Environmental Science & Technology

Article

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Persistence and Potential Effects of Complex Organic Contaminant Mixtures in Wastewater-Impacted Streams

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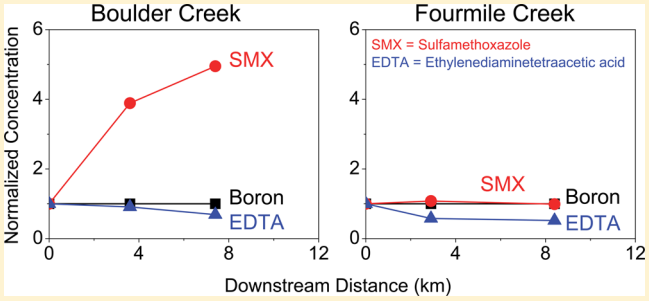
Supporting Information

ABSTRACT: Natural and synthetic organic contaminants in municipal wastewater treatment plant (WWTP) effluents can cause ecosystem impacts, raising concerns about their persistence in receiving streams. In this study, Lagrangian sampling, in which the same approximate parcel of water is tracked as it moves downstream, was conducted at Boulder Creek, Colorado and Fourmile Creek, Iowa to determine in-stream transport and attenuation of organic contaminants discharged from two secondary WWTPs. Similar stream reaches were evaluated, and samples were collected at multiple sites during summer and spring hydrologic conditions. Travel times to the most downstream (7.4 km) site in Boulder Creek were 6.2 h during the summer and 9.3 h during the spring, and to the Fourmile Creek 8.4 km downstream site times were 18 and 8.8 h, respectively. Discharge was measured at each site, and integrated composite samples were collected and analyzed for >200 organic contaminants including metal complexing agents, nonionic surfactant degradates, personal care products, pharmaceuticals, steroidal hormones, and pesticides. The highest concentration (>100 μg L^{-1}) compounds detected in both WWTP effluents were ethylenediaminetetraacetic acid and 4-nonylphenolethoxycarboxylate oligomers, both of which persisted for at least 7 km downstream from the WWTPs. Concentrations of pharmaceuticals were lower (<1 μg L^{-1}), and several compounds, including carbamazepine and sulfamethoxazole, were detected throughout the study reaches. After accounting for in-stream dilution, a complex mixture of contaminants showed little attenuation and was persistent in the receiving streams at concentrations with potential ecosystem implications.



INTRODUCTION

Wastewater treatment plant (WWTP) effluents are complex mixtures of biologically active organic chemicals, including pharmaceuticals and personal care products, many of which have been detected in receiving streams.[1−6] The potential impact of these contaminants on aquatic ecosystems is illustrated by adverse effects of hormonally active chemicals, such as 17β-estradiol and 4-nonylphenol, on the reproductive health of fish.[7−10] With increasing demands on water resources, reuse of WWTP effluent to augment water supplies will become more frequent,[11] and there is a need to better understand in-stream transport of WWTP-derived contaminants. Water-quality impacts downstream from a WWTP are determined by mixing (dilution) with upstream water, hydraulic residence times (HRT), and in-stream attenuation processes.[12,13]

Multiple biotic and abiotic reactions occur simultaneously in heterogeneous streams, making it difficult to define all essential biogeochemical variables. In-stream tracer experiments produce composite "pseudo-first-order" attenuation rates[14−17] that do not define specific transformation processes such as sorption, biodegradation, volatilization, and photolysis.[18−20] Tracer tests can be conducted using direct dosing, in which chemicals are added to the stream as short-term pulses,[16,17,21] or using intrinsic tracers introduced by point-source inputs.[5,14,15]

This study evaluates the occurrence, transport, and potential ecological impact of a broad suite of organic contaminants in two geographically and hydrologically distinct streams at the point of the first major WWTP discharge. Lagrangian sampling was used to track specific parcels of water as they moved downstream, allowing determination of in situ attenuation. Spatiotemporal variability was characterized under different flow regimes using mass-balance and error analysis. This

Received: September 22, 2012
Revised: December 14, 2012
Accepted: December 24, 2012
Published: February 11, 2013

ACS Publications

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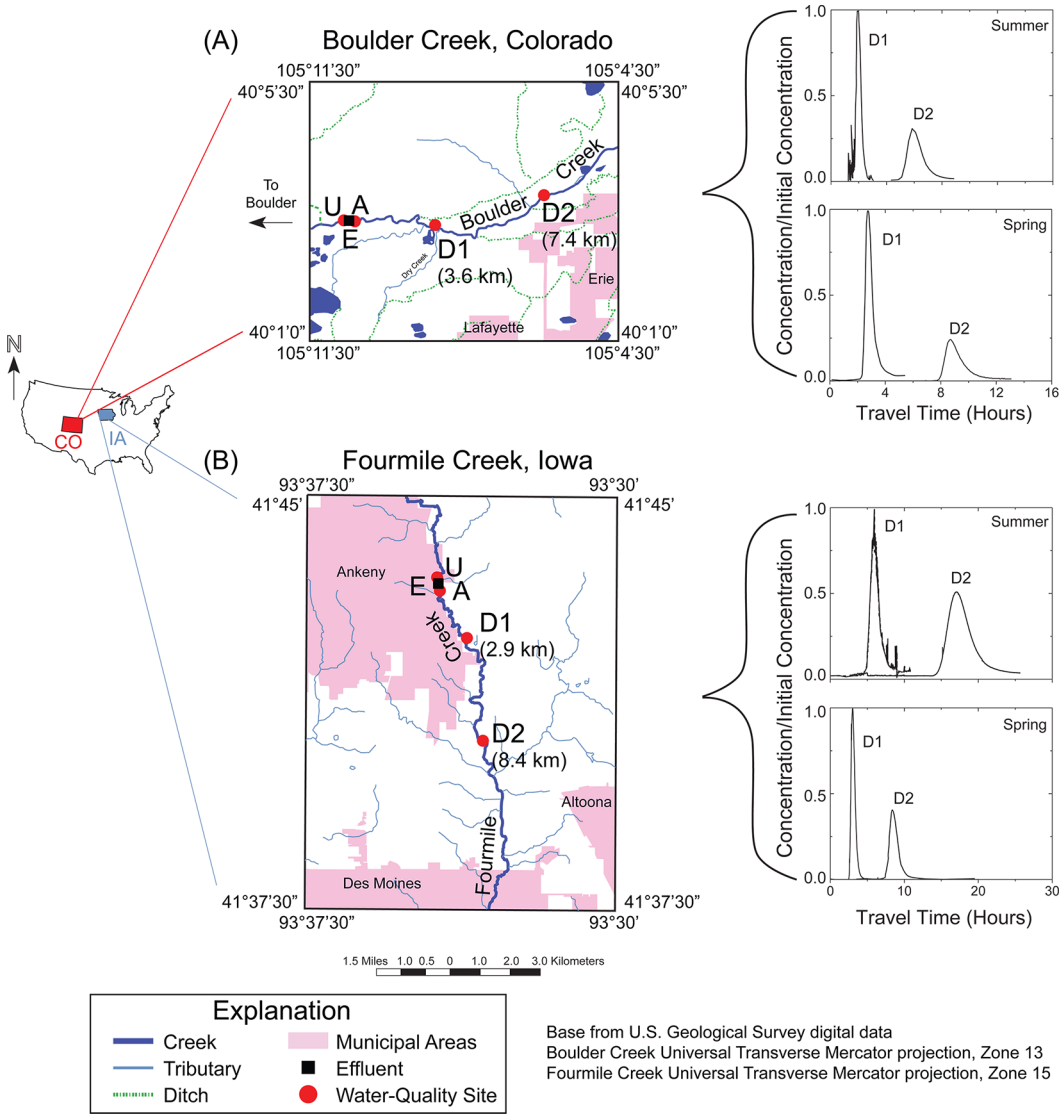

Figure 1. Locations of the (A) Boulder Creek, Colorado and (B) Fourmile Creek, Iowa study reaches investigated during the summer 2003 and spring 2005 Lagrangian samplings. Also shown are rhodamine WT breakthrough curves. [U, sites upstream from wastewater treatment plants (WWTP); E, WWTP effluents; A, calculated mixing zones; D1 and D2, sites downstream from WWTP with distances given in km].

integrated approach provides hydraulic control through stream-discharge and velocity measurements, which, combined with composite sampling and comprehensive chemical analysis, allows assessment of attenuation and potential ecosystem impacts. A companion paper reports on 58 inorganic constituents studied during the same set of field experiments.[5]

SITE DESCRIPTION

Boulder Creek, Colorado and Fourmile Creek, Iowa (Figure 1) were selected to evaluate in-stream persistence of WWTP-derived contaminants because they are headwater-urban streams, and the study reaches encompass the transition from "background" to "wastewater-impacted" streams. Differences between the two sites include the types of wastewater treatment and hydrology.

Boulder Creek runs through the City of Boulder, Colorado, and transects a gradient from pristine snowmelt conditions in the upper watershed to wastewater-impacted conditions in the lower basin.[4,22] The study reach (Figure 1A) extends from 0.1 km above the City of Boulder's WWTP to approximately 7.4 km downstream (hydraulic gradient varies from 0.0031 to 0.0039). Basin area upstream from the WWTP is 790 km^2 and population density during 2000 was 220 people/km^2. The Boulder WWTP discharged an annual average of 0.74 m^3 s^{-1} of effluent following secondary treatment by trickling filter/solids contact with chlorination (chlorine gas) and dechlorination (sodium bisulfite). There are three diversion canals and one small tributary in the reach downstream from the WWTP.

Fourmile Creek, located near Ankeny, Iowa, drains a predominantly agricultural watershed, and the study reach (Figure 1B) extends from 0.1 km above the City of Ankeny's WWTP to approximately 8.4 km downstream (hydraulic gradient varies from 0.0007 to 0.0013).[23] Basin area upstream from the WWTP is 160 km^2 and population density during 2000 was 170 people/km^2. The Ankeny WWTP discharged an annual average of 0.21 m^3 s^{-1} of secondary effluent treated by fine-bubble aeration with ultraviolet light disinfection.

METHODS

Lagrangian Sampling and Chemical Analysis. Lagrangian sampling, in which a parcel of water is tracked as it moves downstream under natural-gradient conditions, was combined with integrative cross-sectional sampling to determine in situ attenuation of organic contaminants. This approach minimizes the effects of temporal variations in chemical loading by incorporating stream discharge measurements[24] and transport times determined from rhodamine WT tracer tests[5,21,25] into the experimental design. Sampling was coordinated to capture summer low-flow conditions (August−September 2003) on the falling limb of the annual stream hydrograph, and spring low-flow conditions (March−April 2005) on the rising limb of the hydrograph, periods when dilution of WWTP effluent by streamwater was low.

Boulder Creek and Fourmile Creek had four sampling sites (Figure 1): 0.1 km upstream from the WWTPs (U), the WWTP effluents (E), and two sites downstream from the WWTPs (D1 and D2). Sampling at the downstream sites was based on transport times determined from tracer tests (Table 1), adjusted for differences in flow between the tracer tests and samplings. Equal-width-depth integrated composite-water samples[26] were collected using clean protocols and stainless steel/Teflon equipment.[27] At each downstream site, sampling was conducted over the period of time required to capture >75% of the initial parcel of water. Due to dispersion, sampling times increased from sites D1 to D2. A minimum of five cross-sectional samples were collected as the parcel of water passed by the sampling point and composited in a 19-L glass carboy before splitting into subsamples for individual chemical analysis using a Teflon churn and cone splitter. Details on sample collection procedures and analytical methods (seven organic and two inorganic) used in this study are summarized in the Supporting Information (SI) and elsewhere.[28]

Hydraulic Analysis. Understanding in-stream attenuation requires accurate determination of hydrological variables, such as discharge volumes and flow velocities, in order to constrain dilution and transport times. Two approaches were used to assess the Lagrangian sampling data: normalized removal relative to a conservative tracer, and quantitative mass flow and error analysis. Generally, a longitudinal profile consisting of U, E, D1, D2, and a calculated mixing zone site A located 0.1 km downstream from E (Figure 1) was used to evaluate contaminant loss or gain along the flow path. Concentrations at mixing zone site A were calculated from concentrations and discharges at sites U and E, and define the upstream boundary condition for the WWTP-impacted reach. Boulder Creek discharge and contaminant concentrations were measured at U, E, D1, and D2, but not at A. Fourmile Creek discharge and contaminant concentrations were measured at U, A, D1, and D2, but not at E (determined by difference between U and A).

Normalized Removal. Normalized removal (Rem_{norm}) calculations used measured concentrations of the contaminants and the conservative tracer boron, similar to the approach used by Schreiber and Mitch.[29]

$$Rem_{norm} = (C_i/C_{boron,i})/(C_{i,A}/C_{boron,A}) \quad (1)$$

where C_i is measured target compound concentration (μg L^{-1}) at site i, $C_{boron,i}$ is measured boron concentration at site i, and $C_{i,A}$ and $C_{boron,A}$ are calculated concentrations at site A.

Mass Flow. The following equations are presented in a generalized form to accommodate the variety of field conditions encountered. Measured in-stream mass flows (M_i; g d^{-1}) were defined as the product of C_i and measured discharges (Q_i; m^3 s^{-1}) at each sampling site, and were compared to calculated mass flows (Θ_i; g d^{-1}), which are a function of calculated discharges (Ω_i; m^3 s^{-1}) at each sampling site.

Downstream Site 1. At site D1, Θ_{D1} is the sum of the mass flow at site A, inflows, and outflows along the stream segment multiplied by a discharge discrepancy parameter ($e_i = Q_i/\Omega_i$), to correct Θ_{D1} for variations in discharge:

$$\Theta_{D1} = (\Theta_A + \sum_{i=1}^{n} C_{in,i}Q_{in,i} - \sum_{j=1}^{m} C_{out,j}Q_{out,j})e_{D1} \quad (2)$$

Table 1. Hydrological Data for the Boulder Creek (BC), Colorado, and Fourmile Creek (FC), Iowa Summer 2003 and Spring 2005 Tracer Experiments and Lagrangian Samplings[a]

site	distance from WWTP discharge (km)	stream discharge (m^3 s^{-1})	flow velocity (m s^{-1})	hydraulic retention time (h)
Boulder Creek				
August 21, 2003: Tracer Experiment				
BC-D1	3.6	2.97	0.48^t, na^m	2.1
BC-D2	7.4	0.94	0.33^t, na^m	6.2
April 16 to 17, 2005: Tracer Experiment				
BC-D1	3.6	2.60	0.32^t, na^m	3.1
BC-D2	7.4	1.29	0.22^t, na^m	9.3
September 3, 2003: Lagrangian Sampling				
BC-U	−0.1	1.62	0.27^m	
BC-E	0	0.92	0.31^m	
BC-D1	3.6	2.41	0.36^m	
BC-D2	7.4	1.84	0.60^m	
April 19, 2005: Lagrangian Sampling				
BC-U	−0.1	1.69	0.28^m	
BC-E	0	1.11	0.29^m	
BC-D1	3.6	2.60	0.45^m	
BC-D2	7.4	1.29	0.45^m	
Fourmile Creek				
July 30 to 31, 2003: Tracer Experiment				
FC-D1	2.9	0.33	0.13^t, 0.09^m	6.4
FC-D2	8.4	0.24	0.13^t, 0.30^m	18
March 3 to 4, 2005: Tracer Experiment				
FC-D1	2.9	1.09	0.26^t, 0.36^m	3.1
FC-D2	8.4	1.12	0.27^t, 0.43^m	8.8
August 5, 2003: Lagrangian Sampling				
FC-U	−0.1	0.04	0.04^m	
FC-E	0	na	na	na
FC-D1	2.9	0.17	0.07^m	
FC-D2	8.4	0.16	0.23^m	
March 8, 2005: Lagrangian Sampling				
FC-U	−0.1	0.62	0.22^m	
FC-E	0	na	na	na
FC-D1	2.9	0.92	0.27^m	
FC-D2	8.4	0.90	0.20^m	

[a]U, upstream from wastewater treatment plant (WWTP); E, WWTP effluent; BC-D1, BC-D2, FC-D1, and FC-D2, sampling sites downstream from the WWTP; *t*, reach-integrated flow velocities determined from tracer test hydraulic retention times; *m*, instantaneous flow velocity determined by flow meter during discharge measurement; na, not analyzed.

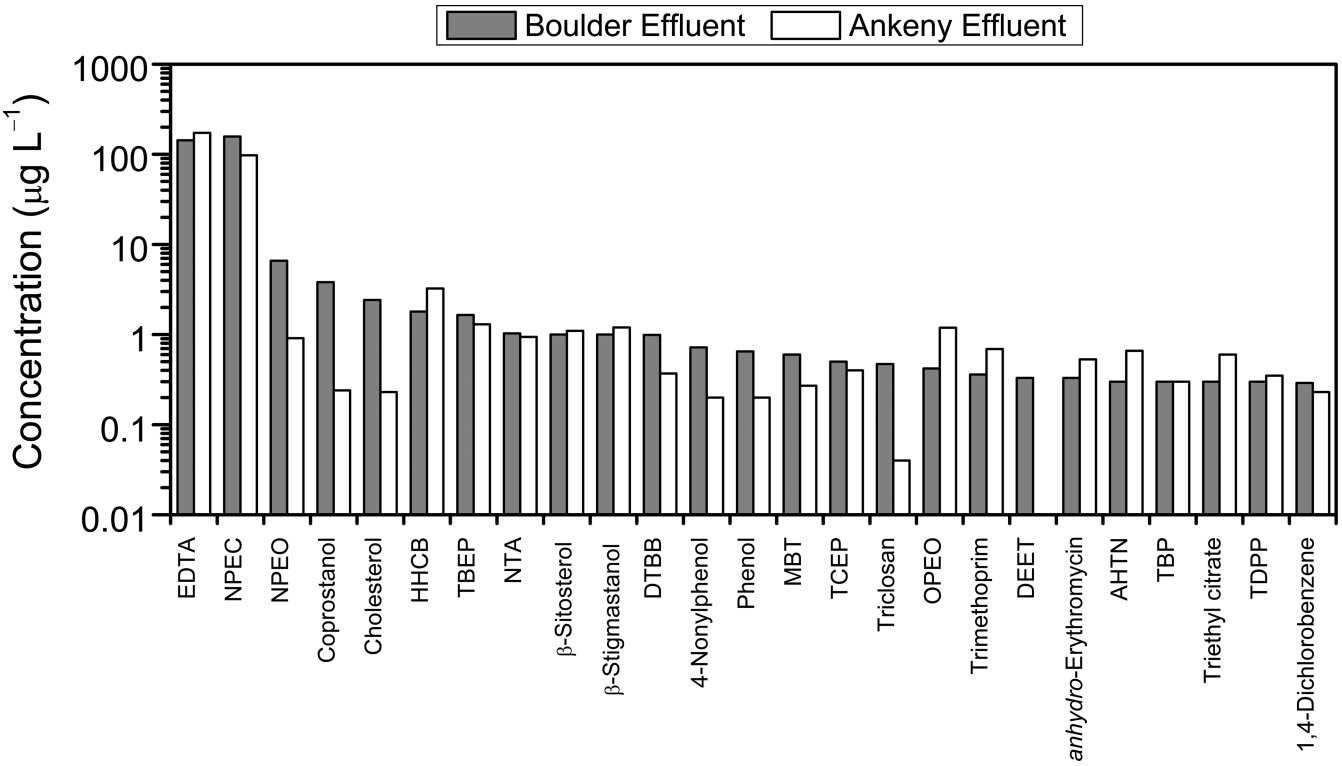

Figure 2. Twenty-five highest concentration organic compounds detected in replicate samples from the Boulder, Colorado and Ankeny, Iowa wastewater treatment plant (WWTP) effluents during the spring 2005 sampling. All compounds detected in the WWTP effluents are summarized in Table SI-8. The complete data set for all compounds and samples analyzed in this study is presented elsewhere.[28] [Compounds ranked from highest to lowest concentration in the Boulder effluent; AHTN, 7-acetyl-1,1,3,4,4,6-hexamethyl-5,6,7,8-tetrahydronaphthalene; DEET, *N,N*-diethy-*meta*-toluamide; DTBB, 2,6-di-*tert*-butyl-1,4-benzoquinone; EDTA, ethylenediaminetetraacetic acid; HHCB, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[γ]-2-benzopyran; MBT, 5-methyl-1H-benzotriazole; NTA, nitrilotriacetic acid; NPEC, 4-nonylphenolethoxycarboxylic acids; NPEO, 4-nonylphenolethoxylates; OPEO, 4-*tert*-octylphenolethoxylates; TBP, tris(butyl)phosphate; TBEP, tris(2-butoxyethyl)phosphate; TCEP, tris(2-chloroethyl)phosphate; TDPP, tris(dichloroisopropyl)phosphate].

where Θ_A is mass flow at site A (sum of U and E mass flows), $C_{in,i}$ is inflow concentration at location i, $C_{out,j}$ is outflow concentration at location j, $Q_{in,i}$ is measured inflow at location i, $Q_{out,j}$ is measured outflow at location j, and n and m are the number of inflows and outflows in the stream reach.

At site D1, Ω_{D1} is defined as the sum of discharges along the stream segment:

$$\Omega_{D1} = \Omega_A + \sum_{i=1}^{n} Q_{in,i} - \sum_{j=1}^{m} Q_{out,j} \tag{3}$$

where Ω_A is calculated discharge at site A (sum of U and E discharges).

Downstream Site 2. At site D2, Θ_{D2} and Ω_{D2} are defined as

$$\Theta_{D2} = (C_{D1}Q_{D1} + \sum_{i=1}^{n} C_{in,i}Q_{in,i} - \sum_{j=1}^{m} C_{out,j}Q_{out,j})e_{D2} \tag{4}$$

and

$$\Omega_{D2} = Q_{D1} + \sum_{i=1}^{n} Q_{in,i} - \sum_{j=1}^{m} Q_{out,j} \tag{5}$$

In-Stream Gains and Losses. Error analysis[30] was applied to assess the significance of mass loss or gain at the downstream sites. Generally, results are defined in terms of a mass flow discrepancy ratio (R_i) ± overall uncertainty (E_i). R_i is defined as

$$R_i = \frac{M_i/\Theta_i}{(1 + e_i + \varepsilon_{i,Cl})/3} \tag{6}$$

where $\varepsilon_{i,Cl}$ (= M_i/Θ_i) is a mass flow discrepancy parameter for the "conservative" element chloride. The $\varepsilon_{i,Cl}$ parameter is similar to e_i and measures deviation from ideal chloride mass flow corrected for discharge discrepancies. E_i is defined by

$$E_i = \sqrt{\mu_i^2 + \mathrm{var}(1, e_i, \varepsilon_{i,Cl})} \tag{7}$$

where var is variance, and μ_i is a ratio of uncertainty similar to the relative standard deviation and allows uncertainties to be compared on an equal basis by

$$\mu_i = \frac{m_i}{M_i} = \frac{\sqrt{q_i^2C_i^2 + c_i^2Q_i^2 + c_i^2q_i^2}}{M_i} \tag{8}$$

where m_i is measurement uncertainty in mass flow at site i, q_i is uncertainty in Q_i (8%, based on stream conditions and replicate cross-sectional measurements[27]), and c_i (relative standard deviation of replicate sample measurements) is uncertainty in C_i. If $|R_i - 1| > 2E_i$, in-stream gain or loss between sites was considered to be significant (similar to standard deviation analysis[30]). To be included in the mass flow and error analysis, a contaminant had to be detected in all replicate samples from the downstream sites at concentrations >3× the detection limit.

Attenuation Rates and Zone-of-Influence. In-stream "pseudo-first-order" attenuation rates were determined for

contaminants that met the >3× the detection limit by log–linear regression of contaminant mass and downstream transport times. Monte Carlo simulations of 10 000 slopes with errors in transport times and concentrations were used to assess uncertainties in mass recovery and error propagation. The attenuation rates and transport times were used to calculate downstream zones-of-influence, defined as the transport distance required to achieve 90% reduction of concentrations at site A (4.5 half-lives). The zone-of-influence assumes no additional contaminant loading other than the WWTP discharge.

Hazard Quotients. Potential ecosystem risks from individual contaminants can be estimated using the hazard quotient (HQ).[31]

$$HQ = MEC/LC50 \text{ or } MEC/NOEC \quad (9)$$

where MEC is maximum measured environmental concentration ($\mu g\ L^{-1}$), LC50 is 50% lethal effect concentration ($\mu g\ L^{-1}$), and NOEC is no-observable effect concentration ($\mu g\ L^{-1}$) for the most sensitive aquatic test species (bacteria, plants, invertebrates, and vertebrates). A HQ value <1 indicates limited potential ecosystem risk (with no additional safety factor) whereas a HQ >1 indicates potential risk.

■ RESULTS AND DISCUSSION

The integrated cross-sectional-stream sampling, discharge measurements, and comprehensive chemical analyses allowed internally consistent comparisons within and between the streams. Despite efforts to conduct true "Lagrangian" sampling, logistical limitations and unpredictable hydrological and anthropogenic variables add uncertainty to tracking the same parcel of water downstream. For example, timing of sampling with respect to the diurnal variability in WWTP flow and composition is important.[5]

Stream Hydraulics. Table 1 shows the differences in hydraulic parameters between Boulder Creek and Fourmile Creek, including discharge volumes and transport velocities. The impact of WWTP discharge on stream hydrology is illustrated by calculated hydraulic mixing ratios at site A. Discharge at Boulder Creek site U during the summer sampling was 40× greater than at Fourmile Creek site U. There was little difference (<5%) in Boulder Creek site U discharge between the summer and spring samplings, and hydraulic mixing ratios indicate that 36% and 40% of the streamflow at site A was WWTP effluent. Discharge at site D1 was similar during the summer and spring samplings, but site D2 had 69% and 50% less flow than site D1 due to diversions. The flow loss resulted in increased hydraulic retention times.

Fourmile Creek hydraulic mixing ratios indicated that 81% and 28% of streamflow at site A was WWTP effluent during the summer and spring samplings. In contrast to Boulder Creek, discharge at Fourmile Creek site U during the spring sampling was 16× greater than during the summer sampling (Table 1), indicating unstable hydraulic conditions (poststorm flow regression). The companion paper[5] reported in-stream loss of inorganic tracers during the spring sampling, indicating more complex hydrogeochemical conditions.

Effluent and Stream Chemistry. The municipal WWTPs used different secondary-treatment processes and the Boulder effluent was less nitrified than the Ankeny effluent.[5] However, the occurrence and concentrations of organic contaminants in the two effluents showed general agreement (Figure 2; Table SI-8).[28] Chemical mixing ratios using the conservative inorganic tracer boron[5,29] indicate that Boulder Creek site A was 32% and 36% effluent during the summer and spring samplings, respectively, and that Fourmile Creek was 79% and 21% effluent, similar to the hydraulic mixing ratios. The chemical data can be visualized using a two-member (sites U and E) mixing model and plotting organic contaminant concentrations against boron concentrations (Figure 3). If concentrations at

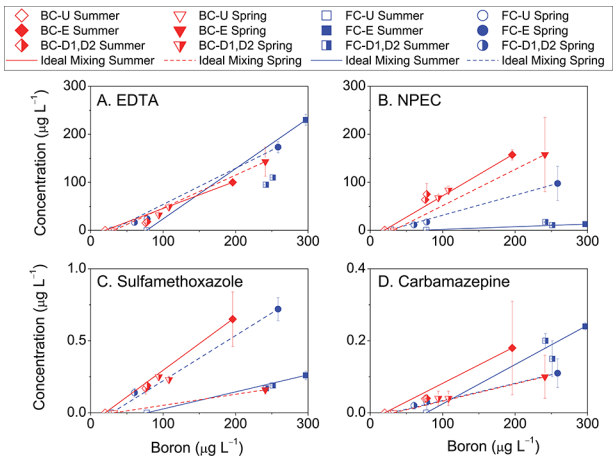

Figure 3. Concentrations of (A) ethylenediaminetetraacetic acid (EDTA), (B) 4-nonylphenolethoxycarboxylic acids (NPEC), (C) sulfamethoxazole, and (D) carbamazepine plotted against boron concentrations for the summer 2003 and spring 2005 Lagrangian samplings in Boulder Creek (BC), Colorado and Fourmile Creek (FC), Iowa. [U, upstream from wastewater treatment plant (WWTP); E, WWTP effluent; D1 and D2, downstream from WWTP; error bars represent one standard deviation (triplicate analysis) or one percent difference (duplicate analysis): vertical error bars represent organic contaminant uncertainty and horizontal error bars (too small to see) represent boron uncertainty].

the downstream sites plot near the mixing line, there is little attenuation. Despite efforts to minimize uncertainty, there is variability associated with this (or any) field study because it is not possible to control all relevant factors. The combined uncertainty in sampling the same parcel of water and analytical variability are indicated by error bars.

On the time-scale of the Lagrangian experiments, many of the >200 organic contaminants had little attenuation relative to boron during transport. Boulder Creek and Fourmile Creek are relatively shallow, and the attenuation that was observed represents interactions among the water column, suspended sediments, bed sediments, and macrophytes.[13−18] Because of low concentrations in the WWTP effluents, several contaminants were diluted below detection limits at the D1 sites and mass flow/error analysis could not be performed. For the subset of organic contaminants that met the >3× detection limit criteria, there was general agreement between normalized-removal and mass flow results (Table 2), although only the mass flow/error-analysis data have statistical significance.

Consumer-Product Contaminants. The high use amino-polycarboxylic chelating agent ethylenediaminetetraacetic acid (EDTA)[32] is resistant to biodegradation[33] and had the greatest concentration of any organic contaminant measured (Figure 2, Table SI-8). These results are consistent with previous studies on Boulder Creek,[4,22] other sites in the U.S.,[2,6] and a recent European survey that showed EDTA to be one of the most abundant organic contaminants in surface waters.[34] Although nitrilotriacetic acid (NTA) is used in greater quantities than

Table 2. Mass Flow Discrepancy Ratios (R_i) and Overall Uncertainty (E_i) between Measured and Calculated Values, and Normalized Removal for Select Organic Contaminants Detected at the Downstream Sites (D1 and D2) in Boulder Creek, Colorado (BC) and Fourmile Creek, Iowa (FC) during the Summer 2003 and Spring 2005 Lagrangian Samplings[a]

location and sampling	D1			D2		
	R_i	E_i	Rem_{norm}	R_i	E_i	Rem_{norm}
Boulder Creek – Summer 2003						
boron	0.94	0.11	1.00	0.89	0.22	1.00
caffeine	0.81	0.27	0.86	0.78	0.57	0.73
ciprofloxacin	0		0.00	na	na	0.00
1,7-dimethylxanthine	0		0.00	na	na	0.00
anhydro-erythromycin	0.54	0.15	0.57	0.64	0.44	0.42
erythromycin	0.45	0.13	0.48	1.19	0.47	0.54
ethylenediaminetetraacetic acid	0.44	0.12	0.46	1.19	0.43	0.53
nitrilotriacetic acid	0.99	0.20	1.05	1.82	0.48	1.52
4-nonylphenolethoxycarboxylic acids[a]	1.15	0.14	1.22	1.22	0.27	1.40
4-nonylphenolethoxylates[a]	0		0.00	na	na	0.00
4-*tert*-octylphenolethoxylates[a]	na	na	0.00	na	na	0.00
ofloxacin	0		0.00	na	na	0.00
sulfamethoxazole	0.73	0.34	0.77	1.14	0.94	0.85
tetracycline	0		0.00	na	na	0.00
tylosin	0		0.00	na	na	0.00
Boulder Creek – Spring 2005						
boron	0.98	0.08	1.00	0.89	0.16	1.00
carbamazepine	0.95	0.10	0.98	1.04	0.21	1.08
ciprofloxacin	0		0	na	na	0
1,4-dichlorobenzene	1.04	0.29	1.06	0		0
diphenhydramine	0.58	0.10	0.60	0.86	0.31	0.58
erythromycin	2.14	0.09	2.20	0.51	0.11	1.45
anhydro-erythromycin	0.94	0.12	0.96	0.82	0.25	0.91
ethylenediaminetetraacetic acid	0.89	0.23	0.91	0.62	0.45	0.69
4-nonylphenolethoxycarboxylic acids[a]	0		0	na	na	0
4-*tert*-octylphenoldiethoxylates[a]	0		0	na	na	0
ofloxacin	2.44	0.10	2.50	0.49	0.11	1.58
sulfamethoxazole	3.79	0.21	3.89	1.30	0.17	4.95
trimethoprim	0.62	0.09	0.63	0.62	0.24	0.48
Fourmile Creek – Summer 2003						
boron	1.01	0.08	1.00	0.96	0.09	1.00
carbamazepine	0.80	0.10	0.79	1.30	0.37	1.07
cimetidine	0		0.00	na	na	0.00
codeine	0.50	0.12	0.49	0.95	0.48	0.48
cotinine	0.54	0.11	0.54	1.09	0.46	0.61
diphenhydramine	0		0.00	na	na	0.00
anhydro-erythromycin	na		na	0		na
ethylenediaminetetraacetic acid	0.61	0.10	0.60	0.86	0.11	0.54
nitrilotriacetic acid	0.76	0.10	0.75	1.46	0.11	1.14
4-nonylphenolethoxycarboxylic acids[a]	1.06	0.10	1.05	1.58	0.11	1.71
ofloxacin	0		0.00	na	na	0.00
sulfamethoxazole	0.95	0.15	0.94	0.90	0.10	0.88
Fourmile Creek – Spring 2005						
boron	0.84	0.10	1.00	0.80	0.09	1.00
ciprofloxacin	0		0.00	na	na	0.00
cotinine	0		0.00	na	na	0.00
1,4-dichlorobenzene	1.02	0.12	1.22	0.39	0.12	0.60
diphenhydramine	0		0.00	na	na	0.00
erythromycin	0.73	0.16	0.87	0.62	0.12	0.68
anhydro-erythromycin	0.55	0.16	0.65	0.72	0.12	0.59
ethylenediaminetetraacetic acid	0.48	0.13	0.58	0.72	0.31	0.52
nitrilotriacetic acid	0.86	0.12	1.02	0.89	0.21	1.15
4-nonylphenolethoxycarboxylic acids[a]	0		0.00	na	na	0.00
4-nonylphenolethoxylates[a]	0.60	0.36	0.72	0.69	0.23	0.62
4-*tert*-octylphenolethoxylates[a]	0.93	0.16	1.11	0.45	0.28	0.63
ofloxacin	0.22	0.13	0.26	0.60	0.09	0.20
sulfamethoxazole	0.90	0.16	1.08	0.73	0.16	0.99
triclosan	1.70	0.29	2.03	0		0.00
trimethoprim	0.64	0.14	0.76	0.56	0.12	0.54

[a]Normalized removal, Rem_{norm}, calculated according to eq 1; R_i values calculated according to eq 6; E_i values calculated according to eq 7; orange cells have significantly lower (± two E_i) mass flows (in-stream loss); pink cells have significantly higher (± two E_i) mass flows (in-stream gain); na, not applicable due to detection limits; 0, indicates mass removal to below detection limit; concentration errors were determined using the standard deviation of replicate analysis; [a], sum of 1−4 ethylene oxide oligomers.

EDTA, due to its greater biodegradability,[33,35] concentrations of NTA were 100× lower than EDTA in the WWTP effluents. EDTA and NTA are water-soluble polyacids,[36] and depending on the form of the aqueous complex (a function of initial commercial product and water chemistry) have diverse environmental behaviors.[32,35,37] EDTA concentrations at the downstream sites generally plot below the mixing line (Figure 3A), with greater attenuation in the summer than in the spring sampling. Mass flow analysis generally showed significant attenuation between sites A and D1 (Table 2). Although

resistant to biodegradation, EDTA undergoes photolysis depending on metal speciation (Fe[III]-EDTA half-life is on the-order-of minutes) and stream conditions.[38] Water depths (<1 m) for both streams were similar during the summer samplings, but EDTA attenuation was greater in Boulder Creek than in Fourmile Creek which had less light penetration due to greater turbidity.[28]

The next most abundant contaminants in the WWTP effluents (Figure 2, Table SI-8) were 4-nonylphenolethoxycarboxylic acids (NPEC; 1−4 ethylene oxide, EO, oligomers - predominantly 1 and 2 EO) derived from the degradation of 4-nonylphenolpolyethoxylate nonionic surfactants.[39] Measured concentrations are in the range previously reported for Boulder Creek[4,22] and for other WWTP effluents and impacted surface waters.[2,6,39−41] In contrast to EDTA, downstream NPEC concentrations in Boulder Creek plotted above the mixing line (Figure 3B), suggesting in-stream gain, possibly the result of biodegradation of longer-chain NPEO degradates.[39]

Other alkylphenol contaminants including 4-nonylphenol, 4-nonylphenolethoxylates (NPEO; 1−4 EO oligomers), 4-*tert*-octylphenol, and 4-*tert*-octylphenolethoxylates (OPEO; 1−4 EO oligomers) were detected in the WWTP effluents and stream waters (Figure 2, Table SI-8). The concentration trend was NPEC ≫ NPEO > 4-nonylphenol > OPEO > 4-*tert*-octylphenol, and reflects differences in water solubility (acid to polar to neutral), use patterns, and transformation during WWTP treatment and stream transport. Because 4-nonylphenol is relatively hydrophobic (octanol/water partition coefficient, log K_{ow} = 4.2)[39] it can sorb to sediments and biofilms[20] (storage rather than elimination) as well as bioaccumulate in fish.[42] However, the low suspended sediment concentrations in the stream waters (5.0−34 mg L^{-1})[28] limit the mass of hydrophobic contaminants such as 4-nonylphenol that can be removed by sorption.[43] Previous studies on Boulder Creek showed 4-nonylphenol concentrations in the bed sediments are orders-of-magnitude greater than in the water[20] and that aerobic biodegradation was significant in the sediments but not the water.[19,44] In contrast to ionic EDTA and NPEC, which have high water solubilities and low vapor pressures, 4-nonylphenol also is susceptible to removal by volatilization.[17] 4-Nonylphenol and NPEO were detected in the WWTP effluents and the first downstream sites in Boulder Creek and Fourmile Creek (Table SI-8), suggesting in-stream persistence. There appeared to be greater attenuation of NPEO than 4-nonylphenol.

The polycyclic musk fragrances 7-acetyl-1,1′,3,4,4′,6-hexamethyl-5,6,7,8-tetrahydronaphthalene (AHTN) and 1,3,4,6,7,8-hexahydro-4,6,6′,7,8,8′-hexamethylcyclopenta-[γ]-2-benzopyran (HHCB) have widespread occurrence in surface waters[3] and were detected in the WWTP effluents and downstream sites at concentrations similar to the alkylphenols (Figure 2, Table SI-8). AHTN and HHCB (log K_{ow} = 5.4 and 5.3, respectively) are more hydrophobic than 4-nonylphenol and tend to sorb to sediments and bioaccumulate.[45,46] Measured polycyclic musk concentrations are in the range previously reported for Boulder Creek[4,22] and other sites.[3,6] Concentrations of HHCB were greater than AHTN in the WWTP effluents, and both compounds persisted at all of the downstream sites (Table SI-8).

The corrosion inhibitor 5-methyl-1H-benzotriazole represents a class of contaminants with high polarity (log K_{ow} = 1.9) and potential anionic characteristics (the NH-acidic heterocycle has an acid disassociation constant, pK_a = 8.2−8.5) that are mobile in the aquatic environment.[47] Measured WWTP-effluent and stream concentrations of 5-methyl-1H-benzotriazole are consistent with values previously reported for Boulder Creek[4,22] and other sites in the U.S. and Europe.[3,47−49] This hydrophilic compound was detected in both WWTP effluents and persisted through the downstream study reaches (Figure 2, Table SI-8).

Another class of contaminants detected in the WWTP effluent and stream samples is organophosphate compounds used as flame retardants and plasticizers, including triphenylphosphate, tris(2-butoxyethyl)phosphate, tris(2-chloroethyl)-phosphate, and tris(dichloroisopropyl)phosphate (Figure 2, Table SI-8). These chemicals share the phosphate group's H-bonding basicity, and log K_{ow} values vary depending on the esterified moieties [triphenylphosphate = 4.6; tris(2-butoxyethyl)phosphate = 3.8; tris(2-chloroethyl)phosphate = 1.4; and tris(dichloroisopropyl)phosphate = 3.7].[50] Although their sorption and biodegradation characteristics vary, the alkyl and chlorinated organophosphorous esters are widespread in the environment and concentrations reported here are consistent with values reported for the U.S. and Europe.[3,50,51] The poorly degradable and hydrophilic organophosphate esters had relatively high concentrations in the WWTP effluents and were persistent in both streams.

Triclosan is an important antimicrobial consumer product chemical, and because it is hydrophobic (log K_{ow} = 4.8), removal during wastewater treatment occurs primarily by sorption[52] and it can accumulate on sediments downstream from WWTP discharges.[53] However, the ionized form of triclosan (pK_a = 8.1) can be present in wastewater-impacted streams. Triclosan concentrations measured in this study were in agreement with previously reported values for Boulder Creek[4,22] and other locations.[3,52] Triclosan was detected in both of the WWTP effluents and persisted at the Boulder Creek and Fourmile Creek downstream sites (Figure 2, Table SI-8).

Pharmaceutical Contaminants. Fourteen antibiotics were detected in the Boulder and Ankeny WWTP effluents (Table SI-8),[28] but only ofloxacin and sulfamethoxazole were present in both effluents during both samplings. Similar antibiotic detections have been reported for other locations.[3,54] Concentrations of sulfamethoxazole at the downstream sites plotted near the mixing line (Figure 3C) during the summer Boulder Creek and the summer and spring Fourmile Creek samplings, suggesting conservative behavior. However, downstream sulfamethoxazole concentrations plotted above the mixing line during the spring Boulder Creek sampling, suggesting in-stream gain (also indicated by mass flow analysis, Table 2). Ofloxacin had significant in-stream removal during the summer Boulder Creek and both Fourmile Creek samplings, and as observed with sulfamethoxazole, there was significant downstream loading during the spring Boulder Creek sampling. Although a downstream antibiotic source in Boulder Creek was not identified, there is a small tributary between E and D1 that was not sampled. Alternatively, retransformation of glucuronide- and acetyl-metabolites is a potential in-stream source of sulfamethoxazole.[55] The persistence of sulfamethoxazole is consistent with its limited sorption (typically occurs as a soluble anion, pK_a = 5.7), biodegradation, and photolysis with respect to the time-scale of these experiments.[56−58] For example, the half-life for sulfamethoxazole in laboratory water/sediment systems was 17 days[55] and in outdoor microcosms was 19 days.[56]

Table 3. Summary of Maximum Measured Environmental Concentrations (MEC) for Organic Contaminants Detected in the Wastewater Treatment Plant Effluents and Downstream Samples during the Spring 2005 Boulder Creek, Colorado and Fourmile Creek, Iowa Samplings (Table SI-8; ref 28) Grouped by Analytical Method[a]

	effluent MEC (μg L^{-1})	stream MEC (μg L^{-1})	LC50 or NOEC (μg L^{-1})	effluent HQ	stream HQ
Acidic Organic Compounds					
ethylenediaminetetraacetic acid	170	48	1,000	0.17	0.048
nitrilotriacetic acid	1.0	0.5	1,500	0.00069	0.00036
4-nonylphenolmonoethoxycarboxylic acid	81	44	1,000	0.081	0.044
4-nonylphenoldiethoxycarboxylic acid	79	39	990	0.080	0.039
Neutral Organic Compounds by CLLE					
bisphenol A	0.084	0.035	0.06	1.4	0.58
1,4-dichlorobenzene	0.29	0.11	0.22	1.3	0.52
N,N-diethyl-*meta*-toluamide	0.33	0.19	521	0.00064	0.00037
5-methyl-1H-benzotriazole	0.60	0.21	22,000	0.000027	0.0000094
4-nonylphenol	0.72	0.34	6.0	0.12	0.056
4-nonylphenolmonoethoxylate	4.7	0.48	280	0.017	0.0017
4-nonylphenoldiethoxylate	1.3	0.22	148	0.0090	0.0015
4-*tert*-octylphenol	0.19	0.08	6.1	0.032	0.013
4-*tert*-octylphenolmonoethoxylate	0.13	0.02	7,070	0.000018	0.0000028
4-*tert*-octylphenoldiethoxylate	1.1	0.29	7,070	0.00016	0.000041
triclosan	0.47	0.15	0.7	0.67	0.21
Neutral Organic Compounds by SPE					
acetylhexamethyltetrahydronaphthalene	0.66	0.20	35	0.019	0.0057
hexahydrohexamethylcyclopentabenzopyran	3.3	0.70	68	0.048	0.010
triphenylphosphate	0.10	0.10	1.4	0.071	0.071
tris(2-chloroethyl)phosphate	0.50	0.20	6,300	0.000079	0.000032
tris(dichloroisopropyl)phosphate	0.35	0.10	1,100	0.00032	0.000091
Antibiotic Compounds					
ciprofloxacin	0.48	0.014	106	0.0045	0.00013
erythromycin	0.25	0.20	1,000	0.00024	0.00020
lincomycin	0.007	<0.005	1,000	0.000007	na
ofloxacin	2.2	0.14	5.0	0.44	0.027
sulfamethoxazole	0.72	0.23	3.4	0.21	0.067
trimethoprim	0.69	0.12	1,000	0.00069	0.00012
tylosin	0.021	<0.005	1,000	0.000021	na
Pharmaceutical Compounds					
acetaminophen	0.046	0.052	10	0.0046	0.0052
caffeine	0.10	0.039	1,000	0.00010	0.000039
carbamazepine	0.11	0.038	25	0.0043	0.0015
cimetidine	0.15	0.037	271,000	0.00000055	0.00000014
cotinine	0.066	0.013	1,000	0.000066	0.000013
diltiazem	0.044	0.009	8,200	0.0000053	0.0000010
Steroid and Steroidal Hormone Compounds					
17β-estradiol	0.003	0.002	0.01	0.32	0.16
estrone	0.11	0.036	0.20	0.55	0.18
17α-ethinylestradiol	0.002	0.001	0.00035	5.9	2.1
Pesticide Compounds					
atrazine	0.026	0.036	4.0	0.0064	0.0090
metolachlor	0.075	0.065	6,000	0.000013	0.000011

[a]Also included are values for the 50% lethal concentration (LC50) and no observable effects concentrations (NOEC) for the most sensitive aquatic species from the literature (sources of data given in Table SI-9). Hazard quotients (HQ) are calculated as MEC/NOEC. CLLE is continuous liquid-liquid extraction. SPE is solid-phase-extraction. na is non applicable.

Fifteen pharmaceuticals were detected, including carbamazepine, caffeine and its degradate 1,7-dimethylxanthine, and cotinine (Table SI-8) as have been reported for other locations.[3,54] During the spring Boulder Creek and spring and summer Fourmile Creek samplings, carbamazepine plotted near the mixing line (Figure 3D) indicating minimal in-stream loss; however, the Boulder Creek summer data plot below the line, suggesting in-stream attenuation. Carbamazepine has been shown to be persistent to environmental attenuation processes.[56,57,59,60] Carbamazepine is hydrophilic (log K_{ow} = 2.2) and has little affinity for removal by sorption or for bioaccumulation,[61] and its reported half-life in outdoor microcosms is 82 days.[56] Previous studies on Boulder Creek and Fourmile Creek have shown that biodegradation of caffeine and cotinine was significant in the bed sediments but not in the water column.[62]

Other Contaminants. The biogenic compounds cholesterol and coprostanol were detected in the Boulder WWTP effluent at concentrations similar to NPEO and 4-nonylphenol, and concentrations were lower in the Ankeny effluent (Figure 2, Table SI-8). The biogenic steroidal hormones 17β-estradiol and estrone, and the synthetic hormone 17α-ethynylestradiol, had concentrations that were orders-of-magnitude lower (Table SI-8) than the fecal sterol coprostanol. The downstream persistence of 17β-estradiol and estrone is consistent with previous studies on Boulder Creek and Fourmile Creek which indicated minimal removal in the water column,[63] as well as field studies in Minnesota[17] and France[64] which showed minimal in-stream removal.

The occurrence of pesticides differed from that of consumer products and pharmaceuticals. In the spring sampling, the herbicides atrazine (and its degradate deethylatrazine) and

metolachlor were detected at all of the Fourmile Creek sites (Table SI-8) reflecting intensive herbicide use in this agricultural region[65] and the in-stream persistence of the compounds.

■ IMPLICATIONS

Impact Zone and Water Supply. Although several organic contaminants had rapid in-stream removal (i.e., ofloxacin), many were only partially attenuated at the downstream sites (Tables 2 and SI-9; ref 28). The zones-of-influence for the conservative tracer boron generally extended >100 km downstream from the WWTPs (Table SI-9). The exception was the spring 2005 Fourmile Creek sampling when the complex hydrology resulted in apparent loss of conservative tracers. During the summer 2003 samplings, EDTA had an estimated half-life of 5.7 h in Boulder Creek and 21 h in Fourmile Creek, resulting in zones-of-influence of 44 and 51 km, respectively. In a study on the wastewater-impacted Trinity River in Texas, which is more turbid than Boulder Creek and Fourmile Creek, EDTA persisted for ∼500 km (60% reduction during 13 days of transport).[14] In Boulder Creek and Fourmile Creek, NPEC exhibited a range of behaviors from production to removal, and the zones-of-influence for NPEC and the other NPEO degradation products differed from EDTA due to complex biogeochemical interactions.[17,39] In a study of the Santa Ana River in California, 86% removal of alkylphenol-polyethoxylates degradates was observed after 12.6 km of transport.[40] Sulfamethoxazole was more persistent than EDTA in Boulder Creek and Fourmile Creek, and had half-lives of 18 and 63 h, and zones-of-influence of 140 and 160 km, respectively. A study on Wascana Creek, a wastewater-impacted stream in Saskatchewan, Canada, indicated that sulfamethoxazole concentrations were reduced 80% after 105 km of transport.[54]

The zones-of-influence for organic contaminants in Boulder Creek were evaluated with respect to potential downstream users, including drinking-water utilities.[66] Because of the complexity of Boulder Creek, which is engineered to convey water to many users,[22] water from multiple sources is diverted to drinking water supplies. Although none directly take water from Boulder Creek or Fourmile Creek, there are multiple communities within the ∼100 km zone-of-influence with source waters potentially influenced by "de facto" wastewater reuse. The persistence of complex contaminant mixtures in Boulder Creek and Fourmile Creek is consistent with results from a national survey of U.S. drinking-water sources.[67]

Hazard Quotients. Consumer-product and pharmaceutical compounds are designed for specific physicochemical (e.g., surface tension) or biological (e.g., bacterial toxicity) functions that often require persistence.[68,69] The MECs for contaminants in the WWTP effluents and the first downstream sites and the available NOEC or LC50 values for the most sensitive aquatic species (Table SI-10) were used to calculate HQs for the effluents and streams (Table 3). Of the 38 compounds, ten had effluent HQ values >0.1 (indicating less than a 10-fold safety factor) and six had stream HQs >0.1. 17α-Ethinylestradiol had the greatest effluent HQ, whereas cimetidine had the lowest. As the result of in-stream attenuation, stream HQ values were up to an order-of-magnitude lower than effluent values. Because of the potential diversity of biological effects that can result from exposure to the individual components of the contaminant mixture present in wastewater-impacted streams, different sensitivities of different test species during different life stages, and the diversity of organisms exposed to the streamwater, it is a challenge to assess ecosystem impacts.

EDTA forms water-soluble aqueous complexes and modifies metal transport and toxicity.[35,37] Because EDTA is used in large quantities and persists in the aquatic environment, it occurs at high concentrations, and despite its low toxicity[70] the effluent HQ was 0.17, suggesting potential for ecosystem effects. 4-Nonylphenol has greater toxicity[71] but lower concentrations, and had an effluent HQ of 0.12 (Table 3).

Ecosystem Impacts. The downstream persistence of organic contaminant mixtures indicates that aquatic organisms are exposed to biologically active compounds. Risks of exposure to mixtures in wastewater-impacted streams is not clear.[12] Although experimental conditions used to determine ecotoxicological end points are variable, HQ results indicate that contaminants detected in the Boulder and Ankeny WWTP effluents exhibit a range of toxicities. The effects of individual contaminants on different organisms, the chemical complexity of WWTP effluents, and the species diversity in streams complicate understanding potential risks. Applying the concepts of mode-of-action and chemical additivity,[72] and adverse outcome pathways,[73] provides insight into contaminants likely to impact aquatic ecosystems. For example, endocrine disruption, as represented by estrogen-receptor mode-of-action reproductive effects on fish, is an issue in wastewater-impacted streams.[7−10] Adverse reproductive effects have been attributed to the synthetic hormone 17α-ethynylestradiol[74,75] and the nonionic surfactant degradate 4-nonylphenol.[76] Other contaminants in the WWTP effluents with endocrine disrupting properties include AHTN and HHCB,[77,78] bisphenol A,[79,80] organophosphorous flame retardants,[81] and triclosan.[82] Because estrogenic contaminants have very low effect levels (ng L^{-1}) and the effects are additive, the implications of complex mixtures must be considered when evaluating WWTP-effluent-impacted streams.[83] In contrast to estrogenic contaminants such as 17α-ethynylestradiol, for which fish reproductive effects are the most sensitive biomarkers, other contaminant classes, such as antibiotics, have different modes-of-action. For example, the most sensitive aquatic species for the antibiotic sulfamethoxazole are plants[84] and bacteria[85,86] due to disruption of the biologically conserved folic acid biosynthesis mode-of-action.

This study documents the persistence of a complex mixture of biologically active organic contaminants in two wastewater-impacted streams under different flow regimes. Although the potential ecological and human health impacts of organic and inorganic contaminant mixtures are poorly understood, they will become increasingly important to water-resource managers as reliance on reuse of reclaimed municipal wastewaters increases.[11]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

(1) Descriptions of analytical methods used for measuring organic contaminants and associated quality assurance, (2) tables listing contaminants analyzed and detection limits, (3) table summarizing contaminants detected in the wastewater treatment plant effluents and the first downstream sampling sites during the spring 2005 sampling, (4) table showing attenuation rates, half-lives, and calculated zones-of-influence, and (5) table showing information used to determine hazard quotients for the detected contaminants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the USGS National Research Program and Toxic Substances Hydrology Program. We thank Susan Glassmeyer from the U.S. Environmental Protection Agency for analytical support, as well as the Iowa Policy Project, Iowa Department of Natural Resources, and Iowa Geological Survey. In addition, we thank Floyd Bebler from the City of Boulder and Ken Plager from the City of Ankeny for site access. Use of trade names is for identification only and does not imply endorsement by the U.S. Government.

REFERENCES

(1) Ternes, T. A. Occurrence of drugs in German sewage treatment plants and rivers. *Water Res.* **1998**, *32*, 3245−3260.

(2) Barber, L. B.; Brown, G. K.; Zaugg, S. D. Potential endocrine disrupting organic chemicals in treated municipal wastewater and river water. In *Analysis of Environmental Endocrine Disruptors*; Keith, L. H., Jones-Lepp, T. L., Needham, L. L., Eds.; ACS Symposium Series 747; American Chemical Society: Washington, DC, 2000; pp 97−123.

(3) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000 - A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.

(4) Barber, L. B.; Murphy, S. F.; Verplanck, P. L.; Sandstrom, M. W.; Taylor, H. E.; Furlong, E. T. Chemical loading into surface water along a hydrological, biogeochemical, and land use gradient - A holistic watershed approach. *Environ. Sci. Technol.* **2006**, *40*, 475−486.

(5) Barber, L. B.; Antweiler, R. C.; Flynn, J. L.; Keefe, S. H.; Kolpin, D. W.; Roth, D. A.; Schnoebelen, D. J.; Taylor, H. E.; Verplanck, P. L. Lagrangian mass flow investigations of inorganic contaminants in wastewater-impacted streams. *Environ. Sci. Technol.* **2011**, *45*, 2575−2583.

(6) Barber, L. B.; Brown, G. K.; Nettesheim, T. G.; Murphy, E. W.; Bartell, S. E.; Schoenfuss, H. L. Effects of biologically-active chemical mixtures on fish in a wastewater-impacted urban stream. *Sci. Total Environ.* **2011**, *409*, 4720−4728.

(7) Jobling, S.; Nolan, M.; Tyler, C. R.; Brighty, G.; Sumpter, J. P. Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* **1998**, *32*, 2498−2506.

(8) Vajda, A. M.; Barber, L. B.; Gray, J. L.; Lopez, E. M.; Woodling, J. D.; Norris, D. O. Reproductive disruption in fish downstream of an estrogenic wastewater effluent. *Environ. Sci. Technol.* **2008**, *42*, 3407−3414.

(9) Vajda, A. M.; Barber, L. B.; Gray, J. L.; Lopez, E. M.; Bolden, A. M.; Schoenfuss, H. L.; Norris, D. O. Demasculinization of male fish by wastewater treatment plant effluent. *Aquat. Toxicol.* **2011**, *103*, 213−221.

(10) Barber, L. B.; Vajda, A. M.; Douville, C.; Norris, D. O.; Writer, J. H. Fish endocrine disruption responses to a major wastewater treatment facility upgrade. *Environ. Sci. Technol.* **2012**, *46*, 2121−2131.

(11) National Research Council. *Water Reuse: Expanding the Nation's Water Supply Through Reuse of Municipal Wastewater*; National Academy Press: Washington, DC, 2012.

(12) Brooks, B. W.; Riley, T. M.; Taylor, R. D. Water quality of effluent-dominated ecosystems: Ecotoxicological, hydrological, and management considerations. *Hydrobiologia* **2006**, 365−379.

(13) Gurr, C. J.; Reinhard, M. Harnessing natural attenuation of pharmaceuticals and hormones in rivers. *Environ. Sci. Technol.* **2006**, *40*, 2872−2876.

(14) Fono, L. J.; Kolodziej, E. P.; Sedlak, D. L. Attenuation of wastewater-derived contaminants in an effluent-dominated river. *Environ. Sci. Technol.* **2006**, *40*, 7257−7262.

(15) Radke, M.; Ulrich, H.; Wurm, C.; Kunkel, U. Dynamics and attenuation of acidic pharmaceuticals along a river stretch. *Environ. Sci. Technol.* **2010**, *44*, 2968−2974.

(16) Kunkel, U.; Radke, M. Reactive tracer test to evaluate the fate of pharmaceuticals in rivers. *Environ. Sci. Technol.* **2011**, *45*, 6296−6302.

(17) Writer, J. H.; Ryan, J. N.; Keefe, S. H.; Barber, L. B. Fate of 4-nonylphenol and 17β-estradiol in the Redwood River of Minnesota. *Environ. Sci. Technol.* **2012**, *46*, 860−868.

(18) Lin, A.; Plumlee, M. H.; Reinhard, M. Attenuation of pharmaceuticals and alkylphenol polyethoxylate metabolites during river transport: Photochemical and biological transformation. *Environ. Toxicol. Chem.* **2006**, *25*, 1458−1464.

(19) Writer, J. H.; Barber, L. B.; Ryan, J. N.; Bradley, P. M. Biodegradation and attenuation of steroidal hormones and alkylphenols by stream biofilms and sediments. *Environ. Sci. Technol.* **2011**, *45*, 4370−4376.

(20) Writer, J. H.; Ryan, J. N.; Barber, L. B. Role of biofilms in sorptive removal of steroidal hormones and 4-nonylphenol compounds from streams. *Environ. Sci. Technol.* **2011**, *45*, 7272−7283.

(21) Keefe, S. H.; Barber, L. B.; Runkel, R. L.; Ryan, J. N.; McKnight, D. M.; Wass, R. D. Conservative and reactive solute transport in constructed wetlands. *Water Resour. Res.* **2004**, *40*, W01201, 1−12.

(22) Murphy, S. F., Verplanck, P. L., Barber, L. B., Eds. *Comprehensive Water Quality of the Boulder Creek Watershed, Colorado, during High-Flow and Low-Flow Conditions, 2000*; USGS Water Resources Investigation Report 03-4045; U.S. Geological Survey: Washington, DC, 2003.

(23) Schnoebelen, D. J.; Kolpin, D. W.; Barber, L. B.; Furlong, E. T.; Meyer, M. M.; Skopec, M. Research plan and preliminary results – A field research site for emerging contaminants in Iowa. *J. Iowa Acad. Sci.* **2006**, *113*, 1−9.

(24) Rantz, S. E. et al. *Measurement and Computation of Streamflow. 1. Measurement of Stage and Discharge*; USGS Water-Supply Paper 2175; U.S. Geological Survey: Washington, DC, 1982; pp 1−284.

(25) Kilpatrick, F. A. *Simulation of Soluble Waste Transport and Buildup in Surface Waters Using Tracers*; USGS Tech. Water Res. Invest. Bk. 3 Ch. A20; U.S. Geological Survey: Washington, DC, 1993.

(26) Edwards, T. K.; Glysson, D. G. *Field Methods for Measurement of Fluvial Sediment*. USGS Open-File Report 86-531; U.S. Geological Survey: Washington, DC, 1988.

(27) U.S. Geological Survey. *National Field Manual for the Collection of Water-Quality Data*. USGS Techniques of Water −Res. Invest., Bk. 9, Ch. A1-A1; U.S. Geological Survey: Washington, DC, variously dated. http://pubs.water.usgs.gov/twri9A (accessed March 2003).

(28) Barber, L. B.; Keefe, S. H.; Kolpin, D. W.; Schnoebelen, D. J.; Flynn, J. L.; Brown, G. K.; Furlong, E. T.; Gray, J. L.; Glassmeyer, S. T.; Meyer, M. T.; Sandstrom, M. W.; Taylor, H. E.; Zaugg, S. D. *Lagrangian Sampling of Wastewater Treatment Plant Discharges into Boulder Creek, Colorado and Fourmile Creek, Iowa during the Summer of 2003 and Spring of 2005 − Hydrological and Water-Quality Data*; USGS Open-File Report 2011-1054; U.S. Geological Survey: Washington, DC, 2011.

(29) Schreiber, I. M.; Mitch, W. A. Occurrence and fate of nitrosamines and nitrosamine precursors in wastewater-impacted surface waters using boron as a conservative tracer. *Environ. Sci. Technol.* **2006**, *40*, 3203−3210.

(30) Taylor, J. R. *An Introduction to Error Analysis*; University Science Books: Mill Valley, CA, 1982.

(31) U.S. Environmental Protection Agency. *Guidelines for Ecological Risk Assessment*; Risk Assessment Forum: Washington, DC, 1998.

(32) Nowack, B. Environmental chemistry of aminopolycarboxylate chelating agents. *Environ. Sci. Technol.* **2002**, *36*, 4009−4016.

(33) Egli, T. Biodegradation of metal-complexing aminopolycarboxylic acids. *J. Biosci. Bioeng.* **2001**, *92*, 89−97.

(34) Reemtsma, T.; Weiss, S.; Mueller, J.; Petroviv, M.; Gonzalez, S.; Barcelo, D.; Ventura, F.; Knepper, T. P. Polar pollutant entry into the

water cycle by municipal wastewater: A European perspective. *Environ. Sci. Technol.* **2006**, *40*, 5451−5458.
(35) Alder, A. C.; Siegrist, H.; Gujer, W.; Giger, W. Behavior of NTA and EDTA in biological wastewater treatment. *Water Res.* **1990**, *24*, 733−742.
(36) Fiorucci, A. R.; de Paula, L. R.; Neves, E. A.; Cavalheiro, E. T. G. Solubility of alkali and alkali earth salts of dihydrogen ethylenediaminetetraacetate in aqueous solutions. *J. Chem. Eng. Data* **2002**, *47*, 1510−1513.
(37) Nowack, B.; Xue, H.; Sigg, L. Influence of natural and anthropogenic ligands on metal transport during infiltration of river water to groundwater. *Environ. Sci. Technol.* **1997**, *31*, 866−872.
(38) Kari, F. G.; Giger, W. Modeling the photochemical degradation of ethylenediaminetetraacetate in the River Glatt. *Environ. Sci. Technol.* **1995**, *29*, 2814−2827.
(39) Ahel, M.; Giger, W.; Schaffner, C. Behavior of alkylphenol polyethoxylate surfactants in the aquatic environment. II - Occurrence and transformation in rivers. *Water Res.* **1994**, *28*, 1143−1152.
(40) Gross, B.; Montgomery-Brown, J.; Naumann, A.; Reinhard, M. Occurrence and fate of pharmaceuticals and alkylphenol ethoxylate metabolites in an effluent-dominated river and wetland. *Environ. Toxicol. Chem.* **2004**, *23*, 2074−2083.
(41) Loyo-Rosales, J. E.; Rice, C. P.; Torrents, A. Fate of octyl- and nonylphenol ethoxylates and some carboxylated derivatives in three American wastewater treatment plants. *Environ. Sci. Technol.* **2007**, *41*, 6815−6821.
(42) Rice, C. P.; Schmitz-Afonso, I.; Loyo-Rosales, J. E.; Link, E.; Thoma, R.; Fay, L.; Altfater, D.; Camp, M. J. Alkylphenol and alkylphenol-ethoxylates in carp, water and sediments from Cuyahoga River, Ohio. *Environ. Sci. Technol.* **2003**, *37*, 3747−3754.
(43) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*; Wiley Interscience: New York, 2003.
(44) Bradley, P. M.; Barber, L. B.; Kolpin, D. W.; McMahon, P. B.; Chapelle, F. H. Potential for 4-*n*-nonylphenol biodegradation in stream sediments. *Environ. Toxicol. Chem.* **2008**, *27*, 260−265.
(45) Artola-Garicano, E.; Sinnige, T. L.; van Holsteijn, I.; Vaes, W. H. J.; Hermens, J. L. M. Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (*Chironomus riparius* and *Lumbriculus variegatus*). *Environ. Toxicol. Chem.* **2003**, *22*, 1086−1092.
(46) Gatermann, R.; Biselli, S.; Hühnerfuss, H.; Rimkus, G. G.; Hecker, M.; Karbe, L. Synthetic musks in the environment. Part 1: Species-dependent bioaccumulation of polycyclic and nitro musk fragrances in freshwater fish and mussels. *Arch. Environ. Contam. Toxicol.* **2002**, *42*, 437−446.
(47) Reemtsma, T.; Miehe, U.; Duennbier, U.; Jekel, M. Polar pollutants in municipal wastewater and the water cycle: Occurrence and removal of benzotriazoles. *Water Res.* **2010**, *44*, 596−604.
(48) Giger, W.; Schaffner, C.; Kohler, H-P. E. Benzotriazole and tolytriazole as aquatic contaminants. 1. Input and occurrence in rivers and lakes. *Environ. Sci. Technol.* **2006**, *40*, 7186−7192.
(49) Janna, H.; Scrimshaw, M. D.; Williams, R. J.; Churchley, J.; Sumpter, J. P. From dishwasher to tap? Xenobiotic substances benzotriazole and tolyltriazole in the environment. *Environ. Sci. Technol.* **2011**, *45*, 3858−3864.
(50) Marklund, A.; Andersson, B.; Haglund, P. Organophosphorus flame retardants and plasticizers in Swedish treatment plants. *Environ. Sci. Technol.* **2005**, *39*, 7423−7429.
(51) van der Veen, I.; de Boer, J. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. *Chemosphere* **2012**, *88*, 1119−1153.
(52) Halden, R. U.; Paull, D. H. Co-occurrence of triclocarban and triclosan in U.S. water resources. *Environ. Sci. Technol.* **2005**, *39*, 1420−1426.
(53) Venkatesan, A. K.; Pycke, B. F. G.; Barber, L. B.; Lee, K. E.; Halden, R. L. Occurrence of triclosan, triclocarban, and its lesser dechlorination congeners in Minnesota freshwater sediments collected near wastewater treatment plants. *J. Hazard. Mater.* **2012**, *229/230*, 29−35.
(54) Waiser, M. J.; Humphries, D.; Tumber, V.; Holm, J. Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wascana Creek, Saskatchewan, Canada. *Environ. Toxicol. Chem.* **2011**, *30*, 508−619.
(55) Radke, M.; Lauwigi, C.; Heinkele, G.; Mürdter, T. E.; Letzel, M. Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. *Environ. Sci. Technol.* **2009**, *43*, 3135−3141.
(56) Lam, M. W.; Young, C. J.; Brain, R. A.; Johnson, D. J.; Hanson, M. A.; Wilson, C. J.; Richards, S. M.; Solomon, K. R.; Maybury, S. A. The aquatic persistence of eight pharmaceuticals in a microcosm study. *Environ. Toxicol. Chem.* **2004**, *23*, 1431−1440.
(57) Lam, M. W.; Mabury, S. A. Photodegradation of the pharmaceuticals atorvastatin, carbamazepine, levofloxacin, and sulfamethoxazole in natural waters. *Aquat. Sci.* **2005**, *67*, 177−188.
(58) Ryan, C. C.; Tan, D. T.; Arnold, W. A. Direct and indirect photolysis of sulfamethoxazole and trimethoprim in wastewater treatment plant effluent. *Water Res.* **2011**, *45*, 1280−1286.
(59) Tixier, C.; Singer, H. P.; Oellers, S.; Müller, S. R. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environ. Sci. Technol.* **2003**, *37*, 1061−1068.
(60) Kosjek, T.; Andersen, H. R.; Kompare, B.; Ledin, A.; Heath, E. Fate of carbamazepine during water treatment. *Environ. Sci. Technol.* **2009**, *43*, 6256−6261.
(61) Vernouillet, G.; Eullaffroy, P.; Lajeunesse, A.; Blaise, C.; Gagné, F.; Juneau, P. Toxic effects and bioaccumulation of carbamazepine by biomarkers measured in organisms of different trophic levels. *Chemosphere* **2010**, *80*, 1062−1068.
(62) Bradley, P. M.; Barber, L. B.; Kolpin, D. W.; McMahon, P. B.; Chapelle, F. H. Biotransformation of caffeine, cotinine, and nicotine in stream sediments: Implications for use as wastewater indicators. *Environ. Toxicol. Chem.* **2007**, *26*, 1116−1121.
(63) Bradley, P. M.; Barber, L. B.; Chapelle, F. H.; Gray, J. L.; Kolpin, D. W.; McMahon, P. B. Biodegradation of 17β-estradiol, estrone, and testosterone in stream sediments. *Environ. Sci. Technol.* **2009**, *43*, 1902−1910.
(64) Labadie, P.; Budzinski, H. Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France). *Environ. Sci. Technol.* **2005**, *39*, 5113−5120.
(65) Thurman, E. M; Goolsby, D. A.; Meyer, M. T.; Mills, M. S.; Pomes, M. L.; Kolpin, D. W. A reconnaissance study of herbicides and their metabolites in surface water of the midwestern United States using immunoassay and gas chromatography/mass spectrometry. *Environ. Sci. Technol.* **1992**, *26*, 2440−2447.
(66) U. S. Environmental Protection Agency. Envirofacts. http://oaspub.epa.gov/enviro/sdwis, accessed May 8, 2012.
(67) Focazio, M. J.; Kolpin, D. W.; Barnes, K. K.; Furlong, E. T.; Meyer, M. T.; Zaugg, S. D.; Barber, L. B.; Thurman, E. M. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States − II. Untreated drinking water sources. *Sci. Total Environ.* **2008**, *402*, 201−216.
(68) Fent, K.; Weston, A. A.; Caminada, D. Ecotoxicology of human pharmaceuticals. *Aquat. Toxicol.* **2006**, *76*, 122−159.
(69) Crane, M.; Watts, C.; Boucard, T. Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Sci. Total Environ.* **2006**, *367*, 23−41.
(70) Schmidt, C. K.; Brauch, H. -J. Impact of aminopolycarboxylates on aquatic organisms and eutrophication: Overview of available data. *Environ. Toxicol.* **2004**, *19*, 620−637, DOI: 10.1002/tox.20071.
(71) U.S. Environmental Protection Agency. *Aquatic Life Ambient Water Quality Criteria - Nonylphenol FINAL*; 822-R-05-005; U.S. Environmental Protection Agency: Washington, DC, 2005.
(72) Brian, J.; Harris, C. A.; Scholze, M.; Backhaus, T.; Booy, P.; Lamoree, M.; Pojans, G.; Jonkers, J.; Runnalls, T.; Bonfá, A.; Marcomini, A.; Sumpter, J. P. Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. *Environ. Health Perspect.* **2005**, *113*, 721−728.

(73) Ankley, G. T.; Bennett, R. S.; Erickson, R. J.; Hoff, D. J.; Hornung, M. W.; Johnson, R. D.; Mount, D. R.; Nichols, J. W.; Russom, C. L.; Schmieder, P. K.; Serrano, J. A.; Tietge, J. E.; Villeneuve, D. L. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ. Toxicol. Chem.* **2010**, *29*, 730−741.
(74) Caldwell, D. J.; Mastrocco, F.; Hutchinson, T. H.; Länge, R.; Heijerick, D.; Janssen, C.; Anderson, P. D.; Sumpter, J. P. Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17α-ethinyl estradiol. *Environ. Sci. Technol.* **2008**, *42*, 7046−7054.
(75) Kidd, K. A.; Blanchfield, P. J.; Mills, K. H.; Palace, V. P.; Evans, R. E.; Lazorchak, J. M.; Flick, R. W. Collapse of a fish population after exposure to a synthetic estrogen. *Proc. Natl. Acad. Sci., U. S. A.* **2007**, *104*, 8897−8901.
(76) Schoenfuss, H. L.; Bartell, S. E.; Bistodeau, T. B.; Cediel, R. A.; Grove, K. J.; Zintek, L.; Lee, K. E.; Barber, L. B. Impairment of the reproductive potential of male fathead minnows by environmentally relevant exposures to 4-nonylphenol. *Aquat. Toxicol.* **2008**, *86*, 91−98.
(77) Schreurs, R. H. M. M.; Sonneveld, E.; Jansen, J. H. J.; Seinen, W.; van der Burg, B. Interaction of polycyclic musks and UV filters with the estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR) in reporter gene bioassays. *Toxicol. Sci.* **2005**, *83*, 264−262.
(78) Yamauchi, R.; Ishibashi, H.; Hirano, M.; Mori, T.; Kim, J.-W.; Arizono, K. Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (*Oryzias latipes*). *Aquat. Toxicol.* **2008**, *90*, 261−268.
(79) Crain, D. A.; Eriksen, M.; Iguchi, T.; Jobling, S.; Laufer, H.; LeBlanc, G. A.; Guillette, L. J., Jr. An ecological assessment of bisphenol-A: Evidence from comparative biology. *Reprod. Toxicol.* **2007**, *24*, 225−239.
(80) Wright-Walters, M.; Volz, C.; Talbott, E.; Davis, D. An updated weight of evidence approach to the hazard assessment of Bisphenol A and the derivation a new predicted no effect concentration (Pnec) using a non-parametric methodology. *Sci. Total Environ.* **2011**, *409*, 676−685.
(81) Liu, X.; Ji, K.; Choi, K. Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. *Aquat. Toxicol.* **2012**, *114/115*, 173−181.
(82) Raut, S. A.; Angus, R. A. Triclosan has endocrine-disrupting effects in male western mosquitofish *Gambusia affinis*. *Environ. Toxicol. Chem.* **2010**, *29*, 1287−1291.
(83) Sumpter, J. P.; Johnson, A. C.; Williams, R. J.; Kortenkamp, A.; Scholze, M. Modeling effects of mixtures of endocrine disrupting chemicals at the river catchment scale. *Environ. Sci. Technol.* **2006**, *40*, 5478−5489.
(84) Brain, R. A.; Ramirez, A. J.; Fulton, B. A.; Chambliss, C. K.; Brooks, B. W. Herbicidal effects of sulfamethoxazole in *Lemna gibba*: Using *p*-aminobenzoic acid as a biomarker of effect. *Environ. Sci. Technol.* **2008**, *42*, 8965−8970.
(85) Underwood, J. C.; Harvey, R. W.; Metge, D. W.; Repert, D. A.; Baumgartner, L. K.; Smith, R. L.; Roane, T. M.; Barber, L. B. Effects of the antimicrobial sulfamethoxazole on groundwater bacterial enrichment. *Environ. Sci. Technol.* **2011**, *45*, 3096−3101.
(86) Haack, S. K.; Metge, D. W.; Fogarty, L. R.; Meyer, M. T.; Barber, L. B.; Harvey, R. W.; LeBlanc, D. R.; Kolpin, D. W. Effects on groundwater microbial communities of an engineered 30-day *in situ* exposure to the antibiotic sulfamethoxazole. *Environ. Sci. Technol.* **2012**, *46*, 7478−7486.