Supporting Information

Occurrence and Spatiotemporal Dynamics of Pharmaceuticals in a Temperate-region Wastewater Effluent-dominated Stream: Variable Inputs and Differential Attenuation Yield Evolving Complex Exposure Mixtures

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CONTAINS:

Field site details, chemical details, 14 Supporting Tables, 20 Supporting Figures, Supporting References, 33 pages in total.

SUPPORTING METHODS.

Field site details.

Muddy Creek (Figure S.1) flows through residential area, schools, and a golf course in the path of the study reach. The creek receives agricultural drainage from upstream. Muddy Creek flows into the Iowa River downstream from study site DS2 (approximately 2 km). A U.S. Geological Survey (USGS) gaging station (05454090) is located at site DS2 that provides continuous flow monitoring (https://waterdata.usgs.gov/ia/nwis/uv/?site_no=05454090&PARAmeter_cd=00065,00060). Detailed land use information is in Table S.1. Muddy Creek has a sandy bottom and heavy tree canopy riparian zone (Figure S.3).

Table S.1: Land use information from Muddy Creek.

Station Name	Open Water	Developed	Barren Land (Rock/Sand/ Clay)	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Pasture/ Hay	Cultivated Crops	Wetlands
US1 (05454050)	0.13%	72.5%	0.07%	1.69%	0%	1.61%	2.82%	20.72%	0.44%
Effluent (05454051)	0.13%	72.5%	0.07%	1.70%	0%	1.61%	2.81%	20.69%	0.46%
DS1 (05454052)	0.13%	72.3%	0.07%	2.0%	0%	1.60%	2.86%	20.52%	0.47%
DS2 (05454090)	0.40%	60.0%	0.04%	12.4%	0.02%	2.38%	7.06%	17.45%	0.31%

Data from USGS National Landcover Database.

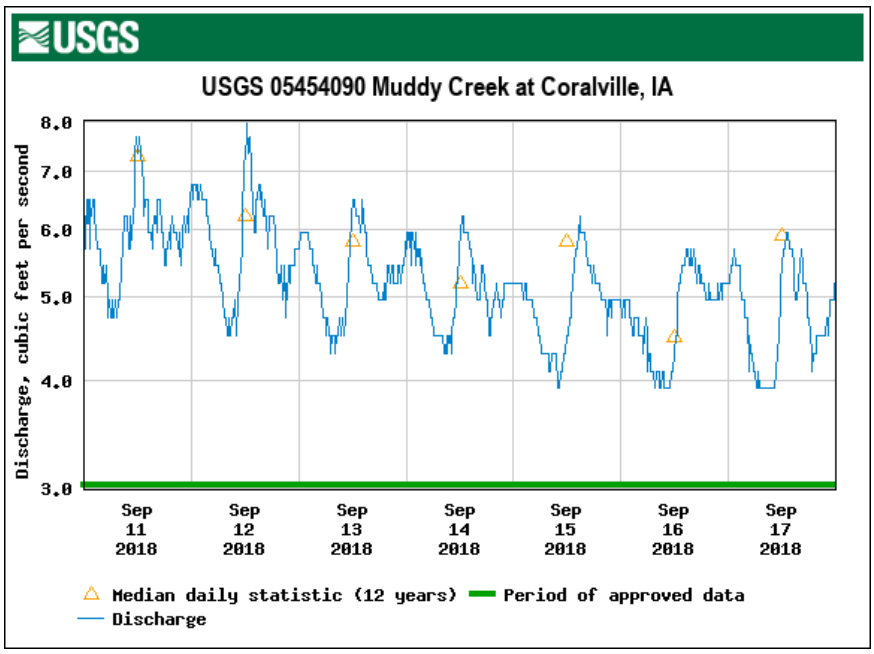

Figure S.1: Example diurnal hydrograph from DS2 during baseflow conditions to demonstrate the diurnal impact of wastewater treatment plant (WWTP) discharge to streamflow at the Muddy Creek study site.[1]

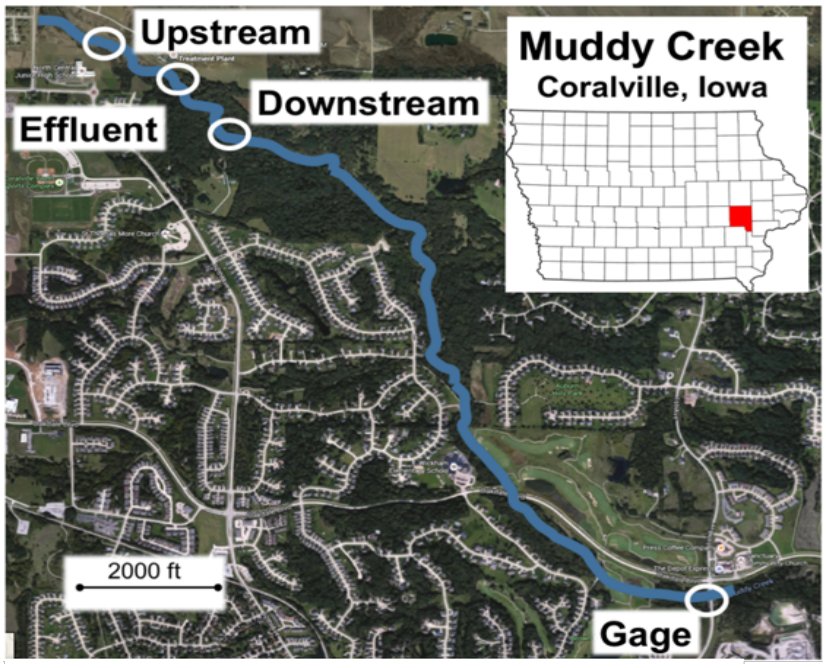

Figure S.2: Sampling map of Muddy Creek, Coralville, Iowa (Latitude 41°42'00", Longitude 91°33'46"). On this map, "Upstream" is sampling site US1, "Downstream" is DS1, and "Gage" is DS2. Background image from Google Maps.

(a) US1 (b) Effluent

(c) DS1 (d) DS2

Figure S.3: Photos of each sampling site. Photos were taken by the authors on May 17th, 2018.

Chemical details.

Monthly method. A slightly updated version of a previously published USGS method[2] was used in this study, which contains 113 chemicals (including 109 pharmaceuticals/degradates and 4 non-pharmaceutical compounds) and 41 surrogates.

Chemical standards used in this method include: 1,7-Dimethylxanthine (p-Xanthine) (CAS 611-59-6), 10-Hydroxy-amitriptyline (CAS 64520-05-4), abacavir (CAS 136470-78-5), acetaminophen (CAS 103-90-2), acyclovir (CAS 59277-89-3), albuterol (CAS 18559-94-9), alprazolam (CAS 28981-97-7), amitriptyline (CAS 50-48-6), amphetamine (CAS 300-62-9), antipyrine (CAS 60-80-0), atenolol (CAS 29122-68-7), atrazine (CAS 1912-24-9), benztropine (CAS 86-13-5), betamethasone (CAS 378-44-9), bupropion (CAS 34911-55-2), caffeine (CAS 58-08-2), carbamazepine (CAS 298-46-4), carisoprodol (CAS 78-44-4), chlorpheniramine (CAS 132-22-9), cimetidine (CAS 51481-61-9), citalopram (CAS 59729-33-8), clonidine (CAS 4205-90-7), codeine (CAS 76-57-3), cotinine (CAS 486-56-6), dehydronifedipine (CAS 67035-22-7), desmethyldiltiazem (CAS 130606-60-9), desvenlafaxine (CAS 93413-62-8), dextromethorphan (CAS 125-71-3), diazepam (valium) (CAS 439-14-5), diltiazem (CAS 42399-41-7), diphenhydramine (CAS 147-24-0), duloxetine (CAS 116539-59-4), erythromycin (CAS 114-07-8), ezetimibe (CAS 163222-33-1), fadrozole (CAS 102676-47-1), famotidine (CAS 76824-35-6), fenofibrate (CAS 49562-28-9), fexofenadine (CAS 83799-24-0), fluconazole (CAS 86386-73-4), fluoxetine (CAS 54910-89-3), fluticasone propionate (CAS 80474-14-2), fluvoxamine (CAS 54739-18-3), gabapentin (CAS 60142-96-3), glipizide (CAS 29094-61-9), glyburide (CAS 10238-21-8), guanylurea (CAS 141-83-3), hexamethylenetetramine (CAS 100-97-0), hydrocodone (CAS 125-29-1), hydrocortisone (CAS 50-23-7), hydroxyzine (CAS 68-88-2), iminostilbene (CAS 256-96-2), ketoconazole (CAS 65277-42-1), lamivudine (CAS 134678-17-4), lidocaine (CAS 137-58-6), loperamide (CAS 53179-11-6), loratadine (CAS 79794-75-5), lorazepam (CAS 846-49-1), meprobamate (CAS 57-53-4), metaxalone (CAS 1665-48-1), metformin (CAS 657-24-9), methadone (CAS 76-99-3), methocarbamol (CAS 532-03-6), methotrexate (CAS 59-05-2), methyl-1H-benzotriazole (CAS 29385-43-1), metoprolol (CAS 51384-51-1), morphine (CAS 57-27-2), nadalol (CAS 42200-33-9), nevirapine (CAS 129618-40-2), nicotine (CAS 54-11-5), nizatidine (CAS 76963-41-2), nordiazepam (CAS 1088-11-5), norethindrone (CAS 68-22-4), norfluoxetine (CAS 56161-73-0), norsertraline (CAS 87857-41-8), norverapamil (CAS 67018-85-3), omeprazole + esomprazole (CAS 73590-58-6; 161796-78-7), oseltamivir (CAS 196618-13-0), oxazepam (CAS 604-75-1), oxycodone (CAS 76-42-6), paroxetine (CAS 61869-08-7), penciclovir (CAS 39809-25-1), pentoxifylline (CAS 6493-05-6), phenazopyridine (CAS 94-78-0), phendimetrazine (CAS 634-03-7), phenytoin (CAS 57-41-0), piperonyl butoxide (CAS 51-03-6), prednisolone (CAS 50-24-8), prednisone (CAS 53-03-2), promethazine (CAS 60-87-7), propoxyphene (CAS 469-62-5), propranolol (CAS 525-66-6), pseudoephedrine + ephedrine (CAS 90-82-4; 299-42-3109), quinine (CAS 130-95-0), ractopamine (CAS 97825-25-7), raloxifene (CAS 84449-90-1), ranitidine (CAS 66357-35-5), sertraline (CAS 79617-96-2), sitagliptin (CAS 486460-32-6), sulfadimethoxine (CAS 122-11-2), sulfamethizole (CAS 144-82-1), sulfamethoxazole (CAS 723-46-6), tamoxifen (CAS 10540-29-1), temazepam (CAS 846-50-4), theophylline (CAS 58-55-9), thiabendazole (CAS 148-79-8), tiotropium (CAS 186691-13-4), tramadol (CAS 27203-92-5), triamterene (CAS 396-01-0), trimethoprim (CAS 738-70-5), valacyclovir (CAS 124832-26-4), venlafaxine (CAS 93413-69-5), verapamil (CAS 52-53-9) and warfarin (CAS 81-81-2).

Note that four non-pharmaceutical compounds (atrazine, methyl-1H-benzotriazole, thiabendazole, and piperonyl butoxide) are included in this method.

Surrogates were used in this method include: acetaminophen-d3 (CAS 60902-28-5), albuterol-d9 (CAS 1173021-73-2), amitriptyline-d3 (CAS 342611-00-1), amphetamine-d6 (CAS 73758-26-6), caffeine (trimethyl-13C3) (CAS 78072-66-9), carisoprodol-d7 (CAS 1218911-16-0), codeine-d6 (CAS 1007844-34-9), cotinine-d3 (CAS 110952-70-0), diazepam-d5 (CAS 65854-76-4), diltiazem-d3 (CAS 1217623-80-7), diphenhydramine-d3 (CAS 170082-18-5), erythromycin-13C-d3 (CAS 959119-26-7), ezetimibe-d4 (CAS 1093659-90-5), fenofibrate-d6 (CAS 1092484-56-4), fexofenadine-d10 (CAS 1215821-44-5), fluoxetine-d6 (CAS 56296-78-7), fluvoxamine-d4 (CAS 1432075-74-5), hydrocodone-d3 (CAS 136765-36-1), hydrocortisone-13C3 (CAS -), ketoconazole-d4 (CAS 1398065-75-2), loperamide-d6 (CAS 1189469-46-2), loratadine-d4 (CAS 381727-27-1), lorazepam-d4 (CAS 84344-15-0), metformin-d6 (CAS 1185166-01-1), methadone-d9 (CAS 1435933-74-6), N-desmethyldiltiazem-d4 (CAS 1217650-51-5), norfluoxetine-d6 (CAS -), oxazepam-d5 (CAS 65854-78-6), oxycodone-d3 (CAS 160227-46-3), promethazine-d6 (CAS 1189947-02-1), propoxyphene-d11 (CAS -), pseudoephedrine-d3 (CAS 284665-25-4), raloxifene-d10 (CAS 82640-04-8), ranitidine-d6 (CAS 1185238-09-8), sulfamethoxazole-(phenyl-13C6) (CAS 1196157-90-0), tamoxifen-d5 (CAS 157698-32-3), temazepam-d5 (CAS 136765-51-0), thiabendazole-d4 (CAS 1190007-20-5), tiotropium-d3 (CAS 1127226-56-5), trimethoprim-d9 (CAS 1189460-62-5) and verapamil-d6 (CAS 1185032-80-7).

Biweekly method. The biweekly method includes 16 chemicals (14 pharmaceuticals/degradates and 2 non-pharmaceutical compounds), 8 surrogates and 1 internal standard. In preliminary research, we collected four water samples on August 1, 2016, and measured for 113 different chemicals (representing 109 pharmaceutical compounds) using an established USGS analytical method for pharmaceuticals.[2] These preliminary results demonstrated that the biweekly pharmaceuticals accounted for >85% of the total pharmaceutical chemical concentration. These biweekly pharmaceuticals/degradates were: metformin, atenolol, bupropion, citalopram, carbamazepine, fexofenadine, venlafaxine, desvenlafaxine, lidocaine, tramadol, methocarbamol, sulfamethoxazole, fluconazole and guanylurea, the primary degradate of metformin.

Biweekly Method Chemicals: Venlafaxine hydrochloride (CAS 99300-78-4), O-desvenlafaine hydrochloride (CAS 300827-87-6), metformin hydrochloride (CAS 1115-70-4), guanylurea (CAS 141-83-3), carbamazepine (CAS 298-46-4), tramadol hydrochloride (CAS 36282-47-0), sulfamethoxazole (CAS 723-46-6), fexofenadine hydrochloride (CAS 153439-40-8), methocarbamol (CAS 532-03-6), lidocaine (CAS 137-58-6), fluconazole (CAS 86386-73-4), bupropion (CAS 31677-93-7), citalopram hydrobromide (CAS 59729-32-7), atenolol (CAS 29122-68-7), caffeine (CAS 58-08-2), 1H-benzotriazole (CAS 95-14-7) and 5-methyl-1H-benzotriazole (CAS 136-85-6) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received.

Metformin-d6 (CAS 1185166-01-1), venlafaxine-d6 hydrochloride (CAS 1062606-12-5), atenolol-d7 (CAS 1202864-50-3), bupropion-d9 (CAS 1189725-26-5), carbamazepine-d10 (CAS 132183-78-9), citalopram-d6 (CAS 1190003-26-9), fluconazole-$^{13}C_3$ and d_4-1H-benzotriazole (CAS 1185072-03-0) from Sigma Aldrich were used as surrogates. Caffeine-$^{13}C_3$ from Sigma Aldrich was used as internal standard. The solvent, methanol, acetonitrile, water, and formic acid were all optima LC-MS grade (Fisher, Fair Lawn, NJ).

The individual standard stock solutions, isotopically labelled surrogate mix solution (contained eight isotopically labelled compounds: metformin-d6, venlafaxine-d6 hydrochloride, atenolol-d7,

bupropion-d9, carbamazepine-d10, citalopram-d6, fluconazole-$^{13}C_3$ and d_4-1H-benzotriazole) that represent a range of chemical properties, as well as internal standard solution (caffeine-$^{13}C_3$), were prepared in methanol and stored at -20°C.

Sample collection and processing.

Biweekly method. Water quality parameter measurement details: During Year 2, dissolved oxygen, pH, water temperature, and conductivity measurements were collected using a HACH HQ40D portable multi meter with an Intellical™ LDO101 electrode for dissolved oxygen, an Intellical™ PHC101 electrode for pH and water temperature, and an Intellical™ CDC401 electrode for conductivity.

SPE method details: A 1-L water sample was measured volumetrically and spiked with 100 µL surrogate mix solution (50 ng of each individual surrogate). The pH of the water samples was adjusted to between 6 and 7 using hydrochloride acid for proper retention during solid phase extraction (SPE). SPE was performed in parallel using both Strata X-CW cartridges (500mg, 6mL, Phenomenex, Torrance, CA, USA) and Oasis HLB cartridges (500mg, 6mL, Waters Corporation, Milford, MA, USA) on a Visiprep SPE manifold with vacuum pump. The cartridges were conditioned with 6 mL methanol followed by 6mL pH 6.5 deionized water. Samples were loaded onto the cartridges at a flow rate of 3-5 mL/min. Each sample bottle was then rinsed with 50mL pH 6.5 deionized water to collect remaining residue. The cartridges were dried under vacuum for 30 min. Cartridges were eluted with 8 mL of a methanol/acetonitrile mixture (20/80, v/v; containing 2% formic acid) by gravity. After elution, the solvent was evaporated to dryness under a gentle stream of nitrogen. The dry residue was reconstituted in 1mL acetonitrile/water (50/50, v/v) and filtered with 0.2µm pore diameter polytetrafluorethylene (PTFE) syringe filter (Advanced Microdevices). Eight hundred µL of the final solution was transferred into a glass screw cap high-performance liquid chromatography (HPLC) vial and 10µL caffeine-$^{13}C_3$ (10 mg/L) was added as an internal standard. Samples were stored at -20°C until analysis, typically within 14 days.

Analytical methods.

Biweekly analytical method chromatography details: An Agilent Eclipse Plus C18 column (4.6×150 mm; 5 µm) coupled with a guard column of the same material, at a flow rate of 0.4 mL/min was used for separation at 50 °C. Eluent A was HPLC grade water with 0.1% formic acid and eluent B was HPLC grade acetonitrile with 0.1% formic acid. The HPLC flow gradient elution used was: 0–3 min 10% B, increased to 45% B within 0.1 min, held to 5 min, then increased to 85% B, held for 1 min, then back to original condition within 0.1 min. The total method was 18 minutes. Samples (composition of 50% acetonitrile and 50% H_2O) were kept in the autosampler tray at 10 °C and the injection volume was 20 µL.

Table S.2: Operating parameters of the triple quadrupole mass spectrometer.

Parameter	Set point
Gas temperature	300 °C
Gas flow	8 L/min
Nebulizer	35 psi
Sheath gas heater	250 °C
Sheath gas flow	9 L/min
Capillary voltage	4000 V

Table S.3: Compound-specific operating parameters of the target dynamic multiple reaction monitoring (MRM) method.

Compound	*Precursor ion (m/z)*	*Quantitative ion (m/z)*	*Qualitative ion (m/z)*	*Retention time (min)*	*Delta retention time (min)*	*Fragmentor (V)*	*Collision energy (V)*	*Cell accelerator voltage (V)*
1H-benzotriazole	120	65	92	10.4	1	75	24/16	7
1H-benzotriazole-d4	124	69	96	10.4	1	120	20/16	7
5-methyl-benzotriazole	134	77	79	11.4	1	80	28/20	7
Atenolol	267	145	74	7.0	2	123	24/20	7
Atenolol-d7	274	145	79	6.9	2	117	24/20	7
Bupropion	240	184	131	10.4	1	86	8/24	7
Bupropion-d9	249	185	131	10.4	1	92	8/28	7
Caffeine	195	138	89	10.0	1	88	6/8	7
Caffeine-c3	198	140	112	10.0	1	123	16/24	7
Citalopram	325	109	262	10.9	1	129	24/16	7
Citalopram-d6	331	109	262	10.9	1	137	24/16	7
Carbamazepine	237	194	179	12.4	1	149	16/36	7
Carbamazepine-c6	243	200	185	12.4	1	132	16/36	7
Desvenlafaxine	264	58	246	9.8	1	105	20/8	7
Fexofenadine	502	466	171	11.3	1	146	28/40	7
Fluconazole	307	238	220	10.5	1	109	12/16	7
Fluconazole-c3	310	241	223	10.5	1	103	12/16	7
Guanylurea	103	60	43	3.6	1	65	8/25	7
Lidocaine	235	86	58	9.9	1	100	12/36	7
methocarbamol	242	118	57	10.8	1	72	4/20	7
Metformin	130	60	71	3.5	1	65	12/24	7
Metformin-d6	136	60	77	3.5	1	63	12/25	7
Sulfamethoxazole	254	92	156	11.6	1	94	24/12	7
Tramadol	264	58	246	10.0	1	94	12/4	7
Venlafaxine	278	58	260	10.3	1	95	16/8	7
Venlafaxine-d6	284	64	266	10.3	1	100	12/8	7

Table S.4: Chemical properties of biweekly pharmaceuticals/degradates investigated in this study.

Chemical	**Category**	**pKa[§]**	**acidic/basic**	**LogKow[§]**	**Biodegradation half-lives (d)[3]**	**Photolysis half-lives (hr)**
Venlafaxine	antidepressant	14.4;8.9	basic	3.28	3.36	51[4]; no direct photolysis[5]; 342±18[6]
Desvenlafaxine	antidepressant	8.9;10.1	basic	0.74	4.66	18[4]
Metformin	antidiabetic	12.3	basic	-2.64	3.98	Very low degradation[7]
Guanylurea[†]	degradate	8.0; 13.5	basic	-2.51	4.10	negligible[8]
Fluconazole	anti-fungal	12.7	basic	0.25	4.46	1.9[9]
Bupropion	antidepressant	7.2	basic	3.85	3.39	Very low degradation[5]
Citalopram	antidepressant	9.5	basic	3.74	3.55	336-1032[10]
Atenolol	beta-blocker	9.6	basic	0.16	3.34	77-730[11]
Carbamazepine	anticonvulsant	15.9	basic	2.45	5.03	84-2100[11]
Tramadol*	pain-relief	9.2, 13.8	basic	3.01	3.35	73[4]
Sulfamethoxazole*	antibiotic	1.9(primary), 6.2(secondary), 0.3(tertiary)	acidic	0.89	3.34	57.6[12]; 134±13[6]
Fexofenadine*	antihistamine	8.8(tertiary amine), 4.3(carboxyl)	amphoteric	2.81	26.2	135±29[6]
Methocarbamol*	muscle relaxant	13.6	basic	0.61	4.48	Not available
Lidocaine*	local anesthetic	7.7;13.8	basic	2.26	3.36	31[4]

(* indicates no associated surrogates; † indicates pharmaceutical degradate; § citation for pKa, logKow: Kim et al.[13])

Table S.5: Method reporting limits (MRLs) of individual compounds analyzed in the biweekly method at University of Iowa. These values are for solid phase extraction (SPE) samples that are enriched by 1000 times. Except for metformin (US1 blank: 3 ng/L; DS1 blank: 54 ng/L; DS2 blank: 66 ng/L), venlafaxine (DS2 blank: 12 ng/L), and fexofenadine (US1 blank: 1 ng/L; Effluent blank: 15 ng/L), no other pharmaceuticals were detected in the field blanks.

Chemical	**MRL (ng/L)**	**Chemical**	**MRL (ng/L)**
Metformin	36	Carbamazepine	37
Guanylurea	26	Fluconazole	96
Desvenlafaxine	36	Methocarbamol	20
Lidocaine	11	Fexofenadine	252
Tramadol	3	Bupropion	41
Venlafaxine	56	Citalopram	72
Sulfamethoxazole	43	1H-benzotriazole	33
5-methyl-1H-benzotriazole	11		

Table S.6: Chemical properties/activity of pharmaceuticals and associated degradate in the effluent and at downstream sites analyzed by the U.S. Geological Survey method.[3]

Parent compound	Active/inactive	degradate	Active/inactive[3]
caffeine	active	1,7-dimethylxanthine	active
Amitriptyline	active	10-hydroxy-amitriptyline	not available
Diazepam	active	Nordiazepam	active
Diltiazem	active	Desmethyldiltiazem	unspecified
Fluoxetine	active	Norfluoxetine	active
Metformin	active	Guanylurea	inactive
Nicotine	active	Cotinine	active
Sertraline	active	Norsertraline	unspecified
Verapamil	active	Norverapamil	active

Table S.7: Laboratory SPE recovery for biweekly method (University of Iowa) including 14 pharmaceuticals/degradates and 2 non-pharmaceutical compounds.

Chemical	Recovery %	Chemical	Recovery %
Metformin	90	Carbamazepine	102
Guanylurea	86	Fluconazole	99
Desvenlafaxine	86	Methocarbamol	94
Lidocaine	97	Fexofenadine	96
Tramadol	89	Bupropion	103
Venlafaxine	87	Citalopram	102
Sulfamethoxazole	88	1H-benzotriazole	95
5-methyl-1H-benzotriazole	94		

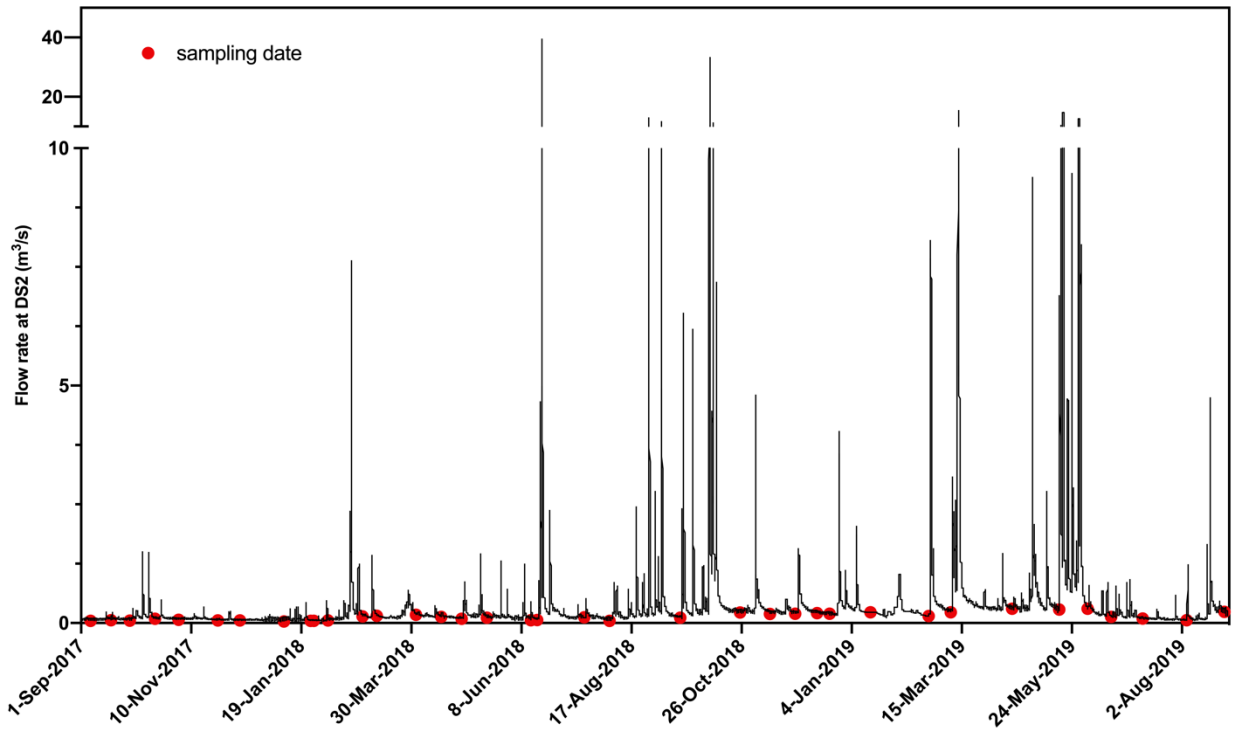

Figure S.4: Two-year diurnal hydrograph at DS2.[1] Red dots represent dates when sampling events occurred (n=37). All samples were collected during baseflow conditions.

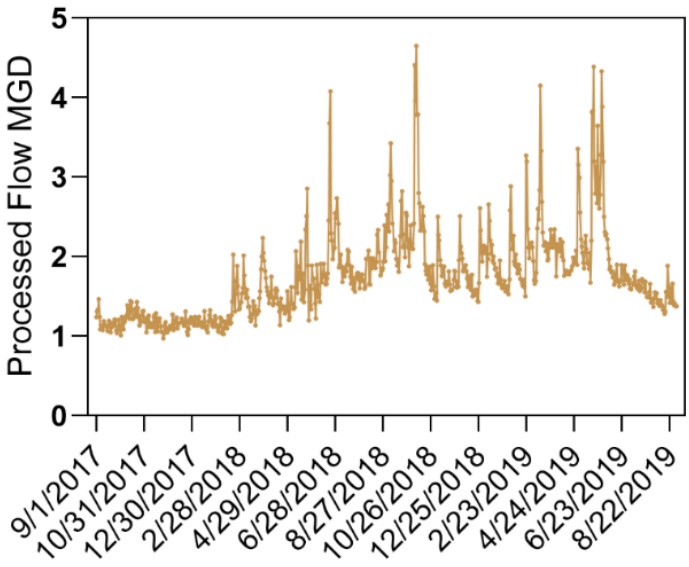

Figure S.5: Wastewater treatment plant (WWTP) daily processed flow discharges during September 2017-August 2019. The discharge remained low and stable from September 2017 to February 2018, then increased from March 2018. The discharge during Year 2 had more fluctuations and had more high discharges compared to Year 1. Data were provided by the North Liberty WWTP.

SUPPORTING RESULTS

Table S.8: Pharmaceutical data comparison between samples processed by monthly method and biweekly method respectively via matched-paired t-tests.

	Effluent		DS1		DS2	
Pharmaceutical / degradate compound	Matched-pair tests $p < 0.05$	Variability %	Matched-pair tests $p < 0.05$	Variability %	Matched-pair tests $p < 0.05$	Variability %
Total biweekly pharmaceuticals/degradates	No	12	No	14	Yes	27
Metformin	Yes	-86	Yes	-95	Yes	-59
Guanylurea	Yes	69	Yes	83	Yes	70
Venlafaxine	Yes	-67	Yes	-74	Yes	-61
Desvenlafaxine	Yes	45	Yes	43	Yes	33
Bupropion	Yes	-90	Yes	-86	Yes	-102
Citalopram	Yes	-93	Yes	-111	Yes	-190
Carbamazepine	Yes	-57	Yes	-57	Yes	-62
Fexofenadine	No	17	No	24	No	3
Fluconazole	Yes	-51	Yes	-50	Yes	-49
Lidocaine	Yes	30	Yes	32	Yes	27
Atenolol	No	19	No	23	No	27
Methocarbamol	Yes	-122	Yes	-49	Yes	-46
Sulfamethoxazole	No	16	Yes	38	Yes	63
Tramadol	Yes	64	Yes	58	Yes	45

Table S.9: Statistical analysis (t-tests of log-transformed concentrations) of monthly measured chemicals by U.S. Geological Survey measured in Year 1between Effluent and DS2 sites. "NA" indicates that the number of detections in the water was insufficient for a particular compound to conduct statistical analysis.

Chemical	**p-value**	**Chemical**	**p-value**
Abacavir	NA	Loratadine	0.023
Acetaminophen	NA	Meprobamate	0.047
Acyclovir	0.128	Metaxalone	0.495
Albuterol	0.0001	Metformin+Guanylurea	0.004
Alprazolam	NA	Methadone	0.054
Amitriptyline+10-Hydroxy-amitriptyline	0.009	Methocarbamol	0.007
Amphetamine	NA	Methyl-1H-benzotriazole	0.003
Atenolol	0.084	Metoprolol	0.0003
Bupropion	<0.0001	Morphine	NA
Caffeine+ 1,7-Dimethylxanthine	0.768	Nicotine+Cotinine	0.154
Carbamazepine	0.002	Omeprazole + Esomeprazole	0.646
Carisoprodol	0.060	Oseltamivir	0.717
Chlorpheniramine	0.160	Oxycodone	0.047
Cimetidine	0.824	Phenytoin	NA
Citalopram	<0.0001	Piperonyl butoxide	NA
Codeine	0.0009	Propranolol	<0.0001
Desvenlafaxine	0.0001	Pseudoephedrine + Ephedrine	0.388
Dextromethorphan	0.0004	Quinine	<0.0001
Diazepam+Nordiazepam	0.534	Ranitidine	0.855
Diltiazem+Desmethyldiltiazem	0.062	Sertraline+Norsertraline	0.352
Diphenhydramine	<0.0001	Sitagliptin	<0.0001
Duloxetine	NA	Sulfamethoxazole	0.176
Erythromycin	NA	Temazepam	0.012
Famotidine	0.639	Theophylline	NA
Fexofenadine	0.002	Tramadol	0.0003
Fluconazole	0.006	Triamterene	<0.0001
Fluoxetine+Norfluoxetine	NA	Trimethoprim	0.568
Fluticasone propionate	NA	Venlafaxine	<0.0001
Gabapentin	0.065	Verapamil+Norverapamil	NA
Hydrocodone	0.059	Warfarin	0.783
Hydroxyzine	NA	Atrazine	0.052
Lamivudine	NA	Thiabendazole	0.0001
Lidocaine	0.0007		

Table S.10: Streamflow during Year 1 monthly samples (unit: m^3/s).[14]

Date	US1	Effluent	DS1	DS2
9/7/2017	0.006	0.078	0.083	0.048
10/2/2017	0.004	0.061	0.065	0.054
11/2/2017	0.002	0.048	0.050	0.072
12/11/2017	0.010	0.085	0.094	0.051
1/8/2018	0.011*	0.092	0.101	0.033
2/5/2018	0.005	0.081	0.086	0.060
3/8/2018	0.053	0.129	0.181	0.146
4/2/2018	0.083	0.100	0.184	0.174
5/1/2018	0.020	0.083	0.102	0.094
6/14/2018	0.007	0.118	0.125	0.057
7/12/2018	0.009	0.107	0.116	0.068
8/1/2018	0.008	0.068	0.076	0.051

*indicates estimated value by U.S. Geological Survey; the streamflow measurement for US1 for January was inadvertently deleted prior to formal documentation and was estimated by using available streamflow measurements from the December 2017 (US1 and DS1) to January 2018 (DS1) and by comparing photos from December 2017 and January 2018 at US1.

Table S.11: The percentage of U.S. Geological Survey (USGS) measured most abundant pharmaceuticals/degradates (biweekly pharmaceuticals/degradates) to USGS measured total 75 chemicals in terms of concentrations. Data from monthly samples analyzed by USGS.[14] Except for the first September event, the biweekly pharmaceutical compounds accounted for 67%-86% of the total pharmaceutical concentrations, indicating that biweekly pharmaceuticals/degradates were the dominant chemicals among all chemicals detected.[14]

Sampling month	Effluent	DS1	DS2
Sep 2017*	47%	48%	83%
Sep 2017	78%	78%	84%
Oct 2017	81%	81%	86%
Nov 2017	86%	85%	86%
Dec 2017	82%	82%	83%
Jan 2018	80%	79%	76%
Feb 2018	81%	81%	78%
Mar 2018	74%	76%	74%
Apr 2018	73%	73%	71%
May 2018	76%	76%	82%
June 2018	83%	82%	77%
July 2018	77%	77%	77%
Aug 2018	67%	67%	73%

*The quality of this set of samples remained questionable due to the shipment delivery delay by the shipping company; thus, additional samples were collected in September for verification purpose.

Table S.12: The pharmaceutical concentrations (in ng/L) and frequency of detection for biweekly pharmaceuticals in Muddy Creek from September 2017 to August 2019. (biweekly data; ND: not detected; s.d.=standard deviation)

	Effluent					DS1					DS2				
Chemical	Max	Min	Mean±s.d.	median	Frequency	Max	Min	Mean±s.d.	median	Frequency	Max	Min	Mean±s.d.	median	Frequency
Metformin	30700	338	2170±4880	1000	100%	28800	223	1790±4580	793	100%	3750	47	830±735	626	100%
Guanylurea	4270	34	773±1020	326	100%	6390	25	804±1410	277	100%	3660	26	612±866	220	100%
Atenolol	1310	ND	149±236	70	97%	430	18	93±83	73	100%	965	17	88±157	46	100%
Tramadol	546	10	117±126	91	100%	567	6	99±119	48	100%	615	2	95±141	44	100%
Venlafaxine	3380	130	1280±723	1130	100%	2610	96	989±626	884	100%	1540	42	536±388	375	100%
Desvenlafaxine	1300	6	264±283	113	100%	998	7	205±236	131	100%	1080	3	168±238	99	100%
Bupropion	1340	29	498±301	485	100%	1040	26	374±237	350	100%	698	24	224±167	169	100%
Carbamazepine	998	31	420±221	361	100%	918	23	325±204	267	100%	710	26	226±174	172	100%
Citalopram	1340	23	521±331	440	100%	984	6	344±229	307	100%	212	2	49±39	34	100%
Fexofenadine	3710	9	1060±960	904	100%	2830	ND	845±819	566	97%	1760	ND	672±547	635	97%
Fluconazole	485	12	224±113	233	100%	376	11	171±97	161	100%	286	9	116±75	106	100%
Lidocaine	379	6	75±85	40	100%	290	ND	59±72	32	97%	325	1	47±64	28	100%
Methocarbamol	735	36	258±234	107	100%	530	16	160±152	88	100%	356	ND	93±86	68	92%
Sulfamethoxazole	2090	29	480±512	308	100%	1570	19	313±362	183	100%	681	ND	167±172	106	97%
Summary	38400	1750	8280±5540	7160	100%	36200	1430	6590±5400	5820	100%	7710	1320	3890±1730	3690	100%

Table S.13: Water temperature (degrees C) at four sampling sites during the two-year sampling periods. Year 1 data were collected by the U.S. Geological Survey[14] and Year 2 data were collected by University of Iowa. The water temperature data are missing during September 2018-December 2018.

Date	US1	Effluent	DS1	DS2
9/7/2017	14.7	21.2	20.4	14.6
9/20/2017	18.7	21.9	21.5	19.6
10/2/2017	16.0	22.0	21.5	16.5
11/2/2017	8.4	20.0	17.4	9.7
12/11/2017	3.2	17.6	15.1	4.3
1/8/2018	3.2	15.8	14.3	0.0
2/5/2018	1.0	14.2	12.2	0.0
3/8/2018	0.4	12.2	7.3	1.4
4/2/2018	1.2	12.3	7.1	2.2
5/1/2018	13.9	14.9	14.7	13.8
6/14/2018	18.4	18.6	18.5	18.2
7/12/2018	21.4	20.3	20.6	22.0
8/1/2018	18.3	20.2	20.1	19.8
1/16/2019	2.5	14.6	9.3	4.0
2/20/2019	1.0	12.7	8.4	1.4
3/8/2019	1.7	12.5	9.5	4.4
4/16/2019	9.3	13.7	11.8	10.1
5/16/2019	14.9	16.0	15.8	15.0
6/3/2019	16.4	16.7	16.1	18.8
7/8/2019	23.9	20.7	21.5	22.0
8/5/2019	21.5	20.8	20.8	21.8
8/29/2019	21.8	20.2	20.7	21.3

Table S.14: Water quality parameters measured by University of Iowa during Year 2. The Year 1 data measured by U.S. Geological Survey (USGS) are published through the USGS data release.[14] Bulk water quality data are missing during September 2018-December 2018.

Date	pH				Conductivity (µS/cm)				Dissolved oxygen (mg/L)			
	US1	Effluent	DS1	DS2	US1	Effluent	DS1	DS2	US1	Effluent	DS1	DS2
2019/1/16	7.6	7.6	7.7	8.2	228	842	1556	1179	11.6	9.5	10.5	12.6
2019/2/20	8.0	7.9	7.9	8.3	1416	1610	1754	1487	11.7	9.3	10.2	12.6
2019/3/8	7.6	7.8	7.9	8.1	857	1933	1543	1332	11.9	9.1	10.7	12.9
2019/4/16	7.8	7.6	7.5	8.1	821	1579	1112	950	9.9	9.7	9.9	10.9
2019/5/16	7.8	7.6	7.7	8.1	729	1259	1259	1062	8.3	8.9	8.8	9.7
2019/6/3	7.5	7.6	7.6	7.6	657	1342	968	804	8.2	9.0	8.7	9.1
2019/7/8	7.8	7.5	7.7	8.1	761	1840	1658	1090	9.5	8.1	8.5	8.2
2019/8/5	7.8	7.9	7.9	8.1	774	1804	1643	1192	6.9	8.6	8.3	8.1
2019/8/29	7.6	7.8	7.8	8.1	718	1782	1459	904	6.7	8.7	8.2	8.1

Supporting Figures

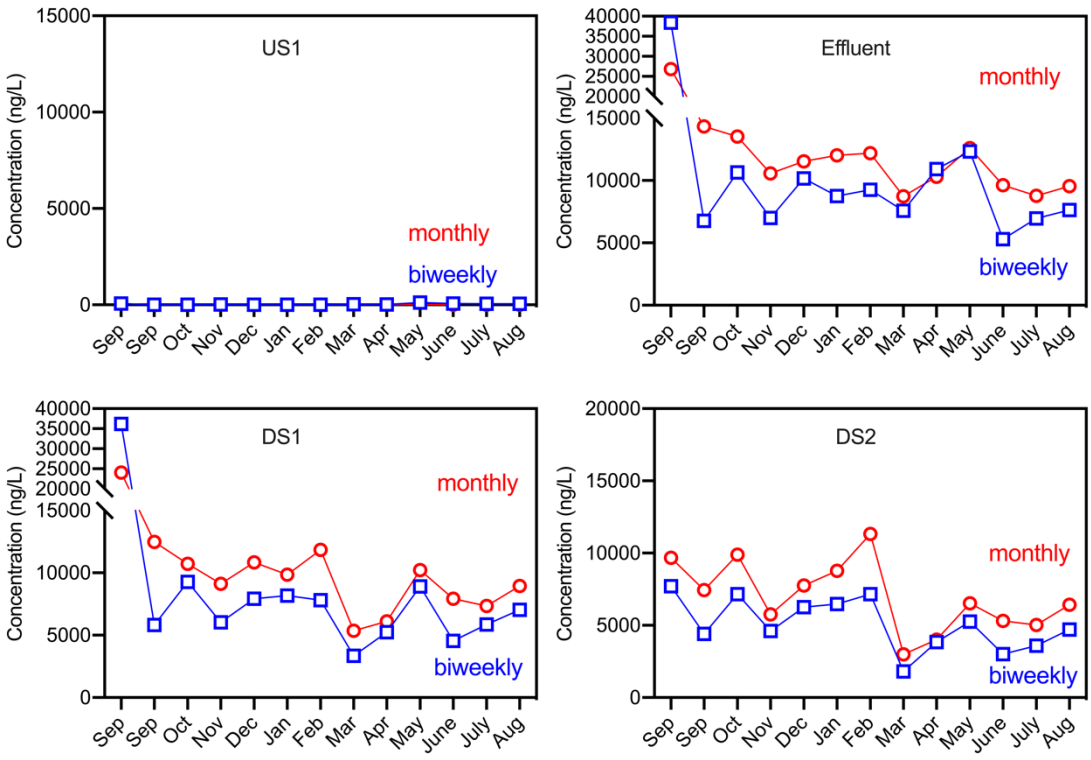

Figure S.6: Matched-paired comparison between biweekly data (blue squares) and monthly data (red circles).[14] (Matched-paired t test, $p<0.05$ for four sites. The percentage of mean differences: US1 = 25%; Effluent = 12%; DS1 = 14%; DS2 = 27%.

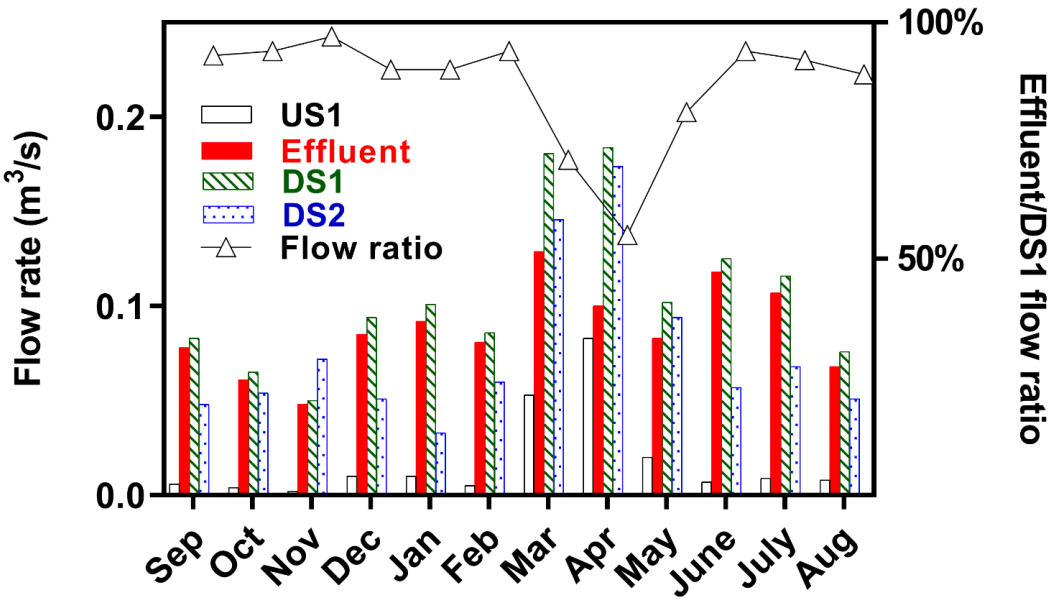

Figure S.7: Streamflow rate variation during Year 1 (September 2017 – August 2018) in Muddy Creek.[14] US1 and DS1 flow were measured via a flow tracker by U.S. Geological Survey.[15] Effluent flow at the specific time of sampling was measured indirectly by subtracting the streamflow measured above from that measured below the WWTP effluent. Streamflow at DS2, located 5.1 km downstream from the effluent outfall, was continuously monitored by the USGS gaging station (05454090).

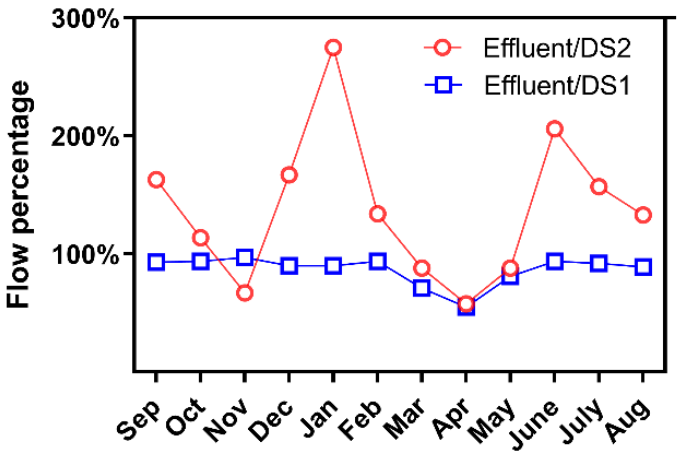

Figure S.8: Wastewater effluent as a fraction of streamflow rate at the DS1 and DS2 compared.[14] The ratios were nearly 100% at DS1, indicating Muddy Creek is a wastewater effluent-dominated stream. The ratios at DS2 above 100% indicated that Muddy Creek is a losing reach and surface water is recharged into shallow groundwater.

Upstream Site (US1) Concentration Data Year 1 (0.1 km from Effluent)

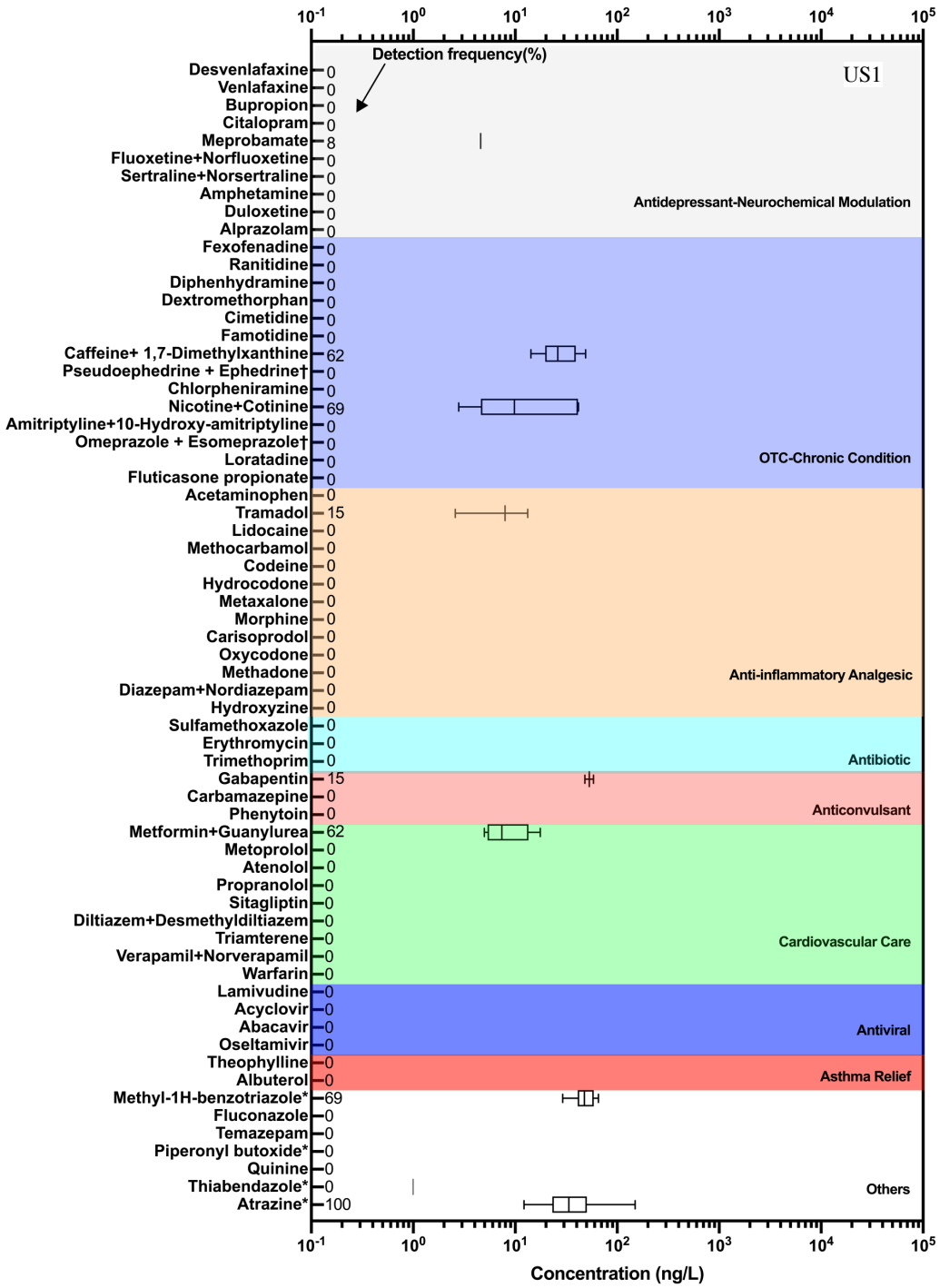

Figure S.9: Box-plot distributions of U.S. Geological Survey measured concentrations for the 74 chemicals (including 70 pharmaceuticals/degradates and 4 non-pharmaceutical compounds) detected in all water samples from US1 (USGS site 05454050; 0.1 km upstream from Effluent) during Year 1 of the study (September 2017–August 2018).[14] Results are sorted by pharmaceutical class and decreasing median concentration from top to bottom within a given class based on data in Effluent samples. *indicates non-pharmaceutical compound, †indicates isomers, "OTC"= "over-the-counter". The box-and-whiskers represent the median, interquartile range, and maximum/minimum values. Nine pharmaceutical degradates were also detected and grouped with their respective parent compounds. The number of pharmaceuticals/degradates detected at US1 was 7 (10% of the total 70 pharmaceuticals/degradates detected among samples from all sites).

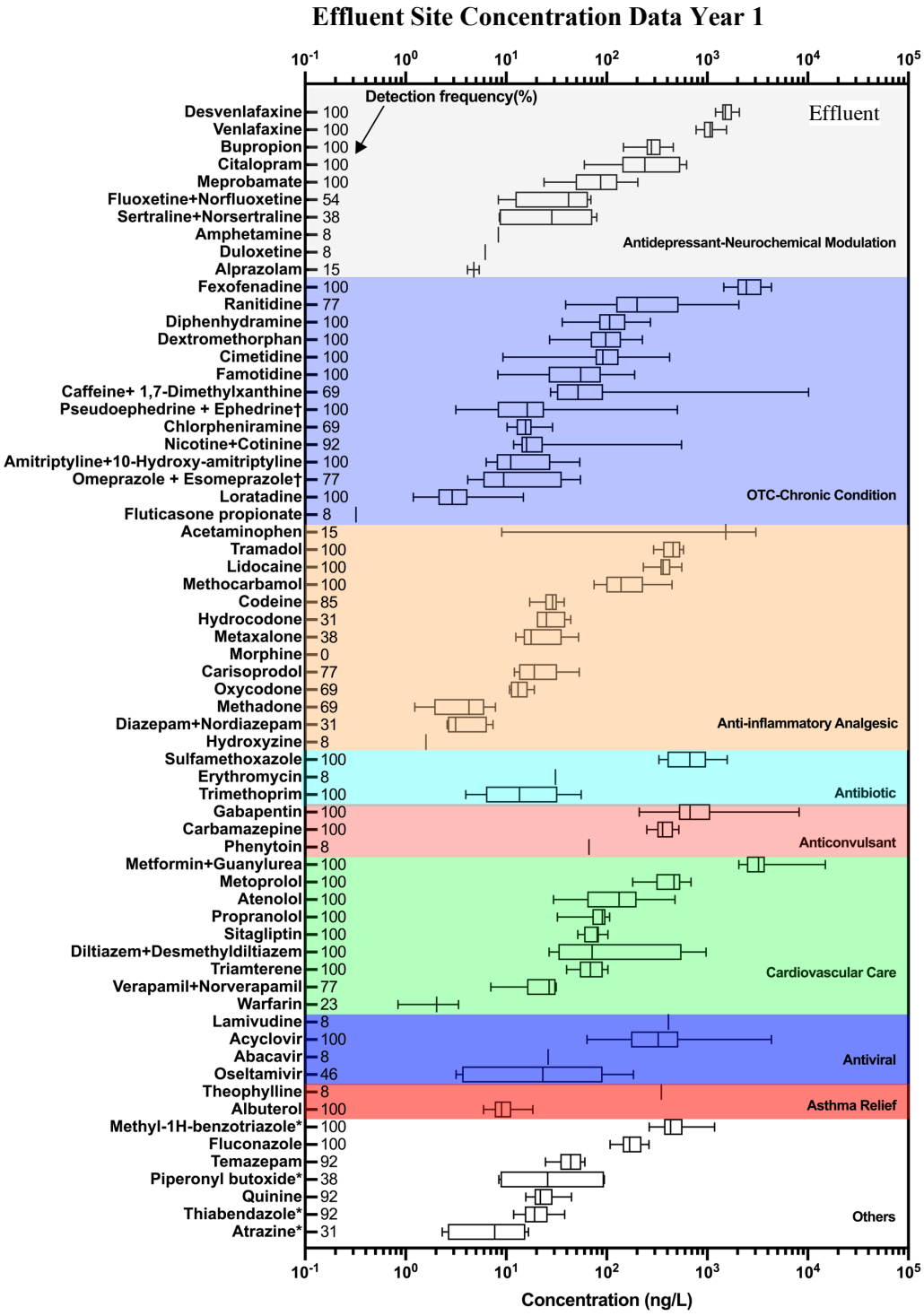

Figure S.10: Box-plot distributions of U.S. Geological Survey measured concentrations for the 74 chemicals (including 70 pharmaceuticals/degradates and 4 non-pharmaceutical compounds) detected in all water samples from Effluent (USGS site 05454051) during Year 1 of the study (September 2017–August 2018).[14] Results are sorted by pharmaceutical class and decreasing median concentration from top to bottom within a given class based on data in Effluent samples. *indicates non-pharmaceutical compound, †indicates isomers, "OTC"= "over-the-counter". The box-and-whiskers represent the median, interquartile range, and maximum/minimum values. Nine pharmaceutical degradates were also detected and grouped with their respective parent compounds. The number of pharmaceuticals/degradates detected in the Effluent was 70 (100% of the total 70 pharmaceuticals/degradates detected among samples from all sites).

Downstream 1 Site (DS1) Concentration Data Year 1 (0.1 km from Effluent)

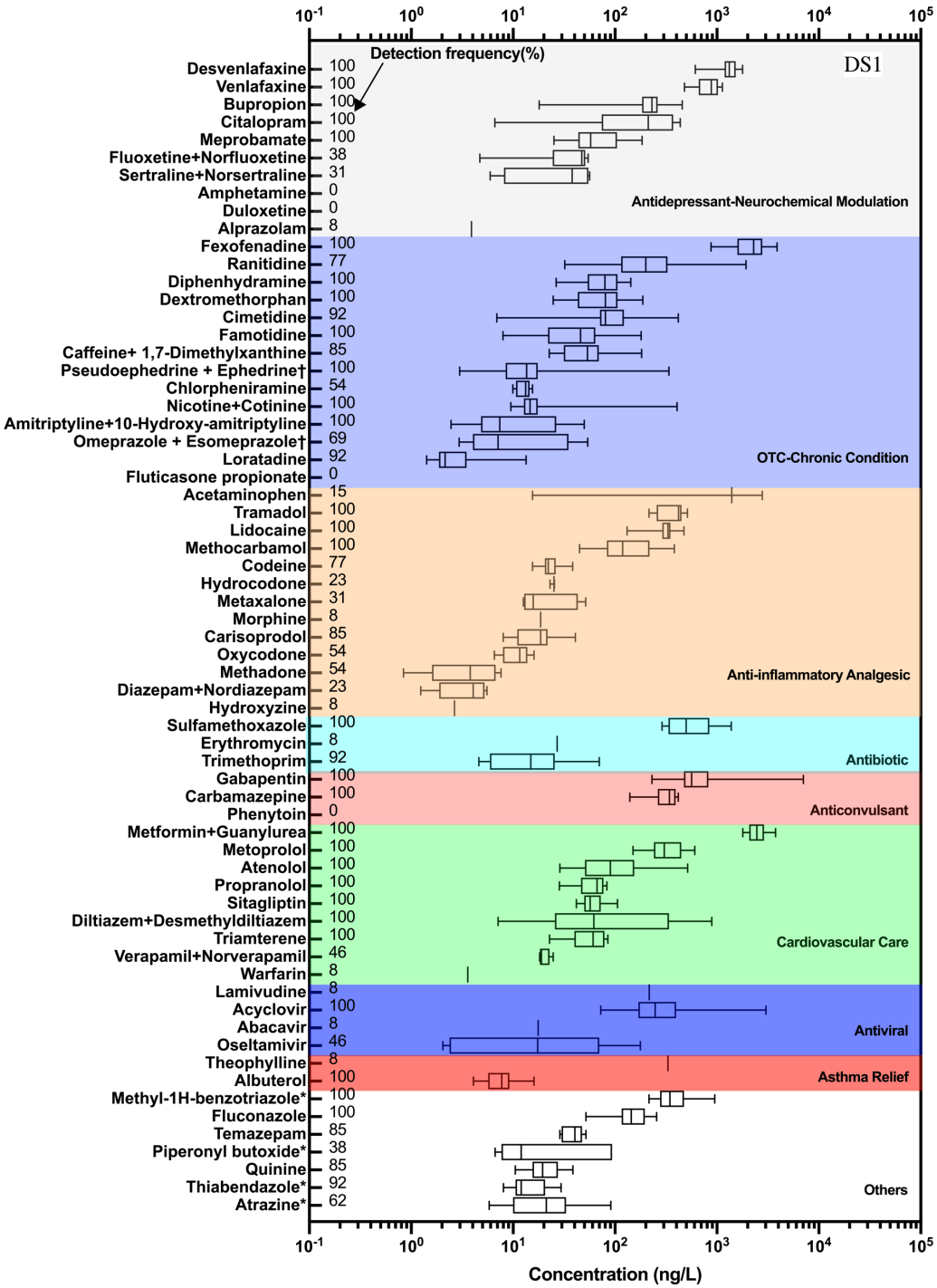

Figure S.11: Box-plot distributions of U.S. Geological Survey measured concentrations for the 74 chemicals (including 70 pharmaceuticals/degradates and 4 non-pharmaceutical compounds) detected in all water samples from DS1 (USGS site 05454052) during Year 1 of the study (September 2017–August 2018).[14] Results are sorted by pharmaceutical class and decreasing median concentration from top to bottom within a given class based on data in Effluent samples. *indicates non-pharmaceutical compound, †indicates isomers, "OTC"= "over-the-counter". The box-and-whiskers represent the median, interquartile range, and maximum/minimum values. Nine pharmaceutical degradates were also detected and grouped with their respective parent compounds. The number of pharmaceuticals/degradates detected at DS1 was 65 (93% of the total 70 pharmaceuticals/degradates detected among samples from all sites).

Downstream 2 Site (DS2) Concentration Data Year 1 (5.1 km from Effluent)

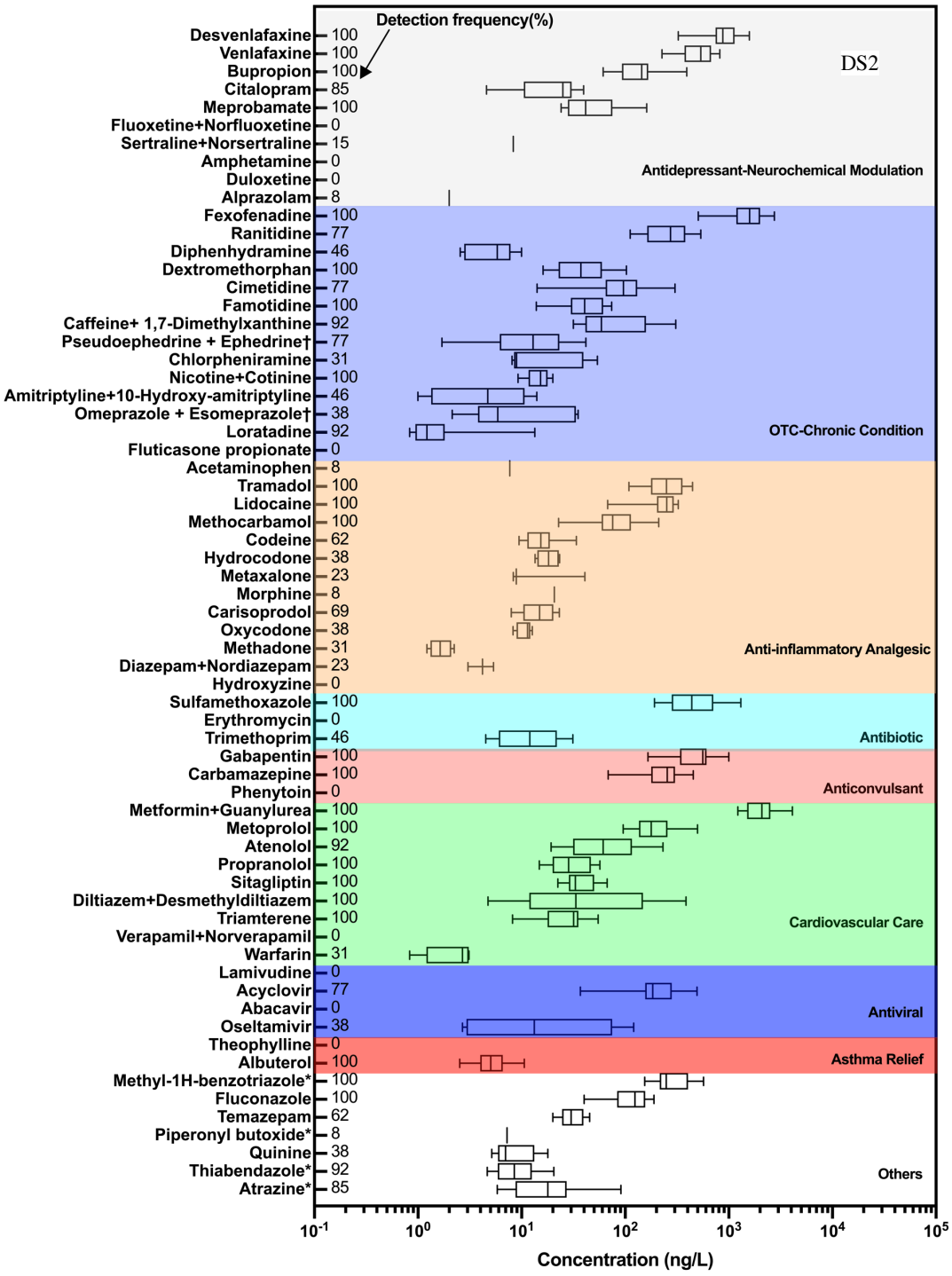

Figure S.12: Box-plot distributions of U.S. Geological Survey measured concentrations for the 74 chemicals (including 70 pharmaceuticals/degradates and 4 non-pharmaceutical compounds) detected in all water samples from DS2 (USGS gaging station 05454090; 5.1 km from effluent) during Year 1 of the study (September 2017–August 2018).[14] Results are sorted by pharmaceutical class and decreasing median concentration from top to bottom within a given class based on data in Effluent samples. *indicates non-pharmaceutical compound, †indicates isomers, "OTC"= "over-the-counter". The box-and-whiskers represent the median, interquartile range, and maximum/minimum values. Nine pharmaceutical degradates were also detected and grouped with their respective parent compounds. The number of pharmaceuticals/degradates detected at DS2 was 55 (78% of the total 70 pharmaceuticals/degradates detected among samples from all sites).

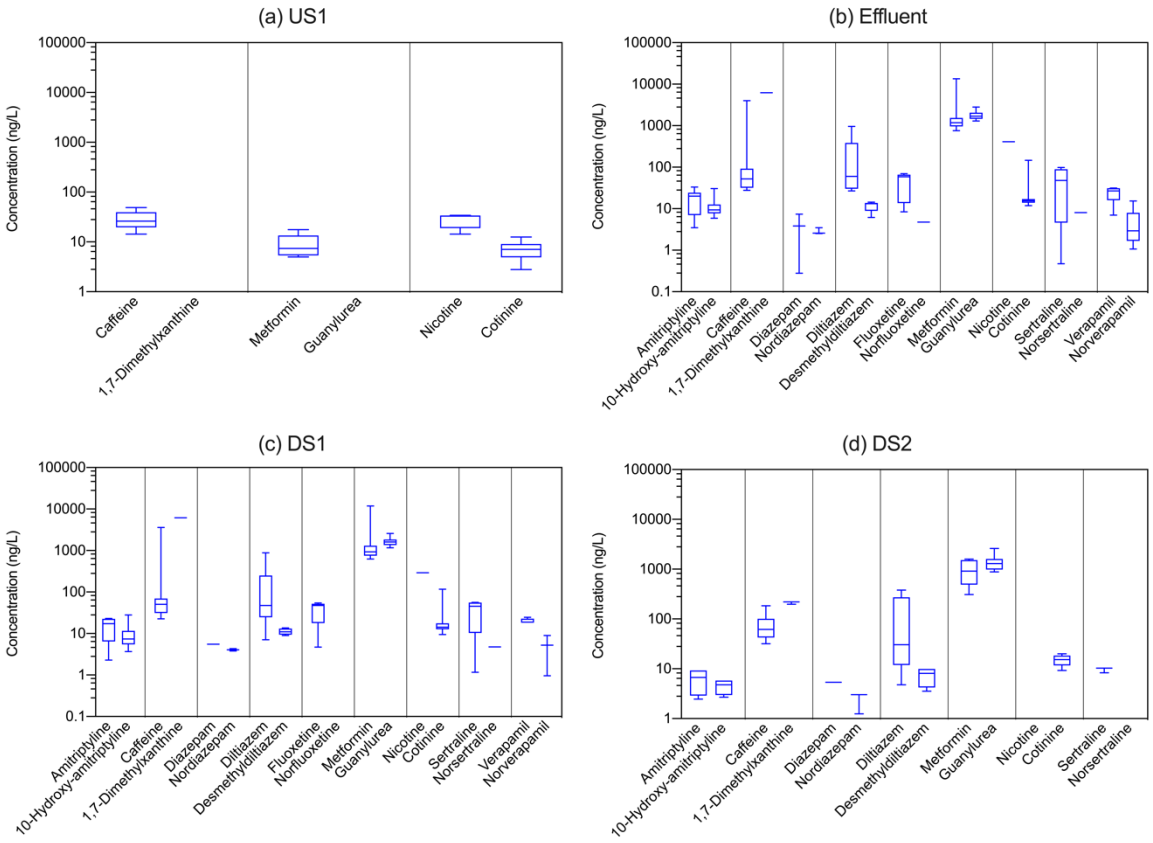

Figure S.13: Occurrence of pharmaceutical parent compounds and associated degradates analyzed via monthly U.S. Geological Survey (USGS) method at four sampling sites.[14] These compounds were grouped as pairs in Figure 1 of the main body text, as is the convention for the monthly established USGS analytical method.[2]

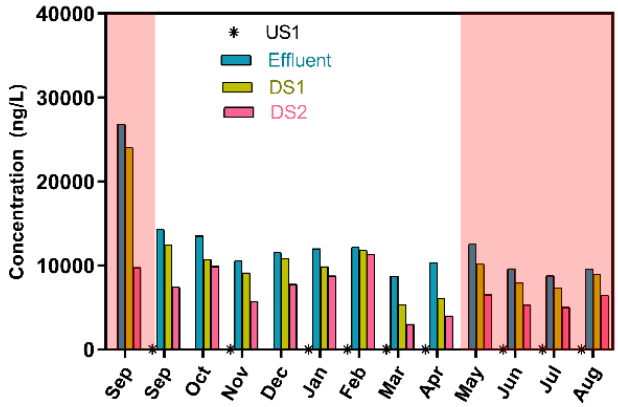

Figure S.14: Spatiotemporal patterns of U.S. Geological Survey (USGS) measured "biweekly" total pharmaceutical data via USGS analytical method during Year 1.[14] Shaded area (pink) was considered warm water condition.

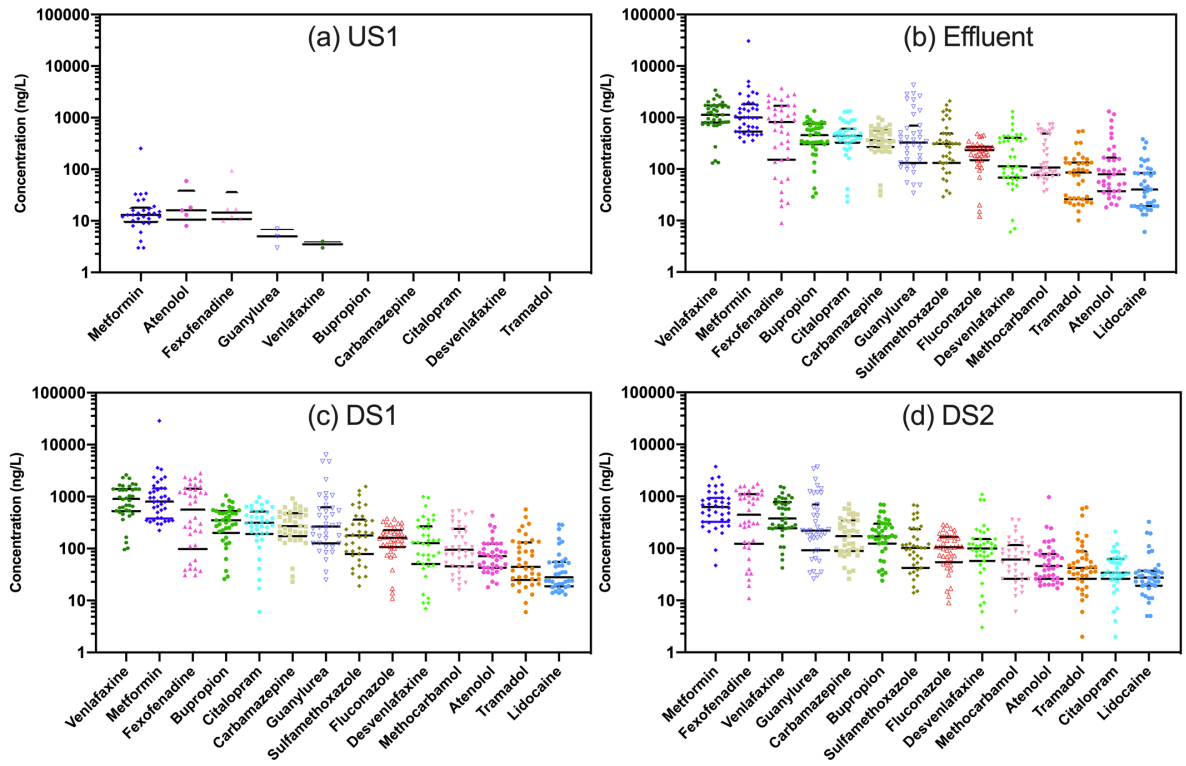

Figure S.15. Occurrence of biweekly method compounds at four sampling sites during 2-year sampling period. Only a few compounds were detected occasionally at US1 (USGS site 05454050), and the pharmaceutical rank order (x-axis) changed from Effluent (05454051) to DS1 (05454052) and to DS2 (05454090), demonstrating differential attenuation behaviors for individual pharmaceutical compounds. The detection frequencies above the minimum reporting limit (MRLs) for each biweekly pharmaceuticals/degradates measured were 100% at effluent and both downstream sites except for: atenolol (effluent: 97%), fexofenadine (DS1: 97%, DS2: 97%), lidocaine (DS1: 97%), methocarbamol (DS2: 92%) and sulfamethoxazole (DS2: 97%). Total concentrations of the biweekly pharmaceuticals ranged 3–268 ng/L (median 16 ng/L) at US1, 1750–38400 ng/L (median 7160 ng/L) at the effluent, 1430–36200 ng/L (median 5820 ng/L) at DS1, and 1320–7710 ng/L (median 3690 ng/L) at DS2 (Table S.12).

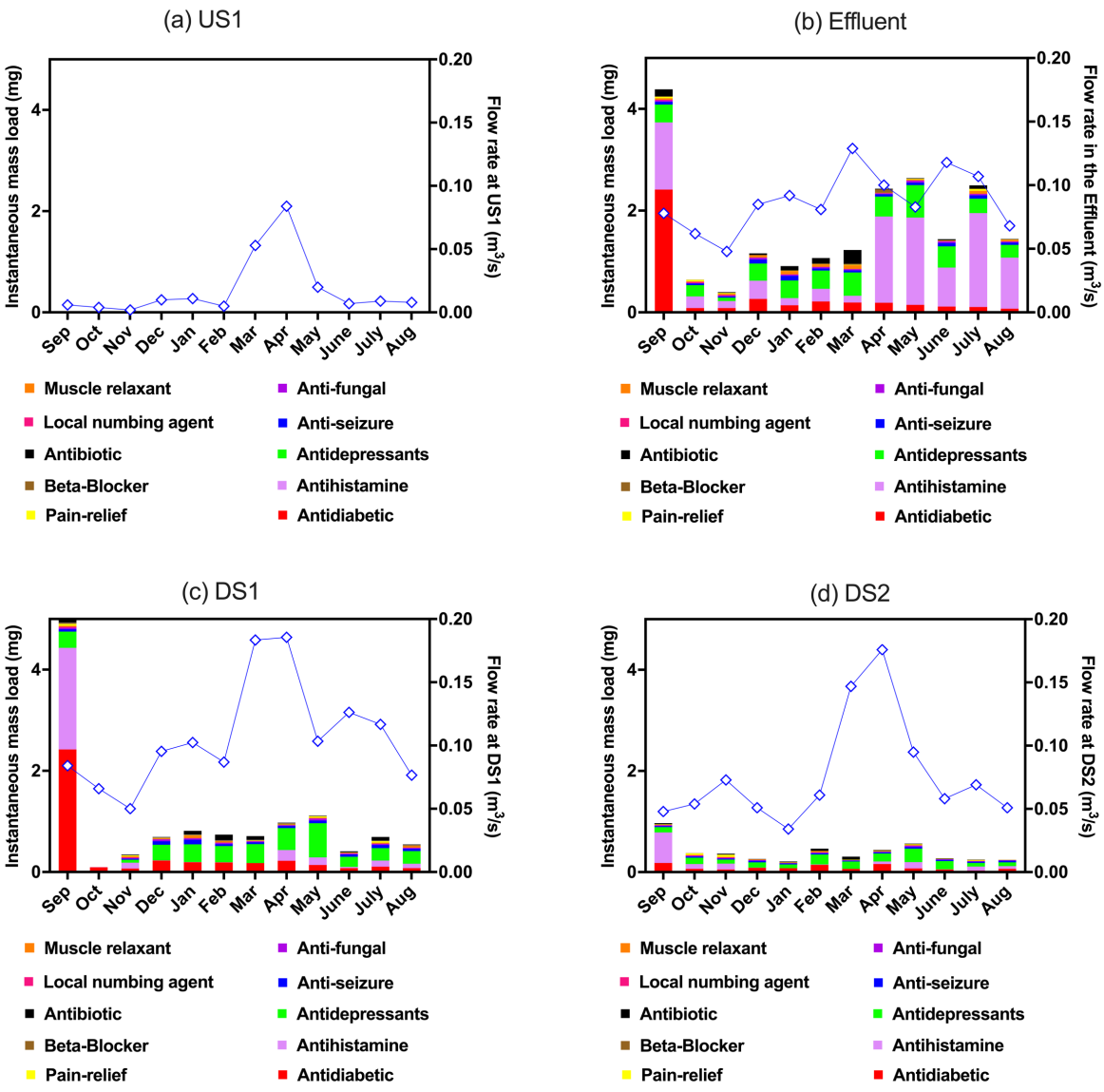

Figure S.16: Comparison of instantaneous mass load calculated based on the measured concentration (mg/L) and measured flow rate (m^3/s) of the most abundant pharmaceutical compounds at (a) US1, (b) Effluent, (c) DS1 and (d) DS2 during Year 1 based on pharmaceutical categories. The flow rate at each site is also shown. All graphs use the same y-axis scale for ease of comparison between sites.

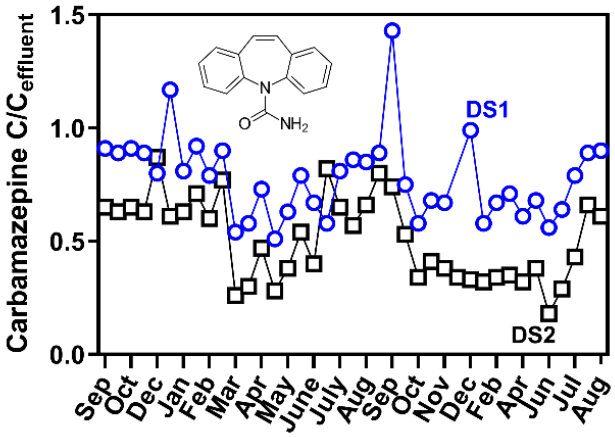

Figure S.17: Attenuation fraction of carbamazepine at DS1 and DS2. Data from biweekly method analyzed by University of Iowa.

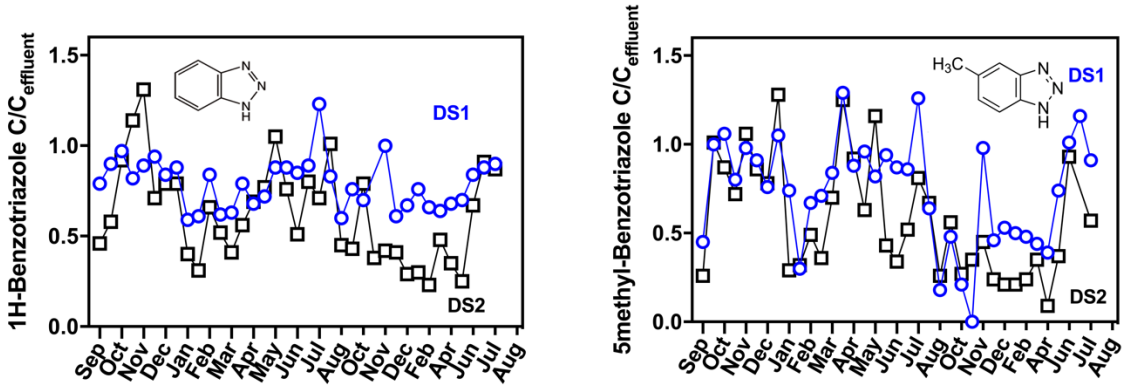

Figure S.18: Attenuation of 1H-benzotriazole (left) and 5-methyl-benzotriazole (right) along the stream. Both 1H-benzotriazole and 5-methyl benzotriazole were detected at US1, ranging from 8–643 ng/L (median 23 ng/L) and 0.23–18 ng/L (median 6 ng/L), respectively, indicating anthropogenic influence at US1. Higher concentrations of 1H-benzotriazole and 5-methyl-benzotriazole, however, ranging from 35–3590 ng/L (median 231 ng/L) and 2–1090 ng/L (median 64 ng/L) occurred in the effluent. The attenuation of 1H-benzotriazole at the DS1 and DS2 sites were 18±14% and 31±25%, respectively. Data from University of Iowa biweekly method.

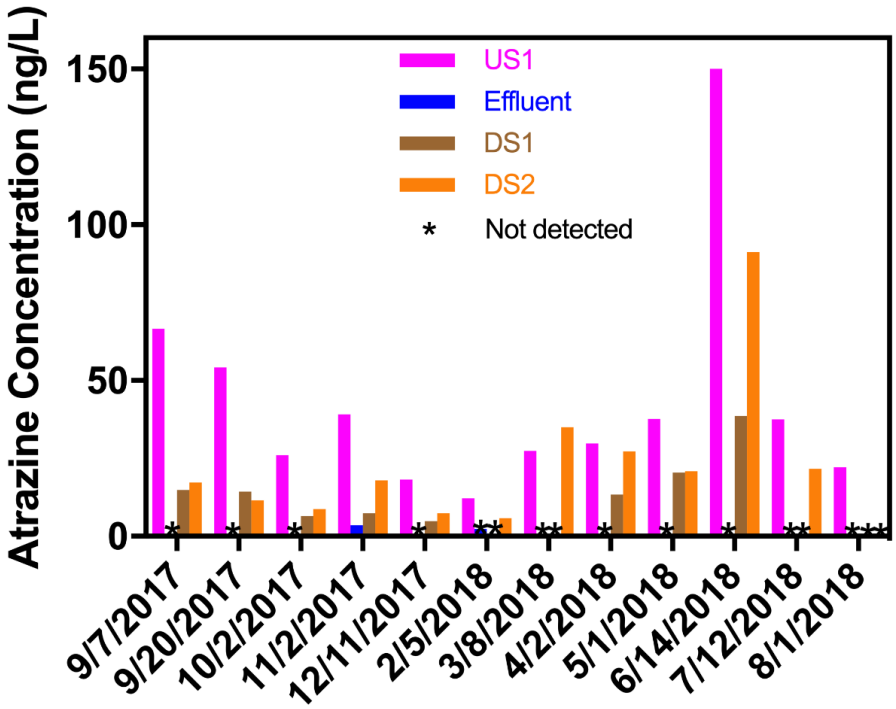

Figure S.19: Atrazine concentration measured by U.S. Geological Survey during Year 1. The detection frequency of atrazine was 100% at US1, 17% in the effluent, 75% at DS1, and 92% at DS2. Atrazine was detected at the greater concentrations at US1 (12–150 ng/L, median = 34 ng/L) compared to the effluent (2–4 ng/L, median = 3 ng/L) and DS1 (5–39 ng/L, median=14 ng/L).[14]

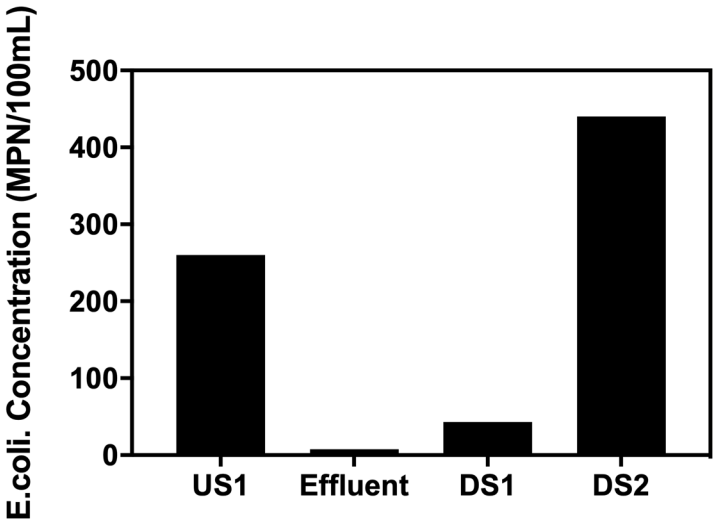

Figure S.20: *Escherichia coli* (*E. coli.*) concentrations measured by U.S. Geological Survey at four sampling sites. Sampling event occurred once on 10/2/2017.[14]

Supporting References

(1) USGS. USGS current conditions for USGS 05454090 Muddy Creek at Coralville, Iowa. https://waterdata.usgs.gov/ia/nwis/uv/?site_no=05454090&PARAmeter_cd=00065,00060. https://doi.org/https://doi.org/10.5066/F7P55KJN.

(2) Furlong, E. T.; Kanagy, C. J.; Kanagy, L. K.; Coffey, L. J.; Burkhardt, M. R. *Determination of Human-Use Pharmaceuticals in Filtered Water by Direct Aqueous Injection–High-Performance Liquid Chromatography/Tandem Mass Spectrometry*; 2014. https://doi.org/http://dx.doi.org/10.3133/tm5B10.

(3) United States Environmental Protection Agency(USEPA). CompTox Chemistry Dashboard. https://comptox.epa.gov/dashboard.

(4) Rúa-Gómez, P. C.; Püttmann, W. Degradation of Lidocaine, Tramadol, Venlafaxine and the Metabolites O-Desmethyltramadol and O-Desmethylvenlafaxine in Surface Waters. *Chemosphere* **2013**, *90* (6), 1952–1959. https://doi.org/10.1016/j.chemosphere.2012.10.039.

(5) Santoke, H.; Song, W.; Cooper, W. J.; Peake, B. M. Advanced Oxidation Treatment and Photochemical Fate of Selected Antidepressant Pharmaceuticals in Solutions of Suwannee River Humic Acid. *J. Hazard. Mater.* **2012**, *217–218*, 382–390. https://doi.org/10.1016/j.jhazmat.2012.03.049.

(6) Blum, K. M.; Norström, S. H.; Golovko, O.; Grabic, R.; Järhult, J. D.; Koba, O.; Söderström Lindström, H. Removal of 30 Active Pharmaceutical Ingredients in Surface Water under Long-Term Artificial UV Irradiation. *Chemosphere* **2017**, *176*, 175–182. https://doi.org/10.1016/j.chemosphere.2017.02.063.

(7) Quintão, F. J. O.; Freitas, J. R. L.; de Fátima Machado, C.; Aquino, S. F.; de Queiroz Silva, S.; de Cássia Franco Afonso, R. J. Characterization of Metformin By-Products under Photolysis, Photocatalysis, Ozonation and Chlorination by High-Performance Liquid Chromatography Coupled to High-Resolution Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2016**, *30* (21), 2360–2368. https://doi.org/10.1002/rcm.7724.

(8) Trautwein, C.; Kümmerer, K. Incomplete Aerobic Degradation of the Antidiabetic Drug Metformin and Identification of the Bacterial Dead-End Transformation Product Guanylurea. *Chemosphere* **2011**, *85* (5), 765–773. https://doi.org/10.1016/j.chemosphere.2011.06.057.

(9) Chen, Z. F.; Ying, G. G.; Jiang, Y. X.; Yang, B.; Lai, H. J.; Liu, Y. S.; Pan, C. G.; Peng, F. Q. Photodegradation of the Azole Fungicide Fluconazole in Aqueous Solution under UV-254: Kinetics, Mechanistic Investigations and Toxicity Evaluation. *Water Res.* **2014**, *52*, 83–91. https://doi.org/10.1016/j.watres.2013.12.039.

(10) Kwon, J. W.; Armbrust, K. L. Degradation of Citalopram by Simulated Sunlight. *Environ. Toxicol. Chem.* **2005**, *24* (7), 1618–1623. https://doi.org/10.1897/04-522R.1.

(11) Yamamoto, H.; Nakamura, Y.; Moriguchi, S.; Nakamura, Y.; Honda, Y.; Tamura, I.; Hirata, Y.; Hayashi, A.; Sekizawa, J. Persistence and Partitioning of Eight Selected Pharmaceuticals in the Aquatic Environment: Laboratory Photolysis, Biodegradation, and Sorption Experiments. *Water Res.* **2009**, *43* (2), 351–362. https://doi.org/10.1016/j.watres.2008.10.039.

(12) Andreozzi, R.; Marotta, R.; Paxéus, N. Pharmaceuticals in STP Effluents and Their

Solar Photodegradation in Aquatic Environment. *Chemosphere* **2003**, *50* (10), 1319–1330. https://doi.org/10.1016/S0045-6535(02)00769-5.

(13) Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2019). PubChem 2019 Update: Improved Access to Chemical Data. *Nucleic Acids Res. 47* (D1), D1102–D1109. https://doi.org/10.1093/nar/gky1033.

(14) Meppelink, S.M., Kolpin, D.W., Lane, R.F., Iwanowicz, L., Zhi, H., LeFevre, G.H., **2020**, Water-quality data for a pharmaceutical study at Muddy Creek in North Liberty and Coralville, Iowa, 2017-2018: U.S. Geological Survey data release, https://doi.org/10.5066/P9WOD2XB.

(15) Oberg, K.; Rehmel, M.; Blanchard, S. F. Office of Surface Water Technical Memorandum 2004.04 - Policy on the use of the FlowTracker for discharge measurements. https://hydroacoustics.usgs.gov/memos/OSW2004-04.pdf (accessed Sep 1, 2017).