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Antidepressant Pharmaceuticals in Two U.S. Effluent-Impacted Streams: Occurrence and Fate in Water and Sediment, and Selective Uptake in Fish Neural Tissue

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Antidepressant pharmaceuticals are widely prescribed in the United States; release of municipal wastewater effluent is a primary route introducing them to aquatic environments, where little is known about their distribution and fate. Water, bed sediment, and brain tissue from native white suckers (*Catostomus commersoni*) were collected upstream and at points progressively downstream from outfalls discharging to two effluent-impacted streams, Boulder Creek (Colorado) and Fourmile Creek (Iowa). A liquid chromatography/tandem mass spectrometry method was used to quantify antidepressants, including fluoxetine, norfluoxetine (degradate), sertraline, norsertraline (degradate), paroxetine, citalopram, fluvoxamine, duloxetine, venlafaxine, and bupropion in all three sample matrices. Antidepressants were not present above the limit of quantitation in water samples upstream from the effluent outfalls but were present at points downstream at ng/L concentrations, even at the farthest downstream sampling site 8.4 km downstream from the outfall. The antidepressants with the highest measured concentrations in both streams were venlafaxine, bupropion, and citalopram and typically were observed at concentrations of at least an order of magnitude greater than the more commonly investigated antidepressants fluoxetine and sertraline. Concentrations of antidepressants in bed sediment were measured at ng/g levels; venlafaxine and fluoxetine were the predominant chemicals observed. Fluoxetine, sertraline, and their degradates were the principal antidepressants observed in fish brain tissue, typically at low ng/g concentrations. A qualitatively different antidepressant profile was observed in brain tissue compared to streamwater samples. This study documents that wastewater effluent can be a point source of antidepressants to stream ecosystems and that the qualitative composition of antidepressants in brain tissue from exposed fish differs substantially from the compositions observed in streamwater and sediment, suggesting selective uptake.

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Introduction

Public interest regarding the presence of pharmaceuticals in the environment is mounting (*1–3*). Antidepressants are one of the most commonly prescribed classes of pharmaceuticals in the United States. In 2007, six of the 200 most widely dispensed pharmaceuticals in the United States were antidepressants, including Lexapro (#3; escitalopram, eutomer of citalopram-CIT), Effexor XR (#12; venlafaxine-VEN), Cymbalta (#21; duloxetine-DLX), Wellbutrin XL (#57; Bupropion-BUP), Paxil CR (#123; paroxetine-PRX), and Zoloft (#159; sertraline-SER) (*4*). This is likely a conservative estimate of prescribing trends because pharmaceuticals that are off-patent, like fluoxetine (FLX), are more difficult to track compared to the patented products. Human pharmaceuticals, including antidepressants, enter wastewater treatment and, subsequently, the environment primarily through domestic waste from human excretion or by direct disposal of unused or expired drugs in toilets (*5*). Recent studies have indicated the presence of selected antidepressants in wastewater effluent (*6, 7, 8*) and river water (*1, 9, 10*). Few data exist regarding the presence of antidepressants in sediment and soil (*11*).

Most antidepressant pharmaceuticals function by inhibiting the neuronal reuptake of catecholamines and/or serotonin in mammals (*12*). Thus, the presence of antidepressants in waters receiving effluent could adversely affect exposed aquatic flora and fauna. Standardized aquatic toxicity tests suggest adverse effects of FLX at concentrations as low as 1 μg/L (*13*). Additional toxicological studies have reported that fluvoxamine (FLV), FLX, and PRX induced spawning in crustaceans and bivalves at concentrations as low as 10^{-10} M, equivalent to about 0.03 μg/L (*14*). The same three antidepressants were toxic to algae at levels ranging from 0.73 to 13.08 mg/L (*15*). Adverse behavioral responses include a decrease in prey capture ability by hybrid striped bass exposed to FLX concentrations ranging from 23.2 to 100.9 μg/L (*16*) and reduced predator avoidance performance in larval fathead minnows exposed to FLX and VEN at individual concentrations ranging from 25 to 250 ng/L and 500 to 5000 ng/L, respectively (*17*).

The few studies of the presence of antidepressants in aquatic organisms outside the laboratory have focused on FLX, SER, and their degradates. Such research has shown that these antidepressants are present in muscle, liver, and brain tissues from native fish populations residing downstream from an effluent outfall in a municipal-effluent dominated stream in Texas (*18, 19*) and in fish sampled from five U.S. effluent-impacted rivers that receive direct wastewater-effluent discharge (*20*).

The purpose of this study was to determine the fate and distribution of a wide range of commonly prescribed

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antidepressant pharmaceuticals and select degradates (Table S1 and Figure S1 of the Supporting Information) in water, sediment, and fish brain tissue as a result of discharge of wastewater effluent into two streams. This is the first study to examine such a broad range of antidepressant compounds from three environmental matrices (water, bed sediment, and tissue) from the same locations.

Materials and Methods

Study Sites. Two streams were selected for study: Boulder Creek in Colorado and Fourmile Creek in Iowa. These streams (1) were single-source, effluent-impacted systems, (2) have background data documenting the input of a wide variety of emerging contaminants from wastewater-treatment plant (WWTP) discharge (*21–23*), (3) have small basins, and (4) have a low-head dam upstream from the WWTP outfalls.

The 1160 km^2 Boulder Creek watershed (Figure S2 of the Supporting Information), located along the Colorado Front Range, varies in elevation from 4120 m at the Continental Divide to 1480 m at the confluence with Saint Vrain Creek (*23*). During the time of the investigation, the Boulder WWTP used secondary treatment by trickling filter/solids contact, nitrification and chlorination/dechlorination. Since 2007, the plant has been upgraded to an activated sludge plant with nitrification/denitrification, and chlorination/dechlorination. The Boulder WWTP discharges an average of 17 MGD (million gallons per day, or 64 million liters per day) and typically contributes less than 10% of streamflow during high-flow conditions (April−July) and more than 75% of streamflow during low-flow conditions (August−March) (*23*).

The 160 km^2 Fourmile Creek watershed (Figure S3 of the Supporting Information) is in central Iowa near Ankeny and varies in elevation from 317 m at its headwaters to 236 m at the confluence with the Des Moines River. The Ankeny WWTP consists of primary clarifiers, fine bubble aeration basins, final clarifiers, and UV disinfection. The Ankeny WWTP discharges an average of 5 MGD (or 19 million liters per day) and typically contributes less than 20% of streamflow during high-flow conditions and more than 80% of streamflow during low-flow conditions. WWTP discharge can contribute up to 100% of streamflow during extreme drought conditions.

Sample Collection. A Lagrangian sampling design was used for water sample collection. Dye-tracing tests were conducted prior to sampling at both field sites to determine the timing of water-sample collection so that the same parcel of water was sampled as it moved downstream under natural flow conditions. In both watersheds, four sites along the stream reach of interest were identified, starting 100 m above the WWTPs (physically separated from WWTP influence by a low-head dam) and extending 8.1 km (Boulder Creek, Figure S2 of the Supporting Information) and 8.4 km (Fourmile Creek, Figure S3 of the Supporting Information) downstream, respectively.

Triplicate water samples were collected from Boulder Creek (−0.1 km [BC 1], WWTP effluent [BC 2], and at 3.7 km [BC 3] and 8.1 km [BC 4] downstream from the Boulder WWTP outfall) in April 2005 and from Fourmile Creek (−0.1 km [FMC 1], WWTP effluent [FMC 2], and at 2.9 km [FMC 3] and 8.4 km [FMC 4] downstream from the Ankeny WWTP outfall) in March 2005, using standard depth- and width-integrated compositing techniques. A replicate sampling campaign, using a simplified depth/width integration sample collection with duplicate samples, was conducted in Boulder Creek in July 2006 and in Fourmile Creek in April 2006. All water samples were stored frozen at −20 °C until analysis to prevent degradation. Streamflow was measured using standard depth, width, and velocity methods (*24*) at the same time water samples were collected. Effluent flow was measured directly at BC 2 and indirectly at FMC 2 by subtracting the streamflow measured above and below the WWTP outfall. Grab samples of bed sediment were also collected from all sampling sites in 2005. The sediment consisted of multiple grab samples of surficial material (to target fined-grained materials) that were composited from a depositional zone in the same general area as the water samples. All sediment samples were stored frozen at −20 °C until analysis.

White suckers (*Catostomus commersoni*) were sampled from both streams by electroshocking with a pulsed direct current. Controls (low-head dams) exist upstream from the effluent discharge and effectively separate the fish populations within each stream. In Boulder Creek, the fish were sampled in 2005 from two sites, BC 1 and BC 2. To collect sufficient fish, electroshocking was started approximately 100 m downstream of each site moving upstream to the site. Fish collections from Fourmile Creek occurred during July 2005 and April 2006 at the FMC 1, FMC 2, and FMC 4 sites. At FMC 1, fish collection started approximately 1700 m upstream of the site, with most of the fish collected during 2005 and all of fish collected during 2006 being above the low-head dam (Figure S3 of the Supporting Information). At FMC 2 and FMC 4, a procedure similar to that used in Boulder Creek was followed. After capture, fish were anesthetized by immersion in tricaine methanesulfonate and sacrificed by rapid decapitation. Brains (male and female fish of varying sizes) were immediately excised, weighed (±0.1 mg), flash-frozen on dry ice, and stored at −80 °C until analysis (for detailed sampling protocols, see Vajda et al. (*25*)).

Analytical Methods. Standards and reagents are described fully elsewhere (*6*) and in the Supporting Information. Solid-phase extraction with Oasis HLB cartridges (Waters, Milford, MA) was used to extract antidepressants from water samples, and recoveries of parent antidepressants from matrix spiking experiments for the individual antidepressants ranged from 72 to 118% at low spiking concentrations (0.5 ng/L) and 70 to 118% at high spiking concentrations (100 ng/L). An Agilent 1100 liquid chromatograph (Santa Clara, CA) directly interfaced to the electrospray ionization (ESI) source coupled to an Applied Biosystems/MDS Sciex 2000 QTrap (Framingham, Mass.) mass spectrometer (LC/MS/MS) was used to analyze water, bed sediment, and brain tissue samples. The QTrap ion source was operated in positive ESI mode, and multiple-reaction-monitoring (MRM) transition mode was employed. Method detection limits for the individual antidepressant compounds ranged from 0.19 to 0.45 ng/L. The limits of quantitation (LOQ) were defined as the concentrations that yielded signal-to-noise values ≥10 within the sample matrix (*6*).

Sediment samples were extracted using accelerated solvent extraction with 70% acetonitrile and 30% water (*11*), followed by instrumental analysis with LC/MS/MS (*6*). Recovery of individual antidepressants added to sediment and corrected for ambient contributions of antidepressants ranged from 32% and 66%. The LOQs ranged from 0.25 to 2.50 ng/g for the individual antidepressants in the sediment extracts.

The white sucker brain-extraction procedure was adapted from Bymaster et al. (*26*). The method was validated with spike and recovery experiments with composite fathead minnow brains, using approximately three brains per analysis for a total of 30−40 mg of brain matter. Briefly, formic acid and acetonitrile were added to the brains and homogenized with an ultrasonic tissue disruptor. The brain sample was then centrifuged, the supernatant evaporated to near dryness, and the extract reconstituted with 0.1% formic acid. The brain extract was analyzed by the same LC/MS/MS instrumental method as the aqueous and sediment samples. The individual antidepressant recoveries ranged from 77 to 97%. The LOQ was 0.015 ng/g for all compounds.

No antidepressants were detected in field or method blanks, and sample replications used to determine the 95% confidence intervals reported are indicated elsewhere in the text. A summary of quality assurance and quality control

TABLE 1. Average Concentrations (ng/L) ± 95% Confidence Interval (n = 3) of Antidepressants in Boulder Creek (BC), April 2005, and Fourmile Creek (FMC), March 2005; the Range of Concentrations at Each Site is in Parentheses

site[a] (streamflow at time of sampleing)	FLX	NFLX	SER	NSER	PRX	CIT	FLV	BUP	VEN	DLX
BC 1 (1690 L/s)	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND
BC 2 (1110 L/s)	9 ± 6 (7.22−11.70)	4 ± 3 (2.68−5.14)	3 ± 4 (2.20−5.15)	5 ± 3 (3.76−6.28)	4 ± 3 (3.38−5.79)	60 ± 20 (52.0−65.6)	0.7 ± 0.3 (0.66−0.90)	50 ± 20 (42.1−56.7)	220 ± 40 (206−241)	<LOQ
BC 3 (2600 L/s)	1 ± 1 (<LOQ−1.50)	1.3 ± 0.3 (1.18−1.40)	4 ± 4 (2.97−5.92)	6 ± 2 (4.88−6.63)	3 ± 1 (2.08−2.87)	70 ± 40 (55.8−86.4)	0.7 ± 0.3 (0.61−0.83)	20 ± 20 (9.1−23.6)	120 ± 40 (73.3−154)	<LOQ
BC 4 (1280 L/s)	<LOQ	1.31 ± 0.03 (1.30−1.32)	2 ± 3 (0.84−3.22)	3 ± 4 (2.51−5.08)	2 ± 2 (1.12−2.57)	50 ± 40 (32.4−69.0)	<LOQ	27 ± 9 (24.2−30.7)	140 ± 40 (119−149)	ND
FMC 1 (620 L/s)	<LOQ	<LOQ	<LOQ	ND	ND	<LOQ	ND	<LOQ	<LOQ	ND
FMC 2 (860 L/s)	4.4 ± 0.5 (4.22−4.59)	1.3 ± 0.4 (1.18−1.49)	2.8 ± 0.4 (2.44−3.14)	2.3 ± 0.6 (2.14−2.57)	0.8 ± 0.2 (0.64−0.82)	30 ± 10 (23.7−31.9)	ND	40 ± 20 (35.7−49.8)	210 ± 40 (188−220)	<LOQ
FMC 3 (920 L/s)	<LOQ	0.96 ± 0.09 (0.93−1.0)	<LOQ	1.8 ± 0.4 (1.65−1.93)	<LOQ	4.9 ± 0.9 (4.58−5.33)	ND	34 ± 7 (31.6−36.6)	140 ± 30 (135−158)	<LOQ
FMC 4[b] (900 L/s)	<LOQ	0.89	<LOQ	1.13	<LOQ	4.53	ND	20	102	<LOQ

[a] Official USGS station IDs and locations are shown in Figures S2 and S3 of the Supporting Information. [b] n = 1; limit of quantitation (LOQ) = 0.5 ng/L, <LOQ = unquantified detect, ND = no detect; a zero was substituted for ND when determining averages.

TABLE 2. Two Replicate Concentrations (ng/L) of Antidepressants in Boulder Creek (BC), July 2006, and Fourmile Creek (FMC), April 2006

site[a] (streamflow at time of sampling)	FLX	NFLX	SER	NSER	PRX	CIT	FLV	BUP	VEN	DLX
BC 1 (2970 L/s)	<LOQ, <LOQ	<LOQ, <LOQ	<LOQ, <LOQ	ND, ND	ND, ND	<LOQ, ND	ND, ND	<LOQ, <LOQ	ND, ND	ND, ND
BC 2 (1940 L/s)	43.2, 39.2	13.6, 13.1	16.4, 19.2	6.2, 5.9	2.2, 3.9	205, 219	4.1, 4.6	213, 227	632, 672	<LOQ, <LOQ
BC 3 (4570 L/s)	7.5, 7.8	2.7, 1.9	8.9, 8.8	5.9, 3.7	3.1, 3.0	13.4, 15.8	<LOQ, <LOQ	76.3, 104	278, 359	<LOQ, <LOQ
BC 4 (2260 L/s)	1.9, 1.6	1.0, 0.9	1.5, 0.7	1.8, 1.1	ND, ND	16.0, 11.7	ND, ND	27.0, 20.5	128, 99.5	<LOQ, <LOQ
FMC 1 (780 L/s)	<LOQ, <LOQ	<LOQ, LOQ	<LOQ, <LOQ	ND, ND	ND, ND	<LOQ, <LOQ	ND, ND	ND, ND	<LOQ, <LOQ	ND, ND
FMC 2 (990 L/s)	29.0, 16.2	2.4, 1.8	37.5, 22.8	26.7, 11.1	5.4, 2.7	159, 85	1.7, 0.8	108, 51.9	690, 371	<LOQ, <LOQ
FMC 3 (1000 L/s)	1.4, 2.1	1.0, 1.3	5.2, 3.4	8.5, 4.3	0.8, 1.0	12.4, 7.4	<LOQ, <LOQ	31.1, 24.1	230, 146	<LOQ, <LOQ
FMC 4 (970 L/s)	0.8, 0.5	1.5, 0.9	2.5, 2.7	8.9, 5.8	<LOQ, <LOQ	5.3, 6.4	<LOQ, <LOQ	25.1, 36.3	176, 261	<LOQ, <LOQ

[a] Official USGS station IDs and locations are shown in Figures S2 and S3 of the Supporting Information. Limit of quantitation (LOQ) = 0.5 ng/L, <LOQ = unquantified detect, ND = no detect.

measures used for this study are provided in the Supporting Information.

Results and Discussion

Streamwater. All 10 target antidepressants were detected in Boulder and Fourmile Creeks, with most stream samples having at least 8 different antidepressants present (Tables 1 and 2). Qualitatively similar antidepressant profiles were observed in both streams. VEN was found in the highest concentration, followed by CIT and BUP (Tables 1 and 2), and were at least an order of magnitude greater than those

TABLE 3. Two Replicate Concentrations (ng/g) of Antidepressants in Boulder Creek Sediment Collected in 2005

site[a]	FLX	NFLX	SER	NSER	PRX	CIT	FLV	BUP	VEN	DLX
BC 1	0.39, 0.68	ND, ND	0.27, 0.37	ND, ND	ND, ND	0.36, 0.53	ND, ND	ND, ND	2.65, 1.62	ND, ND
BC 2	2.05, 7.76	ND, <LOQ	0.88, 5.90	ND, 2.98	0.56, 1.67	0.98, 8.79	ND, ND	ND, ND	6.63, 26.07	ND, ND
BC 3	16.95, 19.37	3.17, 3.01	14.37, 14.43	10.20, 10.70	2.65, 2.99	12.65, 13.01	ND, ND	2.12, 2.05	22.35, 25.29	ND, ND
BC 4	8.67, 14.12	ND, 2.24	12.91, 17.71	9.31, 10.18	3.01, 3.43	12.39, 14.95	ND, ND	1.72, 1.08	22.63, 24.30	ND, ND

[a] Official USGS station IDs and locations are shown in Figure S2 of the Supporting Information; Limit of quantitation (LOQ) = 0.25 ng/g for all analytes, except for NFLX, NSER, and FLV which had LOQ's of 2.50, 0.50, and 1.25, respectively; <LOQ = unquantified detect, ND = no detect.

of FLX and SER. A similar predominance of VEN, CIT, and BUP over more commonly investigated antidepressants was documented in a different effluent-impacted stream and in WWTP effluents (*6*). Typically, the antidepressants found at highest concentrations in the stream samples (VEN, CIT, BUP) were among those most commonly prescribed in the United States in 2005 (*27*) (Table S1 of the Supporting Information). Overall, instantaneous chemical loads (chemical concentration multiplied by streamflow at the time of sampling (Tables 1 and 2) and a unit correction factor) were generally an order of magnitude greater in Boulder Creek (median chemical load = 3470 ng/sec) compared to Fourmile Creek (median chemical load = 445 ng/sec).

Antidepressants were not detected above the LOQ upstream from the WWTP outfalls in either Boulder or Fourmile Creeks (BC 1 or FMC 1, respectively), suggesting that the WWTP effluent from the plant discharging to each stream is the likely route of introduction for antidepressants into that stream. The highest antidepressant concentrations were in the WWTP effluent discharged to both streams and were generally greater in the 2006 sampling compared to 2005 (Tables 1 and 2). The median effluent concentration for the combined antidepressants in 2005 was 4.5 ng/L for BC 2 and 2.6 ng/L for FMC 2, whereas the median effluent concentration for the combined antidepressants in 2006 was 15.6 ng/L for BC 2 and 20.8 ng/L for FMC 2, documenting that antidepressant input from WWTPs can be variable with time. Measurable antidepressant concentrations were also observed downstream from the WWTP outfalls in both streams and generally persisted to the locations farthest from the WWTP outfalls (Tables 1 and 2), documenting that antidepressants can be transported substantial distances in surface water. To better understand the fate of antidepressants once they enter a stream system, concentrations and loads were examined between Site 3 and Site 4 within both Boulder and Fourmile Creeks. Because of the availability of paired observations between these sites, a one-tailed Wilcoxon signed-ranked test (*28*) was used to test for spatial changes in chemical concentrations and loads between these two sampling sites. For this statistical test, the average values from the replicate measurements at Site 4 were subtracted from the average values from the replicate measurements at Site 3 for each antidepressant (Tables 1 and 2). By examining these differences in paired observations, we determined that chemical loads were significantly lower in BC 4 compared to BC 3 in both 2005 ($p = 0.004$) and 2006 ($p = 0.001$), but chemical concentrations were only significantly lower in 2006 ($p = 0.001$). A similar comparison from Fourmile Creek determined significantly lower chemical concentrations ($p = 0.016$) and loads ($p = 0.016$) in 2005, but no significant differences ($p > 0.05$) were determined in 2006. As the flows were similar in Fourmile Creek between these two sampling periods, the observed differences between 2005 and 2006 were not due to differences in water travel times. The greater complexity in the hydrologic system for Boulder Creek (e.g., tributary inflows and artificial diversions and return flows for irrigation purposes) as reflected in larger changes in streamflow compared to Fourmile Creek (Tables 1 and 2, Figures S2 and S3 of the Supporting Information) may have been a contributing factor for the generally greater differences in both chemical concentrations and loads between Sites 3 and 4 in Boulder Creek compared to Fourmile Creek.

The ratios of the degradates NFLX and NSER to their parent compounds may provide additional information regarding antidepressant sources. Both FLX and SER are extensively metabolized in vivo (*29*) and degradate/parent antidepressant ratios on the order of 10 to 20 would be anticipated in wastewater if the efficiency of in vivo metabolism was 90 to 95% and excretion was the sole source of parent compounds and degradates. However Schultz et al. (*6*) observed ratios of degradates to parents <1 for NFLX/FLX and NSER/SER in wastewater influent samples. The degradate-to-parent ratios determined for antidepressants measured in this study are similarly low, although such ratios increased as water parcels moved downstream. For example, the NFLX/FLX ratios for Boulder Creek in 2005 were 0.44, 1.3, and 3.25 for BC 2, BC 3, and BC 4, respectively. A similar trend was observed at Fourmile Creek in 2006 where the NSER/SER ratios for sites FMC 2, FMC 3, and FMC 4 were 0.63, 1.5, and 2.8, respectively.

There are several reasons that these ratios might be lower than expected. For example, the sum of antidepressant metabolism in vivo and during wastewater treatment may be less efficient than the literature suggests. In addition, other sources of the free parent antidepressants may not be accounted for, such as direct disposal of unused or expired drugs to wastewater, or degradation of in vivo conjugated antidepressants to the parent-free form. The method used in this study only measured NFLX and NSER because of a lack of authentic standards (*6*); further degradation to other degradates during wastewater treatment may also occur. The moderate increase in NFLX and NSER ratios after discharge suggests that instream biotic or abiotic mechanisms may be degrading the parent compounds. It also is important to note that NFLX is pharmacologically active (*29*) so that estimating possible biotic effects by these compounds requires concurrent measurement of parents and degradates.

Sediment. With the exception of FLV and DLX, all antidepressants were detected in at least one bed-sediment sample from Boulder Creek in the ng/g range. Poor sample-specific recoveries, observed upon repeated sediment analysis, for Fourmile Creek were deemed inconclusive and were not considered further. Similar to the streamwater data, VEN was measured in the highest concentrations in the Boulder

TABLE 4. Average Concentrations ± 95% Confidence Intervals (ng/g) of Antidepressants in White Sucker Brains collected in Boulder Creek (BC) in 2005 and in Fourmile Creek (FMC) in 2005 and 2006; the Range of Concentrations at Each Site is in Parentheses

site[a]	FLX	NFLX	SER	NSER	PRX	CIT	FLV	BUP	VEN	DLX
BC 1 $n = 4$ (2005)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BC 2 $n = 6$ (2005)	0.6 ± 0.6 (0.293−1.648)	0.9 ± 0.9 (0.216−2.431)	1.5 ± 0.5 (0.987−2.097)	2.5 ± 0.8 (1.426−3.158)	<LOQ	0.07 ± 0.08 (0.024−0.212)	ND	0.05 ± 0.06 (<LOQ−0.145)	ND	ND
FMC 1 $n = 14$ (2005)	<LOQ	<LOQ	ND	0.01 ± 0.02 (ND−0.085)	ND	ND	ND	ND	ND	ND
FMC 2 $n = 20$ (2005)	0.13 ± 0.05 (0.023−0.472)	0.5 ± 0.4 (0.124−3.567)	0.6 ± 0.4 (0.171−4.149)	3 ± 3 (0.776−28.90)	0.02 ± 0.01 (ND−0.113)	0.027 ± 0.008 (ND−0.068)	ND	0.05 ± 0.02 (<LOQ−0.154)	ND	ND
FMC 4 $n = 17$ (2005)	0.14 ± 0.06 (<LOQ−0.364)	0.4 ± 0.1 (0.097−0.886)	0.31 ± 0.08 (<LOQ−0.601)	1.1 ± 0.6 (0.446−5.148)	0.01 ± 0.01 (ND−0.083)	0.01 ± 0.01 (ND−0.072)	ND	0.07 ± 0.05 (ND−0.348)	ND	ND
FMC 1 $n = 20$ (2006)	0.02 ± 0.03 (ND−0.160)	ND	0.005 ± 0.01 (ND−0.099)	0.02 ± 0.05 (ND−0.382)	ND	ND	ND	ND	ND	ND
FMC 2 $n = 20$ (2006)	0.18 ± 0.05 (0.055−0.491)	0.15 ± 0.06 (0.051−0.546)	1.8 ± 0.4 (0.386−4.244)	2.1 ± 0.5 (0.962−6.128)	0.010 ± 0.009 (ND−0.047)	0.04 ± 0.03 (ND−0.195)	ND	0.013 ± 0.009 (ND−0.053)	0.1 ± 0.1 (ND−1.12)	ND
FMC 4 $n = 11$ (2006)	0.13 ± 0.07 (<LOQ−0.391)	0.07 ± 0.04 (<LOQ−0.195)	1.2 ± 0.5 (0.210−2.573)	1.8 ± 0.8 (0.228−4.758)	ND	<LOQ	ND	0.02 ± 0.04 (ND−0.178)	0.02 ± 0.03 (ND−0.131)	ND

[a] Official USGS station IDs and locations are shown in Figures S2 and S3 of the Supporting Information; limit of quantitation (LOQ) = 0.015 ng/g, <LOQ = unquantified detect, ND = no detect; a zero was substituted for ND when determining averages.

Creek bed-sediment samples (Table 3), and FLV and DLX were not detected. However, several differences were noted in antidepressant concentrations between streamwater and bed sediment in Boulder Creek. First, unlike streamwater, four antidepressant compounds were quantified in bed sediment upstream from the Boulder WWTP outfall, suggesting possible upstream contributions of antidepressants to sediment at this sampling location. A small WWTP facility in Nederland, Colorado, discharges effluent 38.5 km upstream from the Boulder WWTP and may provide a source of sediment-associated antidepressants that are not reflected in the water samples collected just upstream from the Boulder WWTP discharge (Tables 1 and 2). Second, and somewhat unexpectedly, the highest antidepressant concentrations in sediment were not found at the WWTP outfall (i.e., BC 2), as was the case with aqueous samples, but were instead substantially farther downstream, such that the apparent spatial pattern of sediment antidepressant concentrations does not reflect the spatial pattern of the overlying stream. There are several possible reasons for this disparity. The transit time for a parcel of water through this reach is on the order of a few hours, whereas the sediment deposits sampled in this study may accumulate over substantially longer periods. Other processes, including discharge of WWTP particulates, partitioning of antidepressants onto sediment, microbial transformation within sediment deposits, and bed-sediment transport, likely act in concert to define the spatial distribution of antidepressants in effluent-impacted stream sediment. Third, the qualitative antidepressant composition pattern observed in sediment, particularly substantially lower CIT and BUP concentrations, is distinctly different from the pattern observed in effluent and stream samples. The observation of comparatively low CIT and undetectable FLV concentrations in Boulder Creek sediment relative to their aqueous concentrations cannot be predicted based on experimentally derived log K_{ow} results (*30*), which would suggest that CIT would be the most abundant antidepressant sediment if hydrophobic partitioning is the primary mechanism controlling liquid−solid distributions.

Finally, whereas concentrations generally decrease with increasing distance from the WWTP outfall in streamwater, observed concentrations of the individual antidepressants actually increased in the bed sediment with increasing distance from the effluent discharge site. Additional research is needed to determine whether this observation occurs consistently or if it is reflective of conditions at the time of sediment sampling.

White Sucker Brains. Eight of the 10 antidepressants measured were detected in white sucker brain tissue from Boulder and Fourmile Creeks (Table 4). Previous research has documented the presence of antidepressants in fish tissue (*18–20*). Overall, levels of antidepressants observed in the white sucker brains at Boulder and Fourmile Creeks were about an order of magnitude lower than reported by Brooks et al. in fish brains collected downstream of a wastewater outfall on Pecan Creek (*18*). It is difficult to make a direct comparison between the two studies because stream and sediment antidepressant concentrations for Pecan Creek were not reported, and the fish species were different (bluegill, channel catfish, and crappie versus white sucker). Average tissue concentrations were of similar magnitude between Boulder and Fourmile Creeks and between the two fish samplings in Fourmile Creek, although sample-to-sample variability is observed, as reflected in the confidence limits reported with the data (Table 4). Nevertheless, results from individual fish brain samples (Supporting Information) demonstrate the distinct compositional pattern of higher concentrations of FLV, NFLX, SER, and NSER, and relatively lower concentrations of other antidepressants when com-

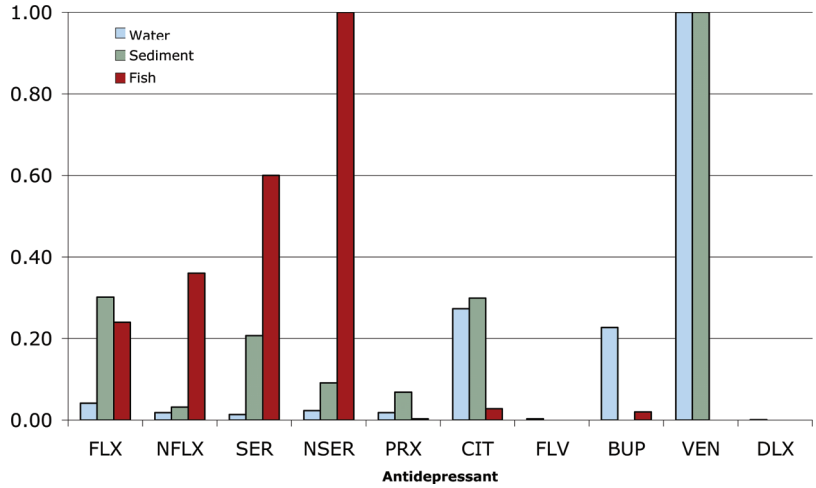

FIGURE 1. Concentrations of antidepressants in water, sediment, and fish from Boulder Creek Site 2 in 2005, normalized to the highest single antidepressant concentration in each sample type. Official USGS station ID and location are shown in Figure S2 of the Supporting Information.

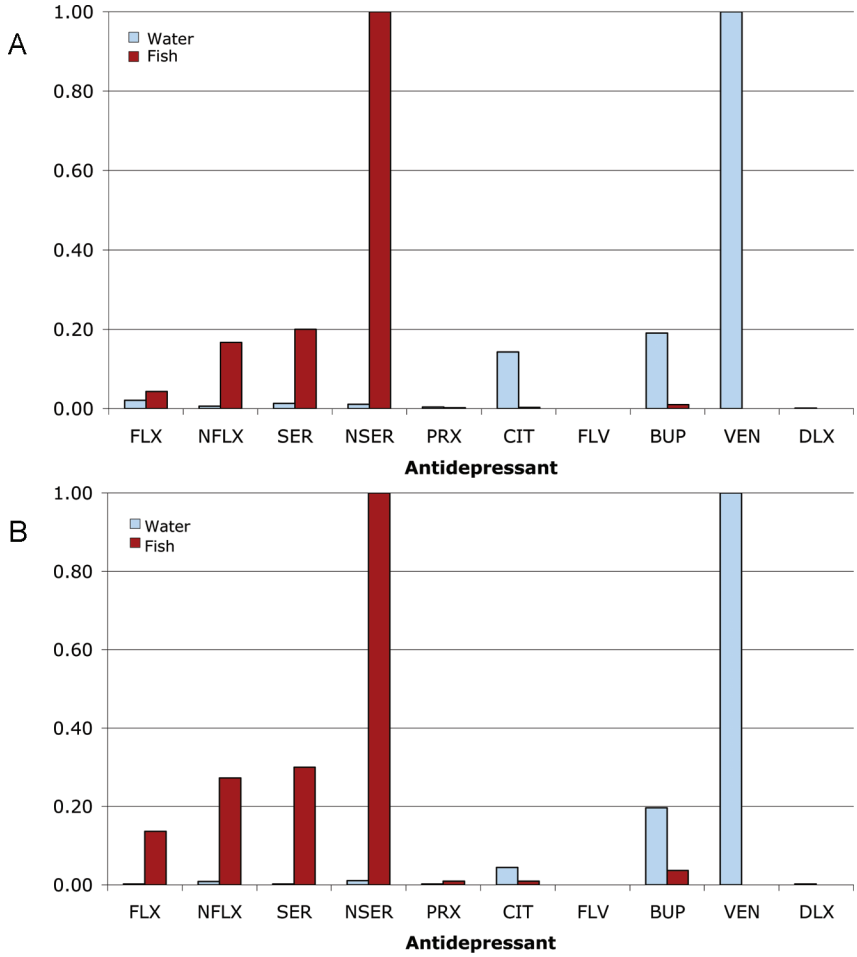

FIGURE 2. A. Concentrations of antidepressants in water and fish from Fourmile Creek Site 2 in 2005, normalized to the highest single antidepressant concentration in each sample type. B. Concentrations of antidepressants in water and fish from Fourmile Creek Site 4 in 2005, normalized to the highest single antidepressant concentration in each sample type. Official USGS station IDs and locations are shown in Figure S3 of the Supporting Information.

pared to water and sediment. These results document that selective antidepressant uptake into brain tissue occurs from environmental exposure via water and/or bed sediment. However, the study was not designed nor can the results be used to determine whether any deleterious effects to the general health and/or reproduction for the native white sucker population has occurred as a result of such exposure.

For a few white sucker from FMC 1, concentrations close to the reporting limit were noted (Table 4). Similar to streamwater, the highest brain tissue concentrations were observed at the stream sites associated with the WWTP outfall, and measurable concentrations were still found even at the sites most distal from the outfall. Differences between tissue antidepressant concentrations were substantially less for sites

FMC 2 and FMC 4 (Table 4), as was observed in streamwater concentrations (Tables 1 and 2).

In stark contrast to both streamwater and bed-sediment samples, substantially different qualitative composition patterns of antidepressants were observed in the brain-tissue samples (Figures 1, 2). Whereas VEN was the dominant antidepressant in both water and bed sediment, little or no VEN was observed in brain-tissue samples. The primary antidepressants in tissue samples were FLX and SER and their degradates NFLX and NSER. In general, the degradates were measured at higher concentrations in individual brain samples than their parent compounds (Supporting Information). Similar observations were made by other studies that examined both FLX and NFLX concentrations in fish tissue, including laboratory studies conducted with Japanese medaka (*Oryzias latipes*) (*31, 32*). In tissues from fish living in waste-dominated streams (*18*), NFLX concentrations exceeded FLX concentrations. The higher observed concentrations of degradates in this current study and previous studies suggest that metabolism of the parent compound may be occurring in fish living in waters containing antidepressants.

The lower observed abundance of VEN in fish neural tissue may be explained in part by hydrophobicity, expressed as the logarithm (log) of the octanol−water partition coefficient K_{ow}. The log K_{ow} of VEN is −0.37 (0.43) (*33*), which is lower than the reported log K_{ow} for FLX and SER, which are 1.22 ± 0.1 (16.6) and 1.37 ± 0.1 (23.4), respectively (*30*). In contrast, citalopram has a log K_{ow} of 1.39 ± 0.1 (24.5). Despite similar octanol−water partition coefficients and significantly higher aqueous concentrations (about an order of magnitude higher) as compared to FLX and SER, the concentrations of CIT observed in the white sucker brains were on average an order of magnitude lower than the levels observed for FLX and SER, suggesting that mechanisms other than hydrophobicity also contribute to the distribution of antidepressants in fish brains, and that, whereas aquatic organisms are exposed to the spectrum of antidepressants in surface water, specific antidepressants are selectively taken up by brain tissues.

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Note Added after ASAP Publication

A pertinent reference, (8), published just prior to the version of this paper published ASAP February 1, 2010, was added to Literature Cited; the corrected version published ASAP February 17, 2010.

Supporting Information Available

Standards, reagents, and specific QA/QC measures used in this study, figures containing the chemical structures of the antidepressant pharmaceuticals, maps of Boulder Creek and Fourmile Creek watersheds; tables are provided that have additional antidepressant information (including the individual antidepressant concentrations observed in the white sucker brains collected from Boulder and Fourmile Creeks). This material is available free of charge via the Internet at http://pubs.acs.org.

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