4 (2/5)	3 (2/5)	1−2 (5/5)
	liver	5 (1/5)	NA (0/5)	NA (0/5)	310−470 (5/5)	3 (3/5)	2−5 (2/5)	1−4 (3/5)
	muscle	NA (1/5)[d]	1−3 (2/5)	NA (0/5)	140−160 (5/5)	1−2 (5/5)	NA (0/5)	1 (5/5)
rudd								
	brain	4 (2/5)	21−24 (3/5)	18 (1/5)	420−680 (5/5)	NA (0/5)	NA (0/5)	4−18 (5/5)
	gonad	2−4 (3/5)	5−9 (2/5)	NA (0/5)	150 (1/5)	NA (0/5)	NA (0/5)	1−2 (5/5)
	liver	20 (1/5)	NA (0/5)	NA (0/5)	300−3000 (5/5)	NA (0/5)	NA (0/5)	3−21 (3/5)
	muscle	1 (1/5)	NA (0/5)	NA (0/5)	110−310 (5/5)	1 (3/5)	NA (0/5)	1 (5/5)
rock bass								
	brain	18 (3/5)	15−130 (4/5)	29 (1/5)	500−1800 (5/5)	NA (0/5)	NA (0/5)	5−29 (5/5)
	gonad	2−3 (4/5)	49 (1/5)	14−15 (2/5)	130−200 (2/5)	NA (0/5)	NA (0/5)	1−9 (5/5)
	liver	9 (3/5)	NA (0/5)	NA (0/5)	770−1600 (4/5)	4 (2/5)	NA (0/5)	1−7 (5/5)
	muscle	NA (3/5)[d]	NA (0/5)	NA (0/5)	94−150 (3/5)	1 (1/5)	NA (0/5)	1 (5/5)
white bass								
	brain	6 (2/5)	18−66 (2/5)	23 (1/5)	510−920 (5/5)	NA (0/5)	NA (0/5)	8−9 (5/5)
	gonad	9 (1/5)	NA (0/5)	2 (1/5)	NA (0/5)	6 (1/5)	1−26 (2/5)	1−2 (5/5)
	liver	1−19 (4/5)	NA (0/5)	NA (0/5)	150−520 (3/5)	NA (1/5)[d]	NA (0/5)	1−15 (5/5)
	muscle	2 (2/5)	3−6 (3/5)	NA (0/5)	140−190 (5/5)	1 (2/5)	NA (0/5)	1−2 (5/5)
white perch								
	brain	NA (0/1)	NA (0/1)	NA (0/1)	840 (1/1)	NA (0/1)	NA (0/1)	4 (1/1)
	gonad	8 (1/1)	1 (1/1)	0.5 (1/1)	NA (0/1)	NA (0/1)	62 (1/1)	8 (1/1)
	liver	NA (0/1)	NA (0/1)	NA (0/1)	1100 (1/1)	11 (1/1)	NA (0/1)	1 (1/1)
	muscle	NA (0/1)	NA (0/1)	NA (0/1)	150 (1/1)	1 (1/1)	NA (0/1)	1 (1/1)
walleye								
	brain	NA (0/5)	NA (0/5)	15−20 (3/5)	350−670 (5/5)	NA (0/5)	NA (0/5)	5−11 (5/5)
	gonad	5	NA	2	200	7−14	46	1−2

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Table 1. continued

species/organ		BAF						
		CIT[b,c]	NFLX[b,c]	SER[b,c]	NSER[b,c]	VEN[b,c]	BUP[b,c]	DPH[b,c]
walleye								
		(1/5)	(0/5)	(2/5)	(1/5)	(3/5)	(1/5)	(5/5)
	liver	3	NA	NA	290	NA	NA	2−4
		(1/5)	(0/5)	(0/5)	(1/5)	(1/5)[d]	(0/5)	(5/5)
	muscle	NA	1	NA	96−330	1	NA	1
		(0/5)	(1/5)	(0/5)	(5/5)	(2/5)	(0/5)	(5/5)
bowfin								
	brain	NA	NA	NA	1500	NA	NA	NA
		(0/1)	(0/1)	(0/1)	(1/1)	(0/1)	(0/1)	(1/1)[d]
	gonad	NA	NA	NA	NA	NA	NA	NA
		(0/1)	(0/1)	(0/1)	(0/1)	(0/1)	(0/1)	(1/1)[d]
	liver	1	NA	NA	260	NA	NA	NA
		(1/1)	(0/1)	(0/1)	(1/1)	(0/1)	(0/1)	(1/1)[d]
	muscle	NA	NA	NA	150	NA	NA	NA
		(0/1)	(0/1)	(0/1)	(1/1)	(0/1)	(0/1)	(1/1)[d]
steelhead								
	brain	NA	NA	NA	160−600	NA	NA	NA
		(0/3)	(0/3)	(0/3)	(3/3)	(0/3)	(0/3)	(3/3)[d]
	gonad	NA	NA	NA	NA	NA	NA	NA
		(1/3)[d]	(0/3)	(0/3)	(0/3)	(0/3)	(0/3)	(2/3)[d]
	liver	17	NA	NA	270−630	NA	NA	1
		(2/3)	(0/3)	(0/3)	(3/3)	(0/3)	(0/3)	(3/3)
	muscle	NA	NA	NA	140−160	1	NA	NA
		(0/3)	(0/3)	(0/3)	(2/3)	(1/3)	(0/3)	(3/3)[d]
yellow perch								
	brain	4	NA	NA	250	NA	NA	NA
		(1/3)	(0/3)	(2/3)[d]	(1/3)	(0/3)	(0/3)	(1/3)[d]
	gonad	1−4	NA	NA	NA	9	30	1−2
		(2/3)	(0/3)	(0/3)	(0/3)	(1/3)	(1/3)	(3/3)
	liver	NA	NA	NA	NA	84−150	NA	1
		(0/3)	(0/3)	(0/3)	(0/3)	(2/3)	(0/3)	(2/3)
	muscle	NA	NA	NA	NA	NA	NA	0.2
		(0/3)	(0/3)	(0/3)	(0/3)	(0/3)	(0/3)	(2/3)

[a]BAF is expressed as the ratio of the analyte concentration in an organ (mg chemical/kg dry weight) to the freely dissolved chemical concentration measured in surface water from Niagara River (mg chemical/L water). NA denotes a sample in which either the chemical was not detected or it was below detection limit. [b]BAFs were determined in individual fish samples for most of the fish species except for yellow perch. BAFs of yellow perch were examined in each group of samples whose muscle, gonad, liver, and brain samples were combined to obtain enough sample mass for chemical analysis (see *Fish sample and organ collection* section in SI). [c]The number in brackets denotes frequency of detection. For example: (2/5) means 2 out of 5 fish have detectable concentrations of the compound in the specified tissue. [d]The target chemicals were detected at concentrations lower that the MQLs.

Cortisol levels increase in fish when under stress; the fish fail to physiologically respond to a threat by lack of cortisol secretion. In addition to antidepressants, the antihistamine diphenhydramine was detected in Niagara River at concentrations up to 252 ng/L and was found to accumulate in all fish organs (0.05−7.3 ng/g dry weight). The presence of diphenhydramine in aquatic environments has been noted to affect the feeding behavior (at 5.6 μg/L, pH 6.5−8.5) and growth (at 49.1 μg/L, pH 6.5−8.5) of fathead minnows (7 days exposure).[47]

The other PPCPs detected in surface water include antimicrobials like erythromycin (2−83 ng/L), sulfamethoxazole (3−260 ng/L), and trimethoprim (3−52 ng/L). The antidiabetic drug metformin was also found (3−20 ng/L). Carbamazepine and dilantin were also found but at very low levels (<3 ng/L). All targeted PPCPs were found at much higher concentrations in the WWTP effluent.

Bioaccumulation of PPCPs, including SSRIs, occurred in all fish species collected in the Niagara River (Table 1). The six SSRIs and two active metabolites that were detected in wastewater effluents (Table S7) and in surface water (Figure 1, Table S13) were also detected in fish samples (Tables S8−S11). The SSRI metabolite norsertraline and the antihistamine diphenhydramine were detected in all fish samples. Some of the target PPCPs were also found in the different organs of the various fish species, but at lower concentrations than the SSRIs. Due to the relatively high concentrations of SSRIs compared to the other PPCPs, the succeeding discussion will focus on the SSRIs. The reader is directed to the SI for details on the levels of the other PPCPs found in the different fish samples. The presence of more than one type of PPCP highlights the need to perform experimental work that better gauge the effects of these pollutants on fish because of possible synergistic and chronic effects that are hard to predict with models.[48]

Norsertraline, the metabolite of sertraline, was found to be the predominant antidepressant observed in all fish organs studied (Figure 2). The highest levels were found at 400 ng/g in brain, 647 ng/g in liver, 44 ng/g in gonad, and 73 ng/g in muscle, all based on dry weight. However, its parent molecule

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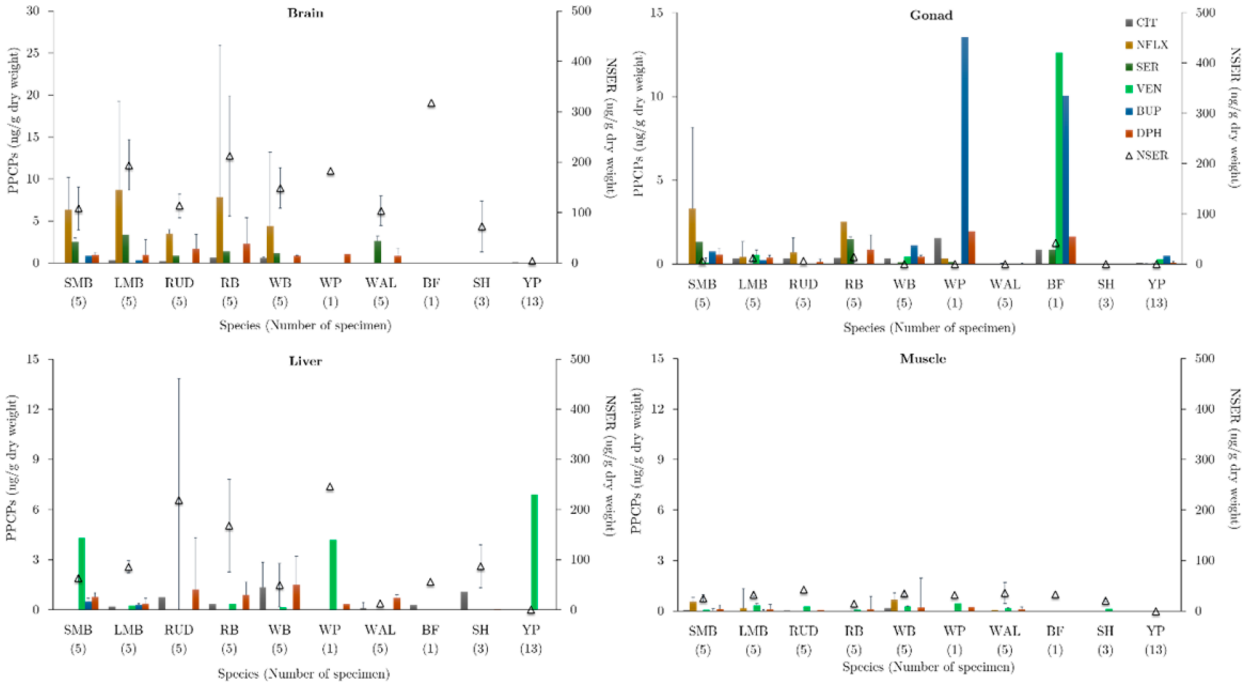

Figure 2. Levels of pharmaceutical residues in organs of fish collected from the Niagara River, shown as bar graph representing the average detected pharmaceutical on the left *Y*-axis for each species. Norsertraline (NSER), the major detected pharmaceutical residue in fish, is presented as triangles with concentrations shown on the right *Y*-axis. Error bars are presented as standard deviation.

sertraline was rarely observed in brain and gonad samples (only up to 17 ng/g) and was not detected in liver and muscle samples. Generally, the SSRI metabolites were detected at higher concentrations than their parent compounds in individual tissue samples. The same trend was observed for sertraline and fluoxetine along with their metabolites, norsertraline and norfluoxetine, in white sucker brains collected in Boulder Creek (Colorado, U.S.A.) and Fourmile Creek (Iowa, U.S.A.).[19] Similar observations were also found for sertraline and norsertraline in plasma, liver, and brain of fish living in an effluent-dominated stream in Matsuyama, Japan.[20] These studies suggest that the SSRIs are metabolized upon uptake in fish, or that the parent SSRIs are excreted faster than the metabolites, or both, since the ratio of metabolite to parent in fish organs were higher than that found in surface water.[20] To support this hypothesis, the ratio of metabolite norsertraline to sertraline was calculated and compared. The ratios are 1.0 in water, 3.2 in fish plasma, 10.7 in fish liver, and 5.8 in fish brain.[20] The differences in ratios suggest three ideas that may warrant further investigation: first, that metabolism of the parent compound occurs in fish tissue after uptake; second, that the depuration rate of the parent compound is faster than that of the metabolites, and third that there is a molecular mechanism behind the preferential accumulation of the metabolites over the parent molecule.

Norfluoxetine was found in fish brain, gonad, and muscle in the range of 0.2−40 ng/g dry weight but not in liver tissues. Alternatively, venlafaxine was observed in gonad, liver, and muscle (up to 57 ng/g dry weight) but was not detected in fish brain. Little to no citalopram and bupropion were observed in the fish organs. These findings suggest that individual SSRIs are taken up at different rates by fish, and that the accumulation of SSRIs into various fish organs is selective. The uptake of these lipophilic drugs can be explained by noting that compounds with log K_{ow} > 3 are introduced into fish bloodstream through the gills and are systemically distributed.[49,50] The Organization for Economic Co-operation and Development considers compounds to be bioaccumulative if they have log K_{ow} > 3.[51]

The bioaccumulative nature of SSRIs and antihistamine diphenhydramine were evaluated and expressed as BAFs. The field-measured BAFs were estimated by calculating the ratio of analyte concentration in each fish tissue to the analyte concentration in surface water collected from the same field location. Even though accumulation of PPCPs by aquatic organisms may be through various routes of exposure such as water, suspended solid, sediment and diet, the ambient PPCP levels in water was used to estimate BAF in the fish samples collected in this study. The US EPA recommends that predicted BAFs using K_{ow} be estimated by using the fraction of freely dissolved chemical in water (f_{fd}), which is derived from the equation below:[52]

$$f_{fd} = \frac{1}{1 + \mathrm{POC} \times K_{ow} + \mathrm{DOC} \times 0.08 \times K_{ow}}$$

We used the national default values of dissolved organic carbon (DOC) and particulate organic carbon (POC) in U.S. surface waters because these values were not measured in Niagara River during the sampling events. Nevertheless, this is a reasonable assumption because only sertraline had a noticeable reduction in the freely dissolved chemical (f_{fd} = 0.875) for fish uptake in the presence DOC and POC in water. The rest of the SSRIs had f_{fd} ranging from 0.954 to 0.996, which did not significantly change the BAF values if partitioning to suspended solids is ignored.

In order to make a conservative approximation, the highest measured surface water concentration of PPCPs during the fall sampling event was used for all BAF calculations. The different levels of SSRIs and the antihistamine observed in the various fish tissues resulted into a wide range of calculated BAFs. For brevity, BAFs are shown as a range of values from the lowest to

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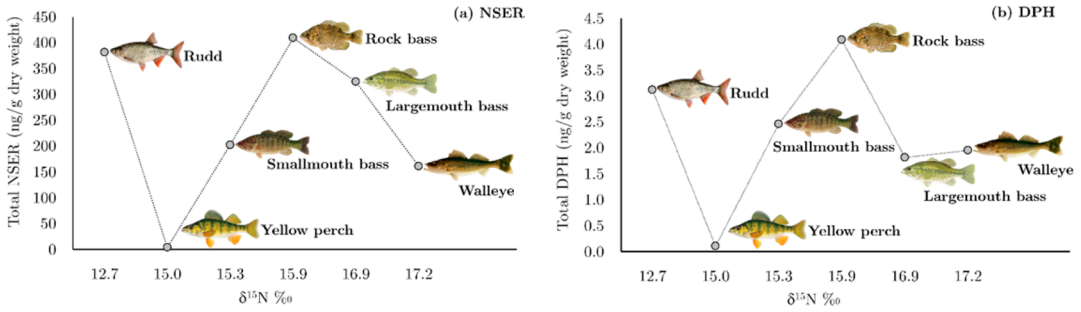

Figure 3. Total concentrations of (a) NSER and (b) DPH related to δ^{15}N. δ^{15}N ‰ in fish increases with carnivory and indicates trophic status.

the highest BAF because reporting an average value could be misleading if samples where no detectable analytes are included in the estimation. The information on the number of fish samples that actually contained detectable levels of SSRIs in each specific organ are also presented in Table 1. For example, in smallmouth bass brain samples, four out of five (4/5) showed measurable levels of norsertraline. Among these samples, the lowest calculated BAF was 465 and the highest BAF was 911; hence this is shown in Table 1 as 465−911.

Of the SSRIs analyzed in this study, only the SSRI metabolite, norsertraline (log K_{ow} = 4.82) presented pronounced bioaccumulation in all of the examined fish organs. However, the accumulation of its parent compound, sertraline (log K_{ow} = 5.29), was barely observed despite its higher log K_{ow} value. This suggests that norsertraline may be formed in the fish by metabolism of accumulated sertraline. In our samples, the ratios of norsertraline to sertraline in water and fish organs were compared to clarify if metabolism occurred in fish, after uptake of the drug. The higher ratios in fish brain (22.8) and gonad (7.4) suggest possible metabolism of SSRIs in the various fish organs. However, further work is needed to support this hypothesis. Also, the fluoxetine metabolite, norfluoxetine (log K_{ow} = 4.18), did not significantly accumulate in fish organs. Organ specific accumulation of SSRIs in fish brain may be explained by the molecule's mode of action and by the high lipid content of this organ, while accumulation in liver is due to the organ's lipid content and being the main site for detoxification. The organ-specific bioaccumulation of SSRIs may be due to differences in the target organ of each pharmaceutical, perfusion rate in each organ, exchange surface area, and substance biotransformation.[16,20,33] The higher fat content and higher perfusion rates in the brain and liver, relative to fish muscle and dischargeable organs like the gonad, favor bioaccumulation of SSRIs in brain and liver.

In this study, norsertraline (log K_{ow} 4.82) exhibited the highest BAF of all the target SSRIs. In addition, in most samples the highest BAF for norsertraline was found in brain, which is the target organ for SSRIs. The most obvious reason for the lowest BAF observed in gonad is because most fish spawn more than once in their lifetime during which their gonads are excised and gonadal tissues are consequently redeveloped. Therefore, if at the time of sampling the organ has just redeveloped, then the amount of accumulated pollutant will be lower compared to intact organs.

Note that accumulated PPCPs in aquatic organisms, including SSRIs in general, are not only acquired from the surrounding media such as water, sediments, and colloids, but also through their diet. Hence, it is expected that there will be differences in bioaccumulation of these compounds in various fish species with different trophic levels and age of the fish. Figure 3a,b shows the relationship between the trophic position (δ^{15}N) and total bioaccumulated norsertraline and diphenhydramine in six fish species.

Although the diet of the common rudd is mostly aquatic vegetation, as indicated by having the lowest δ^{15}N of all the fish studied, the common rudd has one of the highest concentrations of PPCPs. Unlike walleye, a top predator and the most carnivorous of all of the fish studied, diet is not the main pathway for the bioaccumulation of norsertraline and diphenhydramine in the common rudd. As an invasive species to the Great Lakes, the common rudd inhabit marsh areas of the Niagara River where they feed on plants near the sediment in the shoreline.[53] Common rudd can tolerate polluted conditions compared to other fish species and can dominate the bentho-pelagic fish community in the river. The high norsertraline and diphenhydramine concentrations observed in some of the fish studied may be due to direct exposure to WWTP effluent and bioaccumulation of PPCPs rather than ingestion through diet. These chemicals can enter the body through the gill area during exposure, as respiratory uptake critically contributes to the accumulation of chemicals.[54] In addition, organic pollutants could also be taken up from other phases in the surrounding habitat, since antidepressants tend to accumulate in bed sediments and have the affinity to bind to suspended solids.[19,55] Rock bass, smallmouth bass, and largemouth bass feed on a variety of foods like the smaller round goby (*Neogobius melanostomus*) which are benthic and occupy habitats near bulkheads and concrete walls along the upper Niagara River.

Interestingly, yellow perch had very low levels of the target PPCPs, which may be related to their pelagic habits as they feed mostly on zooplankton and also on terrestrial insects that fall into the water. These food sources are typically found in areas away from polluted sites, which may have limited the exposure of yellow perch to WWTP effluents. Yellow perch samples were collected at some of the same sites where the common rudd and rock bass (the species with the highest levels of SSRIs) were collected. In addition, many of the yellow perch samples were collected in the northern sites in Grand Island, where largemouth and smallmouth basses and the common rudd were also collected. However, some of the yellow perch were also collected in the southern part of Grand Island in cleaner sites (Beaver Island and Motor Island) as well as in Strawberry Island (the site of collection for many of the species reported here). Therefore, there is no clear pattern that indicates that the yellow perch were found in cleaner sites exclusively, since many of the polluted fish species were also collected from those sites. Habitat use, distance to effluent, and preferred diet and metabolism most likely were the main factors influencing the low SSRI levels found in yellow perch. In other published

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studies, yellow perch were also found to have low levels of accumulation for other chemicals such as flame retardants.[56] Thus, exposure to polluted habitats rather than bioaccumulation through diet seems to have been the larger factor in the observed chemical contamination of yellow perch.

As a limitation, this work only determined SSRIs and other PPCPs in surface water, but did not examine suspended solids and sediments on which the more hydrophobic PPCPs could be adsorbed. Surface water samples were prefiltered prior to extraction, thus adsorbed chemicals on suspended materials were not included. Interestingly, the polar iodinated compound iopamidol was observed at high concentrations in some brain and gonad tissues (up to 1059 ng/g dry weight in brain and 750 ng/g dry weight in gonad) even though they occurred at low concentrations in surface waters (up to 23 ng/L). Upon close inspection, it can be noticed that there are several structural features of iopamidol that are similar to the structure of the thyroid hormones thyroxine and triiodothyronine. These hormones play a vital role in many developmental processes like brain and body growth, metamorphosis, and reproduction.[57] On the basis of the similarity in their size, we speculate that iopamidol is being transported to the brain and gonadal tissues of fish by the same proteins that transport thyroid hormones. However, this idea warrants further investigation.

Overall, we found the same targeted PPCPs detected in WWTP effluents to be present in the Niagara River water samples, indicating that the local WWTPs discharging into the river and its tributaries are important sources of pharmaceutical pollution in the river. The bioaccumulation of SSRIs and antihistamine DPH in fish from Niagara River, especially in brain tissues, suggests the potential chronic effects of these compounds to the aquatic organisms inhabiting this effluent-impacted river. Results from this study highlight the significance of WWTP effluent on the deterioration of the Niagara River as fish habitat, and the impact of bioaccumulative SSRIs on fish populations and the aquatic community.

The concentrations of PPCPs found in the Niagara River are not unique to this body of water and can be found in many urban and industrial rivers around the world. Rivers have become a conduit for liquid waste disposal and have rarely benefited from an ecological assessment of the impact of such refuse. Perhaps the most insidious form of environmental pollution is the one that alters the behavior of the organisms in that ecosystem, which cease to act according to the impulses that evolution and survival dictate. Antidepressants, anti-inflammatories, analgesics, antiseizure medications, and other pharmacologically active chemicals target the brain and the nervous system of vertebrates, which include the fish that receive this cocktail of drugs 24-h a day, 7-days a week. Our society's reliance on these drugs does seem to be on an increasing trend, so focusing on how to prevent the contamination of the aquatic environment should be a major concern for local governments. It is obvious that improved WWTP processes capable of more effectively removing PPCPs and their metabolites from wastewater are needed to prevent the loss of biological diversity in our lakes and rivers. In addition, other upstream strategies to reduce loadings of PPCPs into the wastewater can be implemented such as urine diversion and pharmaceutical take-back programs, both of which can directly reduce the amounts PPCPs before they enter WWTPs.[58,59] Other potential strategies that are more challenging to realize and execute include redesigning pharmaceuticals such that lower dosages are needed, and that the excreted forms are more biodegradable and pose no ecotoxicity to exposed biota in the environment.[60]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b02912.

Details about the sampling, a thorough explanation of the extraction procedure, and data regarding the pharmaceuticals found in all the fish samples (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS:

ACT	Acetaminophen
ASMX	Acetyl-SMX
BAF	Bioaccumulation factor
BF	Bowfin
BUP	Bupropion
CAF	Caffeine
CBZ	Carbamazepine
CIP	Ciprofloxacin
CIT	Citalopram
DES	Desvenlafaxine
DIC	Diclofenac
DIL	Dilantin
DPH	Diphenhydramine
ERY	Erythromycin
IBU	Ibuprofen
IOPA	Iopamidol
MEP	Meprobamate
MET	Metformin
NFLX	Norfluoxetine
NPX	Naproxen
NSER	Norsertraline
PPCP	Pharmaceuticals and Personal Care Product
PRX	Paroxetine
SER	Sertraline

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SMB	Smallmouth bass
LMB	Largemouth bass
RB	Rock bass
RUD	Common rudd
SH	Steelhead Trout
SMX	Sulfamethoxazole
SSRI	Selective Serotonin Reuptake Inhibitors
TMP	Trimethoprim
VEN	Venlafaxine
WAL	Walleye
WB	White bass
WP	White perch
WWTP	Wastewater Treatment Plant
YP	Yellow perch

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