

Supplementary Information for:

Attenuation of wastewater-derived contaminants in an effluent-dominated river

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Table SI 1. Information about WWTPs sampled.

WWT P	Average Flow			Secondary Treatment	Tertiary/Advanced Treatment
	03/05 $\text{m}^3\text{s}^{-1}(\text{mgd})$	07/05 $\text{m}^3\text{s}^{-1}(\text{mgd})$	09/05 $\text{m}^3\text{s}^{-1}(\text{mgd})$		
1	0.657 (15.0)	0.508 (11.6)	0.499 (11.4)	AS	filtration, chlorination/dechlorination
2	0.618 (14.1)	0.565 (12.9)	0.578 (13.2)	AS	nitrifying activated sludge, filtration, chlorination/dechlorination
3	2.47 (56.5)	2.26 (51.5)	1.93 (44.0)	AS	denitrification, filtration, chlorination/dechlorination
4	5.30 (121)	4.84 (111)	4.77 (109)	AS	filtration, chlorination/dechlorination
5	6.11 (139)	5.99 (137)	5.48 (125)	AS	filtration, chlorination/dechlorination
6	5.97 (136)	5.32 (121)	5.90 (135)	AS /TF	filtration, chlorination/dechlorination

Abbreviations: mgd = megagallons/day; AS = activated sludge; TF = trickling filter

Table SI 2a. Concentration of WWDCs measured in wastewater effluent samples during March 21-30, 2005.

WWTP	1	2	3	4	5	6	Spike Recoveries (%)
Dates Collected ^a	3/23-24/05	3/28-29/05	3/21-22/05	3/29-30/05	3/23-24/05	3/29-30/05	
Hormones (ng/L)							
17a-Estradiol	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n/a
17b-Estradiol	<0.1	<0.1	<0.1	<0.1	<0.1	1.6	n/a
Estrone	<0.1	<0.1	0.5	<0.1	<0.1	3	n/a
Estriol	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n/a
Testosterone	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n/a
Androstenedione	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n/a
Progesterone	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n/a
MedroxyProg.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n/a
Beta Blockers (ng/L)							
metoprolol	257	306	169	440	12	343	95
propranolol	3	9	24	7	<1	22	55
Acidic Pharmaceuticals (ng/L)							
diclofenac	25	<3	6	10	<3	<3	78
gemfibrozil	<3	14	31	22	<3	9	93
ibuprofen	12	<3	<3	149	<3	11	80
indometacine	25	11	9	29	6	8	47
naproxen	41	7	72	67	<3	9	94
ketoprofen	8	<3	4	7	<3	3	54
Organic Iodide (ug/L)	5	6	8	13	9	8	74
Total EDTA (nM)	493	142	264	229	292	173	
FeEDTA (nM)	248	104	196	207	165	183	
spike recoveries (%) (FeEDTA)	43	105	77	84	93	93	

n.b.: duplicates are expressed in parentheses

n/a : spike recoveries were not performed for these analyses

Table SI 2b. Concentration of WWDCs measured in wastewater effluent samples during July 19-20, 2005.

Sample	1	2	3	4	5	6	Spike Recoveries (%)
Dates Collected ^a	7/19/05	7/19/05	7/19/05	7/19/05	7/19/05	7/19/05	
Hormones (ng/L)							
17a-Estradiol	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	138
17b-Estradiol	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	147
Estrone	<0.1	<0.1	1	<0.1	<0.1	<0.1	9.1
Estriol	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	134
Testosterone	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	122
Androstenedione	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	85
Progesterone	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	134
MedroxyProg.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	175
Beta Blockers (ng/L)							
metoprolol	130	176	109 (130)	199	<1	5	48
propranolol	<1	2	<1	3	<1	1	54
Acidic Pharmaceuticals (ng/L)							
diclofenac	33	<10	n/a	<10	<10	<10	<10
gemfibrozil	49	234	n/a	29 (23)	57	18	<10
ibuprofen	41	28	n/a	20 (19)	31	13	<10
indometacine	26	14	n/a	17 (18)	14	14	<10
naproxen	30	46	n/a	21 (22)	63	22	20
ketoprofen	29	<10	n/a	31 (21)	<10	<10	51
Organic Iodide (ug/L)	5.7	6.4	8.4	12	11.2		69
Total EDTA (nM)	1197	426	730	598	651	833	111
FeEDTA (nM)	364	198	623	341	406	635	

n.b.: duplicates are expressed in parentheses

n/a = sample was destroyed during processing

Table SI 2c. Concentration of WWDCs measured in wastewater effluent samples during September 14-15, 2005.

Sample	1	2	3	4	5	6	Spike Recovery (%)	Flow-weighted average concentration
Dates Collected	9/14/05	9/14/05	9/14/05	9/14/05	9/14/05	9/14/05	Lab	
Beta Blockers (ng/L)								
metoprolol	2269	1539	636	921	1132	964 (1203)	73	1070
propranolol	124	47	<10	<10	200	92 (113)	75	93
Acidic Pharmaceuticals (ng/L)								
diclofenac	<10	<10	<10	<10	<10	26	81	8.0
gemfibrozil	34	52 (56)	12	97	27	55	117	52
ibuprofen	60	42 (37)	11	59	<10	403	168	142
indometacine	8	10 (17)	<2	15	<2	<2	48	4.4
naproxen	38	8 (11)	29	90	49	223	97	109
ketoprofen	<10	<10	<10	<10	<10	<10	107	<10
Organic Iodide (ug/L)	5.9 (5.0)	11.7	8.1	11.3	8.3	13	76	10.5
Total EDTA (nM)	1640	540	454	816	677	1641	92	1010
FeEDTA (nM)	723	268	344	538	466	1574		814

n.b.: duplicates are expressed in parentheses

Table SI 3. Concentrations of WWDCs measured in September in the Trinity River.

Site	1				2				3			
Dates Collected ^a	9/12/04	9/13/05	9/14/05	9/15/05	9/12/04	9/13/05	9/14/05	9/15/05	9/12/04	9/13/05	9/14/05	9/15/05
Beta Blockers (ng/L)												
metoprolol	228	571	439	344 (382)	268	326	n/a	376	136	131	220	219
propranolol	16	50	52	24	17	<10	<10	74	<10	<10	<10	<10
Acidic Pharmaceuticals (ng/L)												
diclofenac	<10 (<10)	<10	<10	<10	<10	<10	n/a	<10	<10	<10	<10	<10
gemfibrozil	65 (63)	71	67	50	79	49	n/a	41	49	55	51	57
ibuprofen	64 (56)	74	55	30	76	32	n/a	26	28	21	18	39
indometacine	10 (<5)	<5	11	48	<5	20	n/a	<5	<5	<5	<5	21
naproxen	32 (29)	58	30	32	10	24	n/a	14	8	11	12	24
ketoprofen	<10 (<10)	<10	<10	<10	<10	<10	n/a	<10	<10	<10	<10	<10
Organic Iodide (ug/L)	10.6	13.4	15.6	11.4	11.2 (10.5)	9.4	10.4	n/a	7.7	11.4	11.8	11.9
								n/a				
Total EDTA (nM)	240	327	277	243	220	196	190	216	150	n/a	145	152
FeEDTA (nM)	23	35	46	57	31	33	47	47	34	n/a	54	37

Sample	4				5				Upstrm	Spike Recovery %	
Dates Collected ^a	9/12/05	9/13/05	9/14/05	9/15/05	9/12/05	9/13/05	9/14/05	9/15/05	9/12/05	9/13/05	Field Spike
Beta Blockers (ng/L)											
metoprolol	77	99	122	124	50	82	41	82	<10	<10	89
propranolol	<10	<10	37	35	<10	<10	25	57	<10	<10	79
Acidic Pharmaceuticals (ng/L)											
diclofenac	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	54
gemfibrozil	20	37	37	32	20	17	14	11	nd	nd	91
ibuprofen	8	10	<5	23	8	5	<5	6	<5	<5	119
indometacine	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	54
naproxen	4	9	1	13	4	6	<1	5	<1	<1	76
ketoprofen	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	67
Organic Iodide (ug/L)	10.5	17.4	9.9	11.2	9.8	9.6	11.1	6.6	4.1	3.3	82
Total EDTA (nM)	124	90	79	104	69	543	437	71	<35	<35	96
FeEDTA (nM)	<35	9	<35	13	15	357	345	<35	<35	<35	

n.b.: duplicates are expressed in parentheses

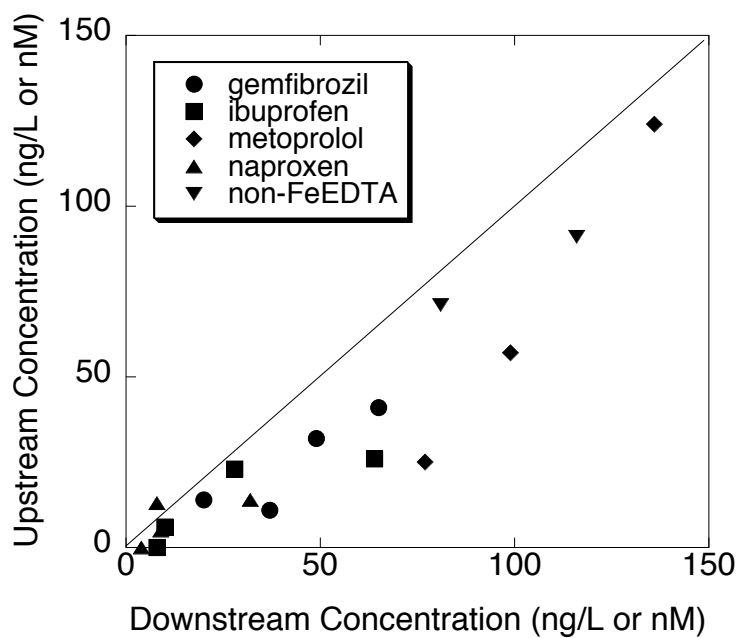


Figure SI 1. A comparison of concentrations of reactive compounds upstream and samples from the Trinity River. Upstream and downstream pairs were defined on the basis of the estimated hydraulic transit time between sampling sites.

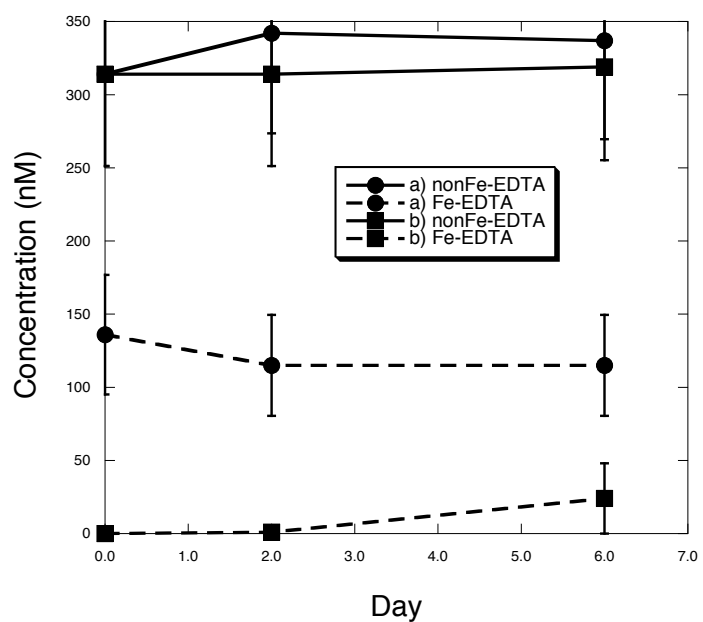
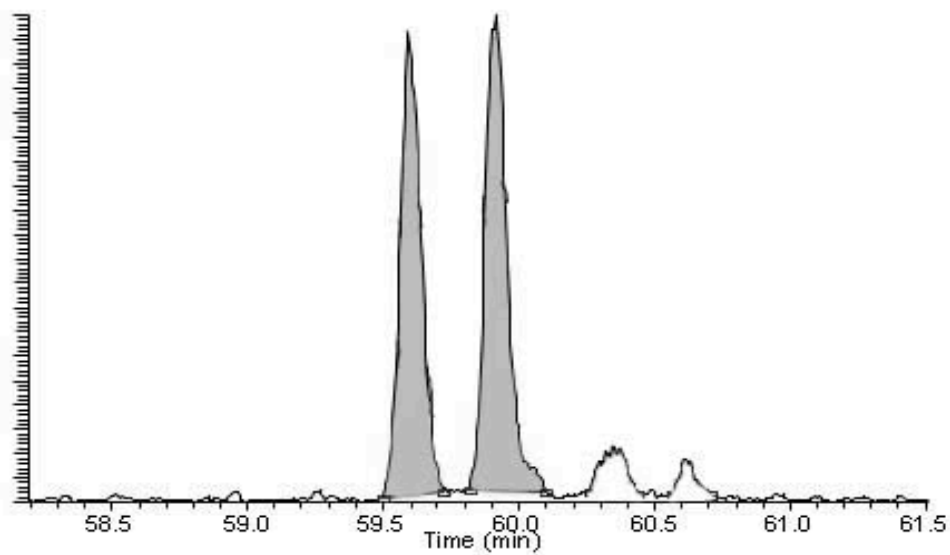


Figure SI 2. Speciation of EDTA with initial concentration of 460 ± 20 during incubation of mixed Trinity River/WWTP effluent samples. a) Experiment immediately put in the dark; b) Experiment initially irradiated to remove FeEDTA.

a)



b)

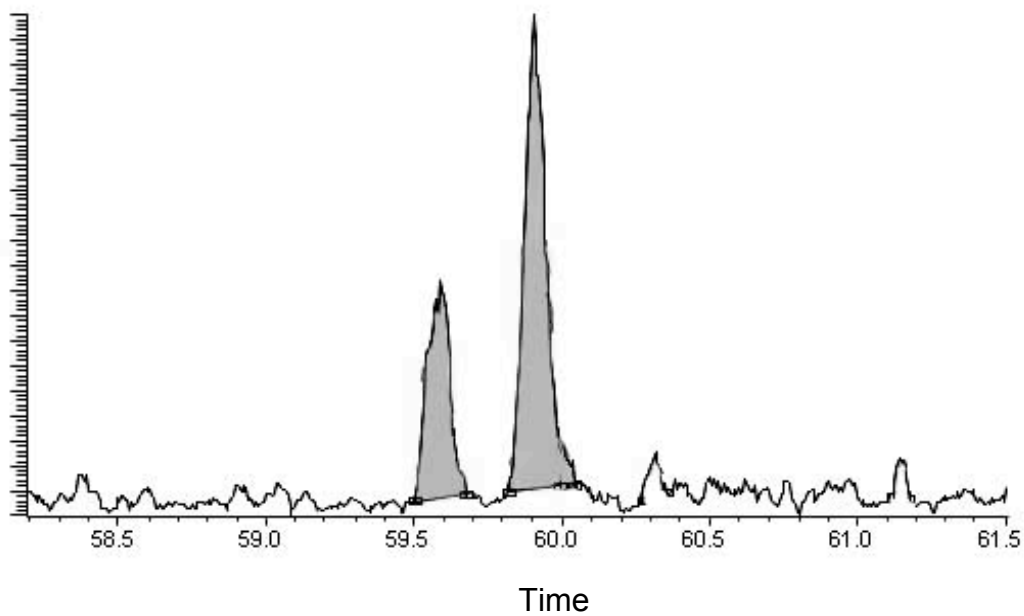


Figure SI 3. Chromatograms of the enantiomers of metoprolol from a) WWTP 2 on Sept 14, 2005 (EF= 0.48) and b) furthest downstream Trinity River sampling site, site 5, on Sept 13,2005 (EF = 0.32)

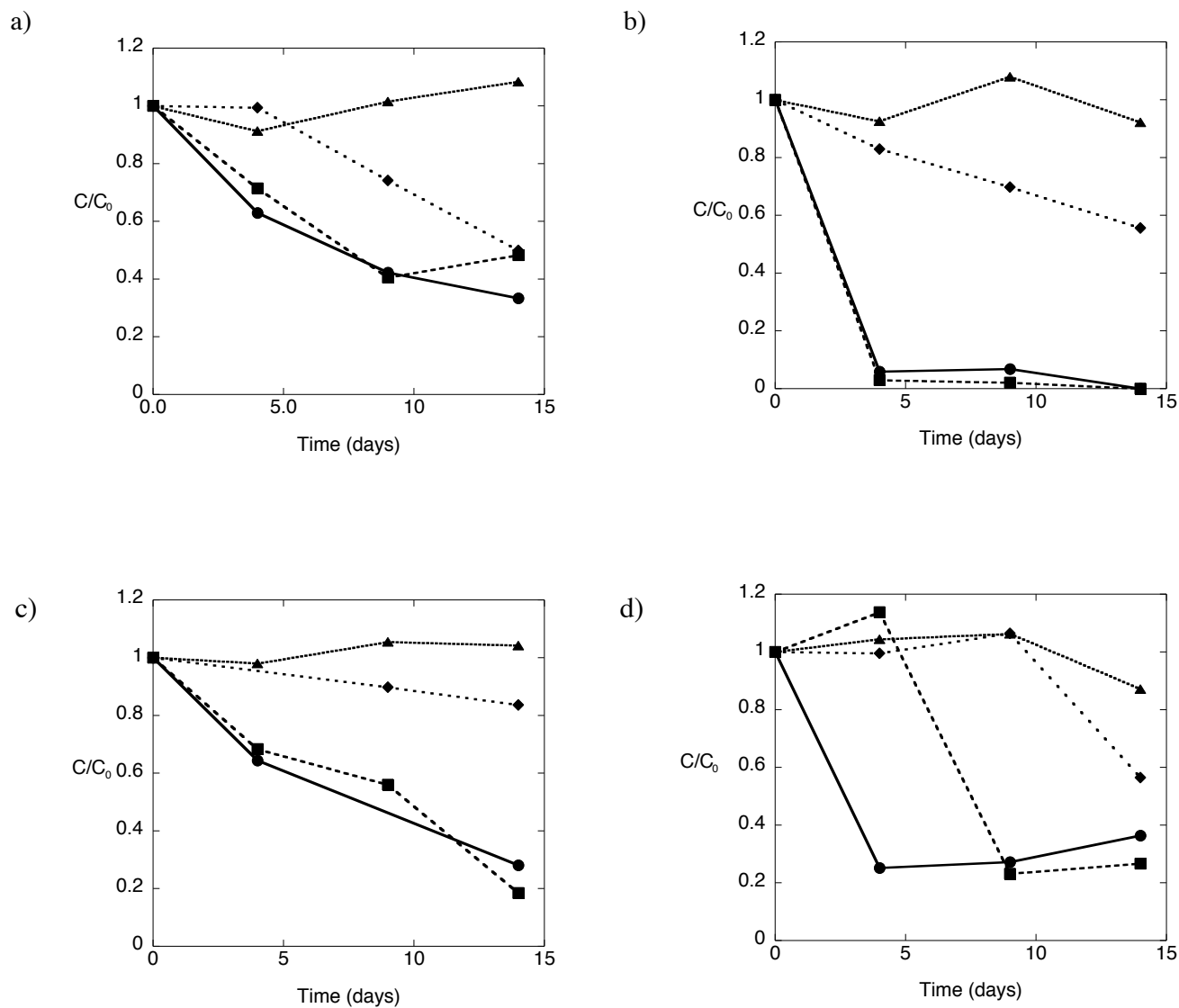
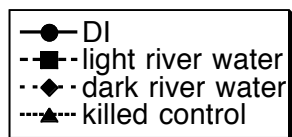


Figure SI 4. Microcosm experiments for a) gemfibrozil; b) naproxen; c) metoprolol and d) ibuprofen. Initial concentrations were 1 $\mu\text{g/L}$ for each compound. DI refers to deionized water control.

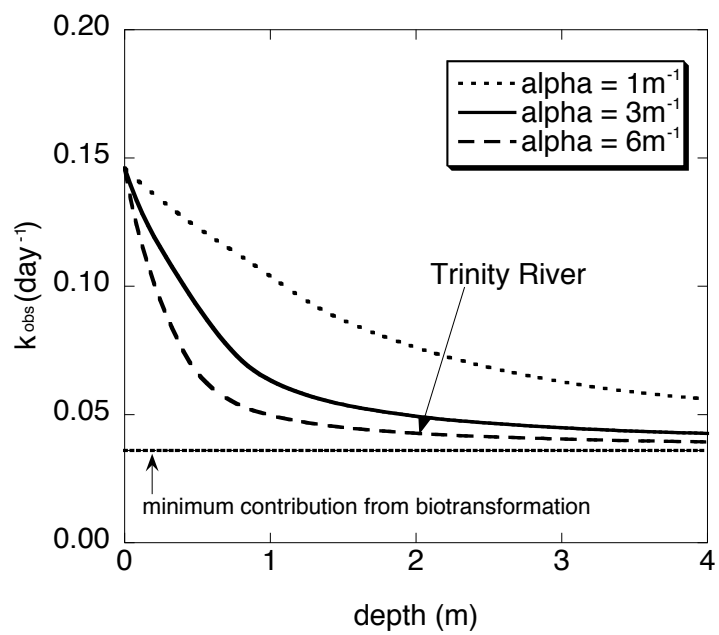


Figure SI 5. Calculated attenuation rates for ibuprofen for mid-September, 33°N.

SI Acidic Pharmaceutical Method

The following method is similar to that published by Oellers et al. for determining concentrations of acidic pharmaceuticals (1).

After filtration of the samples, flurbiprofen (Sigma) was added as a surrogate standard at a final concentration of 500 ng/L. The sample pH was adjusted to less than 2 with concentrated hydrochloric acid. The sample was placed in a silanized glass container connected to the extraction columns with Teflon tubing. The silanized glass extraction columns were packed with 500 mg of ENVI-18 solid phase extraction resin (Supelco) that was rinsed with 10 mL HPLC grade methanol (Fisher Scientific) and 20 mL Nanopure water prior to extraction. The samples were passed through the extraction columns at a flow rate of approximately 10 mL/min by connecting the tubing to a peristaltic pump placed downstream of the extraction column. After passing the sample through the columns, they were rinsed with 20 mL nanopure water and then dried for 10 minutes by pumping air through the resins at the same flow rate.

Following solid phase extraction, the samples were eluted from the resins using 10 mL of HPLC grade methanol (Fisher Scientific). The methanolic extracts were collected in silanized glass test tubes. The extracts then were dried completely by placing them in a vacuum oven at room temperature overnight. Following the drying step, the samples were resuspended in 2 mL of HPLC grade methanol and transferred to 4 mL glass vials. The extracts then were blown to dryness under a gentle stream of high purity nitrogen over a period of approximately 30 minutes. Following blowdown, the samples were derivatized with a diazomethane/diethylether mixture, prepared as described by the manufacturer (Aldrich Chemical Company, Technical Bulletin A1-180). Diazomethane presents some potential safety hazards (e.g., it is a mutagen and can explode upon impact) and should be handled with great care (see Aldrich Chemical Company, Technical Bulletin A1-180 for details). After adding 250 μ L of diazomethane/diethyl ether, the extracts were allowed to react for 2 minutes prior to quenching the excess diazomethane with 10 μ L of a 1:10 acetic acid/acetone mixture. The derivatized samples were again blown to near dryness under a stream of high purity nitrogen and resuspended in 200 μ L of isooctane with 500 mg/L of hexachlorobenzene as an internal standard.

The derivatized acidic drugs were analyzed by GC/MS/MS using a Finnegan GCQ GC/MS/MS system with a 30-meter DB-5 column. Prior to analysis the GC/MS/MS system was optimized by changing the injection port liner, cutting back the first 2-cm of the column when necessary, and cleaning the ion trap, when necessary. The analysis of wastewater extracts led to a decrease in system performance, and it was usually necessary to repeat the cleaning procedure after approximately 200 injections. The analytical conditions used for quantification are listed in Table 1 and the following conditions were used for the oven and mass spectrometer: isothermal at 50°C for 4 min, 20 °C/min to 120°C, 2°C/min to 180 °C, and finally 30°C/min to 290 °C, where it was held for 8 min. The carrier gas used was helium at a flow rate of 1.2 mL/min. Splitless injection of 2 μ L samples was used with a split flow of 50 mL/min and an injection port temperature of 270 °C. The mass spectrometer had a source temp of 200 °C and the transfer line was held at 300°C.

Table SI 4. Conditions used for analysis of acidic drugs.

Compound	Retention time (min)	Parent ion	Product ion	Voltage (V)
Ibuprofen	19.2	161	105, 119	0.75
Gemfibrozil	32.8	143	83	0.75
Flurbiprofen	38.5	258	199	1.0
Naproxen	41.0	244	170, 185	0.75
Ketoprofen	46.3	209	105	0.50
Diclofenac	48.7	214	151, 178	0.75
Indometacine	58.5	371	139, 312	1.0
Hexachlorobenzene	25.8	142, 249, 284*		

*Hexachlorobenzene, the internal standard, was quantified by SIM.

Prior to analysis of samples two standard curves were constructed using a mixed standard of the suite of acidic drugs. The standard curve contained 37.5 µg/L, 75 µg/L, 150 µg/L, 225 µg/L and 300 , 600 µg/L, 900 µg/L and 1200 µg/L. A linear calibration curve was calculated from simple linear regression. Following calibration, a run sequence was used consisting of five standards followed by a randomized mixture of the samples and QA/QC samples. The calibration curve was checked every fifteen samples by running a blank and a reslope standard from the middle of the calibration curve. If the calibration standard disagreed with the standard curve by more than 25% the samples in the following section were rerun.

For the acidic drugs, the target for recoveries was 60-120%, as determined by recovery of flurbiprofen. For good recoveries, the concentrations were corrected for losses in extraction and derivitization. Typical detection limits for acidic drugs in wastewater effluent were around 10 ng/L. These compounds were not detected in blanks.

SI Calculations

a. *Ratio of day-averaged surface solar intensities L(330nm) in Berkeley, CA (October) to the Trinity River (September), neglecting cloud cover, estimated from Appendix Table A-7 and A-8, Leifer (1988) (2):*

$$\begin{aligned}\text{Berkeley: } L(330\text{nm}) &\cong 8.7 \times 10^{-2} \text{ millieinsteins cm}^{-2}\text{day}^{-1} \\ \text{Trinity River (TR): } L(330\text{nm}) &\cong 9.3 \times 10^{-2} \text{ millieinsteins cm}^{-2}\text{day}^{-1}\end{aligned}$$

$$\text{Ratio : } L_{\text{berkeley}}/L_{\text{TR}} = (8.7 \times 10^{-2})/(9.3 \times 10^{-2}) = 0.94$$

b. *Depth of photic zone in Trinity River*

The photic zone is defined as follows:
Transmittance (T)=I/I₀= 0.05 (I=Intensity)
Absorption(A) = -log(T) = 1.30

$$\begin{aligned}A_{330\text{nm}} &= \alpha_{330} z_{\text{photic}} \quad (\alpha_{330}=0.06\text{cm}^{-1}; \text{ the average measured from all Trinity River samples}) \\ z_{\text{photic}} &= A/\alpha_{330} = 1.30/0.06\text{cm}^{-1} = 22 \text{ cm}\end{aligned}$$

Ratio of photic zone to total depth, assuming a mean depth of 2 m in the Trinity River:

$$z_{\text{photic}}/z_{\text{tot}} = 0.22\text{m}/2\text{m} = 0.11$$

c. *Comparison of photodegradation rates in sunlit river water micocosm and in situ, Trinity River*

k_{surf} ≡ surface photodegradation rate constant in Dallas, TX, in September.

S ≡ Screening factor to account for absorption by DOM

α ≡ beam attenuation coefficient (6 m⁻¹ for Trinity River water at 330 nm)

z ≡ depth of photic zone (0.13 m for microcosm, 0.22 m for Trinity River)

$$\begin{aligned}\text{Microcosm: } k_{\text{surf}}(L_{\text{berk}}/L_{\text{TR}})*S & \quad [S = (1-e^{-\alpha z})/\alpha z = (1-e^{-6 \times 0.13})/(6 \times 0.13) = 0.69] \\ &= k_{\text{surf}} (0.94)(0.69) \\ &= 0.65k_{\text{surf}}\end{aligned}$$

$$\begin{aligned}\text{River In Situ: } k_{\text{surf}} (z_{\text{photic}}/z_{\text{tot}})*S & \quad [S = (1-e^{-\alpha z})/\alpha z = (1-e^{-6 \times 0.22})/(6 \times 0.22) = 0.55] \\ &= k_{\text{surf}} (0.11)(0.55) \\ &= 0.061k_{\text{surf}}\end{aligned}$$

Ratio of photodegradation rates in the microcosm (k_{micro}) and the Trinity River (k_{TR}):

$$k_{\text{micro}}/k_{\text{TR}} = 0.65 k_{\text{surf}}/0.061 k_{\text{surf}} = 11$$

d. Calculated EF of metoprolol on day 14 of light nonsterile microcosm experiment

Using data from figure 3 for the field data:

$$\Delta EF/(1-C/C_0) \text{ in river at last sampling point} = (0.50-0.315)/(1-54/1418) = 0.19$$

$$\Delta EF_{\text{microfinal}} = 0.19 \times (1-C/C_0) \text{ in microcosm} = 0.19 \times (1 - 0.84) = 0.03 \quad \text{Ratio : 0.94}$$

e. Calculations for attenuation of naproxen used in Figure 7.

muddy river $\alpha_{330\text{nm}} = 6\text{m}^{-1}$ (measured in Trinity River water)

medium river $\alpha_{330\text{nm}} = 3\text{m}^{-1}$ (estimated) (3)

clear river $\alpha_{330\text{nm}} = 1\text{m}^{-1}$ (estimated)

photolysis rate from deionized water microcosm treatment = 0.37 d^{-1}

adjusted for latitude/season $k_{\text{tex}} = k_{\text{berk}}/\text{ratio} = 0.37/0.94 = 0.39\text{ d}^{-1}$

adjusted to surface intensity (instead of average over 13 cm microcosm depth)

then, for each river, k_{surf} is multiplied by S, where

$$k_{\text{photo}}(z) = S(z)k_{\text{surf}}; \text{ for } z_{\text{tot}} < z_{\text{photic}}$$

and

$$k_{\text{photo}}(z) = (z_{\text{photic}}/z) \times S(z_{\text{photic}}) \times k_{\text{surf}}; \text{ for } z_{\text{tot}} > z_{\text{photic}}$$

finally, for overall rate k_{tot} , expressed on y-axis of Figure 7,

$$k_{\text{obs}} = k_{\text{photo}} + k_{\text{bio}}$$

where the biodegradation rate, $k_{\text{bio}} = 0.041\text{ d}^{-1}$

References

- (1) Ollers, S.; Singer, H. P.; Fassler, P.; Muller, S. R., Simultaneous quantification of neutral and acidic pharmaceuticals and pesticides at the low-ng/l level in surface and waste water. *Journal of Chromatography A* 2001, 911, 225-234.
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- (3) Schwarzenbach, R.; Gshwend, P.; Imboden, D. Chapter 13: Photochemical Transformation Reactions. In *Environmental Organic Chemistry*; John Wiley & Sons, Inc.: New York, 1993; pp 436-484.