SUPPORTING INFORMATION

for the paper

A simple field-based biodegradation test shows pH to be an inadequately controlled parameter in laboratory biodegradation testing

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Table S1: ***List of targeted compounds***

Compound	CAS Number	Molecular Formula
10,11-Dihydro-10,11-dihydroxy carbamazepine	35079-97-1	C15H14N2O3
1H-benzotriazole	95-14-7	C6H5N3
1-Methyl-1H-benzotriazole	13351-73-0	C7H7N3
acetaminophen	103-90-2	C8H9NO2
acridine	260-94-6	C13H9N
anastrozole	120511-73-1	C17H19N5
atenolol	29122-68-7	C14H22N2O3
bezafibrate	41859-67-0	C19H20ClNO4
bicalutamide	90357-06-5	C18H14F4N2O4S
caffeine	58-08-2	C8H10N4O2
carbamazepine	298-46-4	C15H12N2O
carbamazepine-10,11-epoxide	36507-30-9	C15H12N2O2
climbazole	38083-17-9	C15H17ClN2O2
clofibric acid	882-09-7	C10H11ClO3
diclofenac	15307-86-5	C14H11Cl2NO2
fluconazole	86386-73-4	C13H12F2N6O
furosemide	54-31-9	C12H11ClN2O5S
gabapentin	60142-96-3	C9H17NO2
hydrochlorothiazide	58-93-5	C7H8ClN3O4S2
irbesartan	138402-11-6	C25H28N6O
ketoprofen	22071-15-4	C16H14O3
MCPA (2-methyl-4-chlorophenoxyacetic acid)	94-74-6	C9H9ClO3
mecoprop	93-65-2	C10H11ClO3
metformin	657-24-9	C4H11N5
methotrexate	59-05-2	C20H22N8O5
metoprolol	51384-51-1	C15H25NO3
metoprolol acid	56392-14-4	C14H21NO4
O-desmethylvenlafaxine	93413-62-8	C16H25NO2
oxazepam	604-75-1	C15H11N2O2Cl
pravastatine	81093-37-0	C23H36O7
propranolol	525-66-6	C16H21NO2
ranitidine	66357-35-5	C13H22N4O3S
sitagliptin	486460-32-6	C16H15F6N5O
sotalol	3930-20-9	C12H20N2O3S
sulfamethoxazole	723-46-6	C10H11N3O3S
tramadol	27203-92-5	C16H25NO2
triclosan	3380-34-5	C12H7Cl3O2
valsartan	137862-53-4	C24H29N5O3
valsartan acid	164265-78-5	C14H10N4O2
venlafaxine	93413-69-5	C17H27NO2

Table S2: ***Compound-specific method statistics for experimental samples***
Method statistics for samples processed using vacuum-assisted evaporation. 38 of 40 targeted compounds are shown (2 compounds were removed due to evaporation-related problems).

	Compound	MLOQ† (ng/L)	MLOD‡ (ng/L)	Abs. Yield§	Abs. Yield RSD	Matched ISTD?	Ion Mode
Compounds with measurable dissipation	atenolol	0.8	0.8	31%	18%	Y	Pos
	bezafibrate	3.2	1.6	79%	13%	Y	Pos
	caffeine	21.8	10.9	23%	20%	Y	Pos
	diclofenac	0.5	0.5	48%	14%	Y	Neg
	furosemide	1.2	1.2	21%	13%	Y	Neg
	ketoprofen	1.7	0.7	71%	16%	Y	Pos
	MCPA	0.66	0.66	38%	15%	Y	Neg
	metformin	3.7	3.7	33%	17%	Y	Pos
	metoprolol	0.7	0.7	37%	14%	Y	Pos
	propranolol	1.3	1.3	19%	28%	Y	Pos
	sitagliptin	5.5	5.5	23%	24%	N	Pos
	sotalol	1.8	0.9	28%	16%	Y	Pos
	valsartan	0.6	0.6	86%	12%	Y	Neg
Other Quantified Compounds	10,11-Dihydro-10,11-dihydroxy carbamazepine	3.6	1.4	35%	18%	N	Pos
	1H-benzotriazole	4.2	1.7	30%	21%	Y	Pos
	carbamazepine	0.6	0.6	41%	14%	Y	Pos
	carbamazepine-10,11-epoxide	1.5	1.5	34%	18%	N	Pos
	fluconazole	5.6	2.8	44%	17%	Y	Pos
	gabapentin	7.6	1.9	66%	17%	Y	Pos
	irbesartan	12.7	2.5	20%	13%	Y	Pos
	metoprolol acid	0.9	0.9	58%	17%	Y	Pos
	O-desmethylvenlafaxine	0.6	0.6	44%	17%	N	Pos
	oxazepam	0.8	0.4	61%	12%	Y	Pos
	sulfamethoxazole	5.5	2.2	23%	24%	Y	Pos
	tramadol	0.7	0.7	35%	18%	Y	Pos
	venlafaxine	0.7	0.7	34%	18%	Y	Pos
Detected but not Quantified Compounds	acridine	2.8	0.6	44%	17%	N	Pos
	bicalutamide¶	2.0	2.0	12%	132%	Y	Neg
	climbazole	2.5	2.5	10%	66%	Y	Pos
	ranitidine	26.6	13.3	9%	36%	Y	Pos
Targeted but Undetected Compounds	1-Methyl-1H-benzotriazole	3.6	1.4	35%	18%	N	Pos
	acetaminophen	14.9	14.9	17%	16%	Y	Pos
	anastrozole	1.1	0.5	47%	14%	Y	Pos
	clofibric acid	0.7	0.7	36%	15%	Y	Neg
	mecoprop	0.5	0.5	49%	15%	Y	Neg
	pravastatine	12.5	6.3	20%	12%	Y	Neg
	triclosan	34.6	13.9	4%	51%	Y	Neg
	valsartan acid	4.5	1.8	28%	19%	N	Neg

† MLOQ: see paper for definition.

‡ MLOD: see paper for definition.

§ Abs. recovery: Average recovery of the internal standard that was spiked into the sample prior to evaporation. Recovery was determined by quantification against the standard curve and thus includes both losses during evaporation/transfer to the vial and matrix effects affecting signal intensity. For compounds lacking a corresponding internal standard, the recovery for non-matched internal standard is listed.

¶ Bicalutamide was detected above the theoretical MLOQ but extreme variation in recovery made reliable quantification impossible.

Table S3: ***Compound-specific method statistics for abiotic control samples***
Method statistics for the abiotic samples that were directly injected. 38 of 40 targeted compounds are shown (2 compounds were removed from the study due to evaporation-related problems).

	Compound	MLOQ	MLOD	Abs. Yield	Abs. Yield RSD
Quantified Compounds Corresponding to Degraded Compounds in Experiment	atenolol	5.2	5.2	96%	14%
	furosemide	9.58	9.58	52%	14%
	ketoprofen	13.0	6.5	77%	6%
	metformin	26.7	26.7	93%	6%
	metoprolol	9.5	9.5	52%	12%
	sitagliptin	25.2	10.1	99%	8%
	sotalol	5.1	5.1	99%	7%
	valsartan	16.2	16.2	62%	17%
Other Quantified Compounds	10,11-Dihydro-10,11-dihydroxy carbamazepine	37.7	37.7	66%	5%
	1H-benzotriazole	37.7	7.5	66%	6%
	carbamazepine	6.2	6.2	81%	7%
	carbamazepine-10,11-epoxide	8.6	8.6	58%	6%
	gabapentin	84.1	21.0	119%	5%
	metoprolol acid	8.6	4.3	116%	5%
	O-desmethylvenlafaxine	6.1	6.1	82%	6%
	oxazepam	14.5	14.5	69%	8%
	sulfamethoxazole	25.2	10.1	99%	8%
	tramadol	7.5	7.5	66%	5%
	venlafaxine	8.6	8.6	58%	6%
Detected but not Quantified Compounds	caffeine	143.5	35.9	70%	7%
	diclofenac†	7.5	7.5	67%	10%
	fluconazole	30.6	12.2	82%	6%
	MCPA	6.6	6.6	76%	6%
Targeted but Undetected Compounds	1-Methyl-1H-benzotriazole	7.5	7.5	66%	5%
	acetaminophen	72.9	36.5	69%	10%
	acridine	6.1	6.1	82%	6%
	anastrozole	8.9	8.9	56%	10%
	bezafibrate	71.6	71.6	35%	25%
	bicalutamide	36.9	36.9	14%	88%
	climbazole	26.0	26.0	19%	47%
	clofibric acid	7.0	7.0	72%	8%
	irbesartan	62.1	31.1	16%	53%
	mecoprop	6.6	6.6	76%	6%
	pravastatine	352.3	176.1	14%	35%
	propranolol	14.4	14.4	35%	15%
	ranitidine	90.2	90.2	55%	15%
	triclosan	76.5	30.6	33%	26%
	valsartan acid	40.8	16.3	61%	10%

† Found in high levels in the blanks and not considered quantifiable for abiotic samples.

Table S4: ***pH, temperature, dissolved oxygen and conductivity***

Table S4a: pH

	29-Apr	30-Apr	2-May	5-May	9-May	13-May
Field A	7.91	7.94	7.89	7.90	7.86	7.91
Field B	7.93	7.96	7.92	7.93	7.91	7.89
Field C	7.94	7.97	7.93	7.93	7.96	7.96
Lab A	7.81	8.42	8.54	8.64	8.65	8.64
Lab B	7.85	8.44	8.61	8.65	8.65	8.64
Lab C	7.88	8.49	8.59	8.66	8.67	8.67
Fyris River	7.91	7.95	7.80	7.93	7.89	7.85

Table S4b: Temperature (°C)

	29-Apr	30-Apr	2-May	5-May	9-May	13-May
Field A	14.3	13.2	10.9	9.5	10.0	11.3
Field B	14.4	13.3	11.0	9.5	9.8	11.6
Field C	14.4	13.2	10.8	9.5	9.8	11.7
Lab A	13.8	12.6	12.6	12.5	12.5	12.5
Lab B	13.8	12.4	12.5	12.4	12.4	12.5
Lab C	13.9	12.4	12.5	12.5	12.5	12.4
Fyris River	13.6	13.0	11.9	9.6	9.8	12.2

Table S4c: Dissolved oxygen concentration (mg/L)

	29-Apr	30-Apr	2-May	5-May	9-May	13-May
Field A	9.90	9.37	9.26	10.20	10.25	10.39
Field B	9.77	8.87	9.23	10.37	10.70	10.61
Field C	9.71	8.85	9.27	10.25	10.58	9.98
Lab A	9.94	10.35	10.13	10.43	10.48	10.74
Lab B	9.91	10.38	10.16	10.45	10.42	10.63
Lab C	9.91	10.36	10.11	10.43	10.42	10.66
Fyris River	9.90	9.88	9.54	10.54	10.80	10.39

Table S4d: Conductivity (µS/cm)

	29-Apr	30-Apr	2-May	5-May	9-May	13-May
Field A	498	495	492	491	492	493
Field B	497	495	491	492	490	492
Field C	495	495	493	492	491	491
Lab A	500	492	492	498	505	510
Lab B	500	492	493	499	503	506
Lab C	501	492	494	498	516	528
Fyris River	494	509	517	481	529	556

Table S5: ***Dissipation rate constants and biodegradation half-lives***

The compounds showing significant degradation over the course of two weeks are ordered here by ratio of average field to average lab half-life. The errors given are the standard error. Stars denote a significant difference in half-life between lab and field.

Table S5a: Dissipation rate constants from regressions

Compound	k (day^{-1})					
	Field A	Field B	Field C	Lab A	Lab B	Lab C
sitagliptin	-0.097 ± 0.006	-0.072 ± 0.017	-0.104 ± 0.012	-0.042 ± 0.014	-	-0.068 ± 0.006
metformin*	-0.035 ± 0.006	-0.029 ± 0.005	-0.038 ± 0.007	-0.024 ± 0.003	-0.018 ± 0.002	-0.021 ± 0.005
MCPA	-0.178 ± 0.044	-0.147 ± 0.030	-0.131 ± 0.042	-0.129 ± 0.038	-0.162 ± 0.046	-
diclofenac	-0.039 ± 0.004	-0.034 ± 0.006	-0.052 ± 0.005	-0.050 ± 0.004	-0.051 ± 0.006	-0.045 ± 0.002
bezafibrate	-0.093 ± 0.007	-0.109 ± 0.007	-0.145 ± 0.030	-0.143 ± 0.013	-0.128 ± 0.006	-0.138 ± 0.008
furosemide	-0.097 ± 0.007	-0.106 ± 0.008	-0.153 ± 0.011	-0.165 ± 0.034	-0.146 ± 0.030	-0.124 ± 0.016
ketoprofen	-0.115 ± 0.015	-0.123 ± 0.011	-0.155 ± 0.016	-0.221 ± 0.041	-0.110 ± 0.039	-0.212 ± 0.044
valsartan*	-0.097 ± 0.008	-0.101 ± 0.009	-0.115 ± 0.007	-0.160 ± 0.006	-0.165 ± 0.009	-0.139 ± 0.007
caffeine*	-0.105 ± 0.009	-0.107 ± 0.010	-0.121 ± 0.012	-0.180 ± 0.009	-0.192 ± 0.004	-0.165 ± 0.040
propranolol*	-0.054 ± 0.009	-0.033 ± 0.008	-0.047 ± 0.008	-0.100 ± 0.010	-0.124 ± 0.007	-0.113 ± 0.005
sotalol*	-0.035 ± 0.007	-0.029 ± 0.008	-0.048 ± 0.006	-0.104 ± 0.004	-0.111 ± 0.011	-0.092 ± 0.006
atenolol*	-0.126 ± 0.011	-0.124 ± 0.010	-0.156 ± 0.011	-0.422 ± 0.014	-0.342 ± 0.012	-0.400 ± 0.011
metoprolol*	-0.027 ± 0.003	-0.030 ± 0.004	-0.039 ± 0.005	-0.116 ± 0.004	-0.115 ± 0.010	-0.101 ± 0.004

Table S5b: Biodegradation half-lives

Compound	Half-Life (days)†								Ratio
	Field A	Field B	Field C	Lab A	Lab B	Lab C	Avg. Field	Avg. Lab	
sitagliptin	7.2 ± 0.4	9.7 ± 2.2	6.7 ± 0.8	16.6 ± 5.5	-	10.3 ± 0.9	7.8	13.5	0.58
metformin*	19.9 ± 3.2	24.0 ± 3.8	18.1 ± 3.5	29.0 ± 4.0	38.6 ± 5.0	32.6 ± 7.3	20.6	33.4	0.62
MCPA	3.9 ± 1.0	4.7 ± 1.0	5.3 ± 1.7	5.4 ± 1.6	4.3 ± 1.2	-	4.6	4.8	0.96
diclofenac	17.8 ± 2.0	20.1 ± 3.2	13.3 ± 1.4	14.0 ± 1.0	13.6 ± 1.6	15.5 ± 0.7	17.1	14.4	1.19
bezafibrate	7.4 ± 0.5	6.3 ± 0.4	4.8 ± 1.0	4.9 ± 0.4	5.4 ± 0.3	5.0 ± 0.3	6.2	5.1	1.21
furosemide	7.2 ± 0.5	6.5 ± 0.5	4.5 ± 0.3	4.2 ± 0.9	4.7 ± 1.0	5.6 ± 0.7	6.1	4.8	1.25
ketoprofen	6.0 ± 0.8	5.6 ± 0.5	4.5 ± 0.5	3.1 ± 0.6	6.3 ± 2.3	3.3 ± 0.7	5.4	4.2	1.27
valsartan*	7.2 ± 0.6	6.9 ± 0.6	6.0 ± 0.3	4.3 ± 0.2	4.2 ± 0.2	5.0 ± 0.2	6.7	4.5	1.48
caffeine*	6.6 ± 0.6	6.5 ± 0.6	5.7 ± 0.6	3.8 ± 0.2	3.6 ± 0.1	4.2 ± 1.0	6.3	3.9	1.61
propranolol*	12.8 ± 2.1	21.1 ± 5.1	14.7 ± 2.4	6.9 ± 0.7	5.6 ± 0.3	6.2 ± 0.3	16.2	6.2	2.60
sotalol*	19.6 ± 3.7	23.9 ± 6.4	14.4 ± 1.7	6.7 ± 0.2	6.3 ± 0.6	7.5 ± 0.5	19.3	6.8	2.84
atenolol*	5.5 ± 0.5	5.6 ± 0.5	4.4 ± 0.3	1.6 ± 0.1	2.0 ± 0.1	1.7 ± 0.0	5.2	1.8	2.88
metoprolol*	25.3 ± 3.2	22.9 ± 2.8	17.7 ± 2.2	6.0 ± 0.2	6.0 ± 0.5	6.8 ± 0.3	22.0	6.3	3.50

† Half-lives were not calculated for sitagliptin (Lab B) and MCPA (Lab C) due to poor linear fits ($R^2 < 0.7$).

Table S6: *R^2 values from regressions*

	R^2					
Compound	**Field A**	**Field B**	**Field C**	**Lab A**	**Lab B**	**Lab C**
sitagliptin	0.98	0.83	0.95	0.70	0.41†	0.97
metformin	0.91	0.91	0.87	0.93	0.95	0.83
MCPA	0.89	0.89	0.83	0.85	0.92	0.59†
diclofenac	0.95	0.91	0.96	0.98	0.96	0.99
bezafibrate	0.98	0.99	0.92	0.98	1.00	0.99
furosemide	0.98	0.98	0.98	0.89	0.92	0.95
ketoprofen	0.94	0.97	0.97	0.90	0.89	0.89
valsartan	0.98	0.97	0.99	0.99	0.99	0.99
caffeine	0.97	0.97	0.96	1.00	1.00	0.89
propranolol	0.90	0.81	0.90	0.96	0.99	0.99
sotalol	0.87	0.78	0.95	0.99	0.97	0.99
atenolol	0.97	0.97	0.98	1.00	1.00	1.00
metoprolol	0.94	0.94	0.94	1.00	0.98	0.99

† These regressions were removed from the dataset due to poor fit.

Figure S1: ***Modeled effect of 10% leak***

We modeled the impact of a 10% (35 mL) leak in one of the field bottles on the first day to evaluate the impact of the leak observed in the experiment. Since the bottles were underwater, water leaked in rather than out. This assumes the same concentration of the compound in the outside water as was present in the bottle at the beginning of the test. The impact is theoretically greatest for compounds with a short half-life so we used a 3.5 day half-life. The calculated half-life had an error of 0.67% compared to the theoretical one.

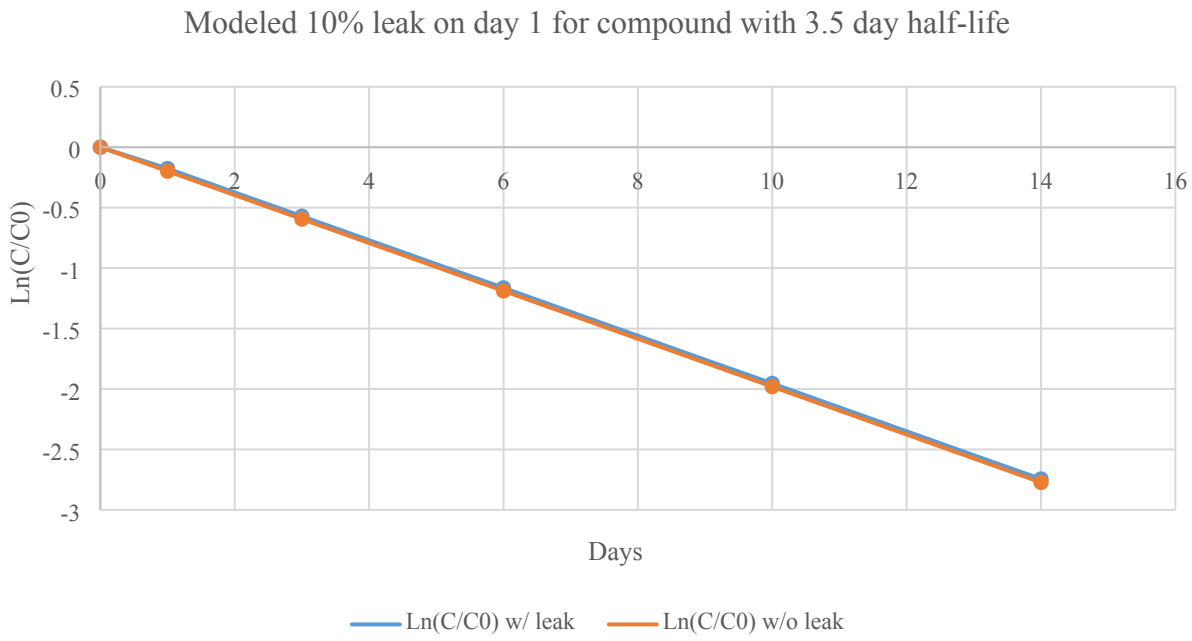


Figure S2: ***Concentration time series for degraded compounds***

One outlier each was removed from the caffeine and the ketoprofen datasets.

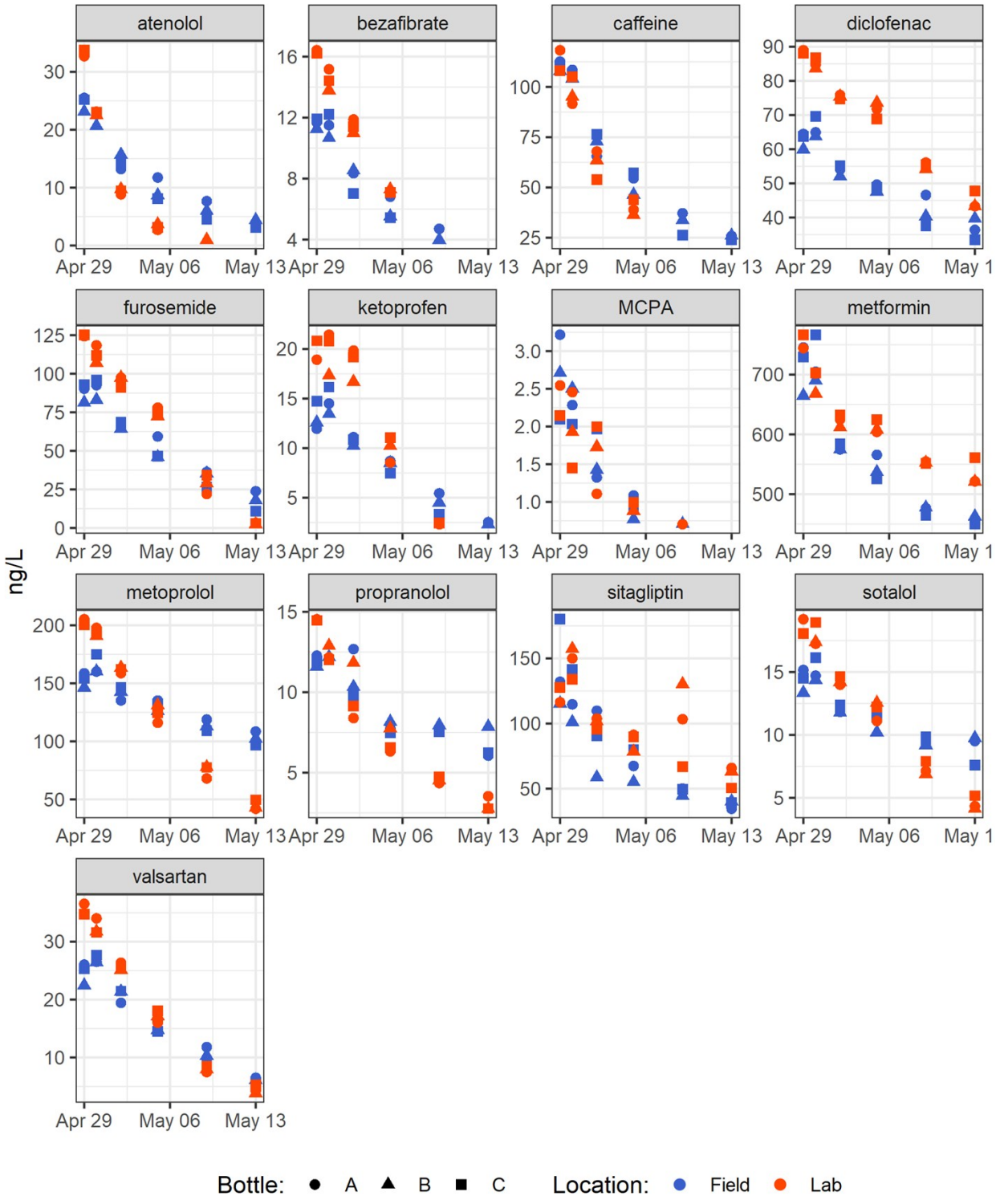
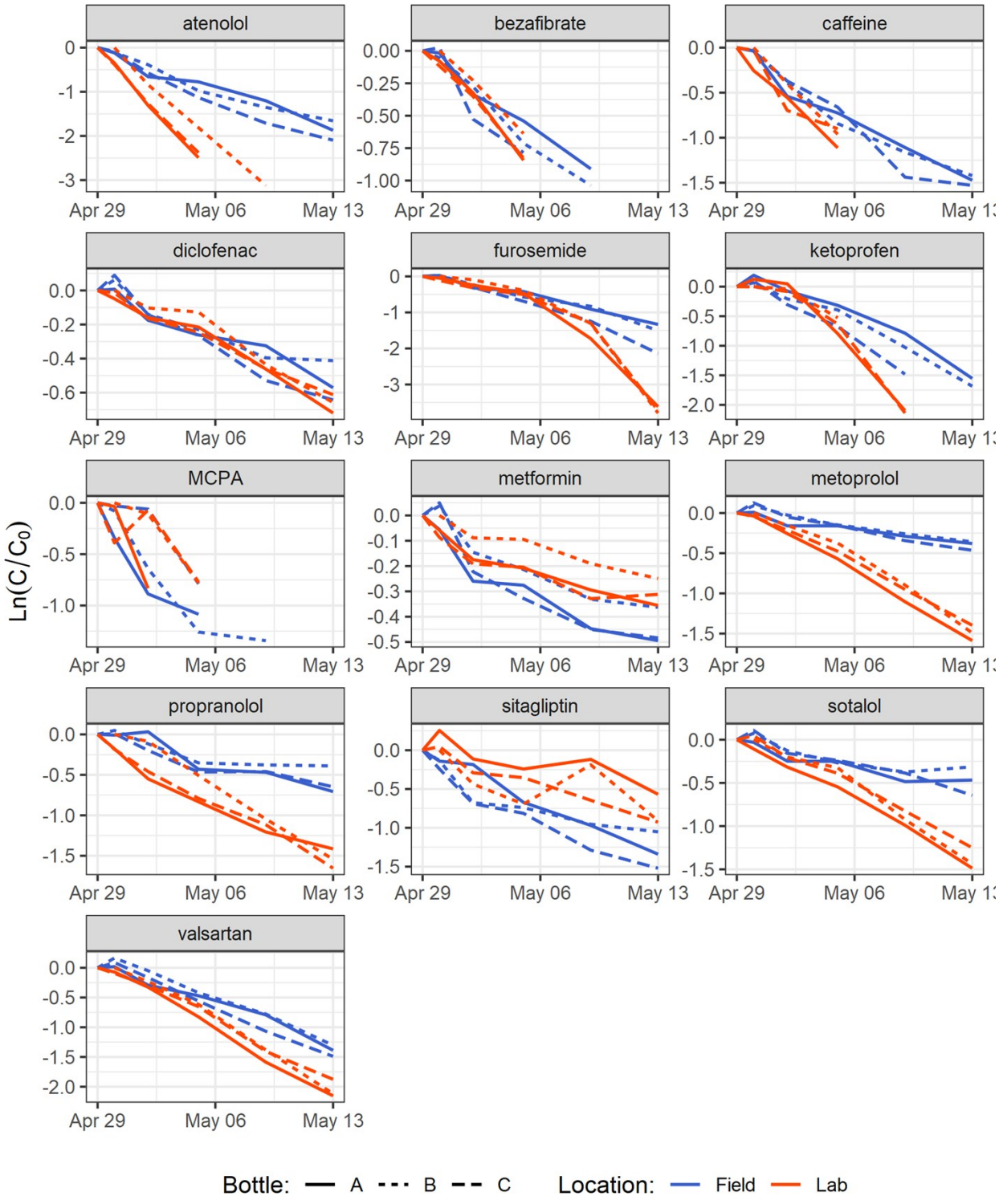


Figure S3: ***Natural logarithm of normalized concentrations for degraded compounds***

One outlier each was removed from the caffeine and the ketoprofen datasets.



Text S1: Calculations of mixing distance for the Fyris River

We assumed a mid-depth, centerline discharge of wastewater effluent and performed calculations according to the EPA's *Handbook: Stream Sampling for Waste Load Allocation Applications* page 3-3.[1] The values x_z and x_y are estimates of the distances where 95% of the vertical and transverse mixing respectively are complete. The expressions for x_z and x_y are simple estimates and must be multiplied by a factor of ft/s to give the appropriate units. Calculations are performed in feet.

$$x_z = 0.1h^2/\varepsilon_z \quad x_y = 0.1w^2/\varepsilon_y$$

$$\varepsilon_z = \beta hU \quad \varepsilon_y = \alpha hU$$

We assume an average river depth *h* of 10 feet, an average river width *w* of 90 feet, $\beta = 0.05$ to 0.07, and $\alpha = 0.3$ to 1.0 (β and α as suggested by EPA). Average river flow rate was 5.3 m^3/s (190 ft^3/s) which gives an average linear velocity *U* of 0.21 ft/s based on the average river cross-section.

This gives an estimate of $\varepsilon_z = 0.11$ to 0.15 ft^2/s and $\varepsilon_y = 0.63$ to 2.1 ft^2/s, giving $x_z = 67$ ft to 90 ft (20 to 27 m) and $x_y = 390$ ft to 1300 ft (120 to 400 m). These are considerably shorter distances than the 1.1 km downstream between wastewater effluent discharge and our sampling point, so we conclude that the effluent was well-mixed into the river at our sampling location.

References

[1] W. B. Mills, G. L. Bowie, T. M. Grieb, K. M. Johnson and R. C. Whittemore, *Stream Sampling for Waste Load Allocation Applications*, U.S. Environmental Protection Agency, Washington, 1986; pp 1-63.