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Fate of Sulfamethoxazole, 4-Nonylphenol, and 17 β -Estradiol in Groundwater Contaminated by Wastewater Treatment Plant Effluent

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Organic wastewater contaminants (OWCs) were measured in samples collected from monitoring wells located along a 4.5-km transect of a plume of groundwater contaminated by 60 years of continuous rapid infiltration disposal of wastewater treatment plant effluent. Fifteen percent of the 212 OWCs analyzed were detected, including the antibiotic sulfamethoxazole (SX), the nonionic surfactant degradation product 4-nonylphenol (NP), the solvent tetrachloroethene (PCE), and the disinfectant 1,4-dichlorobenzene (DCB). Comparison of the 2005 sampling results to data collected from the same wells in 1985 indicates that PCE and DCB are transported more rapidly in the aquifer than NP, consistent with predictions based on compound hydrophobicity. Natural gradient in situ tracer experiments were conducted to evaluate the subsurface behavior of SX, NP, and the female sex hormone 17 β -estradiol (E2) in two oxic zones in the aquifer: (1) a downgradient transition zone at the interface between the contamination plume and the overlying uncontaminated groundwater and (2) a contaminated zone located beneath the infiltration beds, which have not been loaded for 10 years. In both zones, breakthrough curves for the conservative tracer bromide (Br⁻) and SX were nearly coincident, whereas NP and E2 were retarded relative to Br⁻ and showed mass loss. Retardation was greater in the contaminated zone than in the transition zone. Attenuation of NP and E2

in the aquifer was attributed to biotransformation, and oxic laboratory microcosm experiments using sediments from the transition and contaminated zones show that uniform-ring-labeled ¹⁴C 4-normal-NP was biodegraded more rapidly (30–60% recovered as ¹⁴CO₂ in 13 days) than 4-¹⁴C E2 (20–90% recovered as ¹⁴CO₂ in 54 days). There was little difference in mineralization potential between sites.

Introduction

Reliance on wastewater treatment plant (WWTP) effluent for aquifer recharge and supplementing potable water supply (1) increases the need for understanding the occurrence and fate of organic wastewater contaminants (OWCs) in groundwater. Groundwater withdrawals in the United States during 1995 were estimated at 2.9×10^5 m³ d⁻¹ and provided public water supply for over 40 million people (2). Groundwater is a major source of water for irrigation and contributes flow to streams and wetland habitats (3). There is growing concern about potential effects of OWCs on the aquatic environment (4), and their occurrence in WWTP effluents and surface waters is well documented (5–11). Less is known about the occurrence and fate of OWCs in groundwater (12–16).

The chemistry of WWTP effluents is complex and has been linked to reproductive system disruption of fish living in effluent impacted streams (17–20). The most potent estrogenic chemicals in WWTP effluents are steroid hormones such as the female sex hormone 17 β -estradiol (E2), although nonsteroid chemicals such as the nonionic surfactant degradation product 4-nonylphenol (NP) also have estrogenic activity (21). Other OWCs, such as the antibiotic sulfamethoxazole (SX), have widespread occurrence (8, 9, 22, 23), and there is concern about their ecological effects (24) and development of antibiotic resistance in microbes (25).

This study was conducted at the U.S. Geological Survey's Cape Cod Toxic Substances Hydrology research site, an intensively investigated plume of WWTP effluent contaminated groundwater (26, 27). The plume contains OWCs including NP, the solvent tetrachloroethene (PCE), the disinfectant 1,4-dichlorobenzene (DCB), the anionic surfactant linear alkylbenzene sulfonate (LAS), and many other compounds (12, 28–30). A 3-tiered approach was used to assess OWCs in the Cape Cod groundwater contamination plume: (1) resampling monitoring wells 20 years after their original sampling in 1985, (2) conducting natural gradient in situ tracer experiments using SX, NP, and E2, and (3) conducting laboratory sediment microcosm experiments using NP and E2.

Methods

Study Site. The extensive groundwater contamination plume (Figure 1A) at the Cape Cod research site (26) results from 60 years of continuous rapid infiltration disposal of WWTP effluent (about 4.6×10^7 m³ of effluent was discharged between 1936 and 1995). The unconfined glacial outwash aquifer consists of moderately heterogeneous, fine to coarse grained sand and gravel, with groundwater flow to the southwest at a velocity of 0.42 m d⁻¹, a hydraulic gradient of 1.5 m km⁻¹, a hydraulic conductivity of 110 m d⁻¹, a porosity of 0.39, and a longitudinal dispersivity (for a 300-m long tracer experiment) of 0.96 m (31, 32). Hydraulic properties vary as a function of spatial scale (33). The aquifer sediments are relatively unweathered (deposited about 15 000 years ago in the most recent glacial advance) and composed primarily of quartz and feldspars with a complex suite of accessory minerals (including glauconite, biotite, amphiboles,

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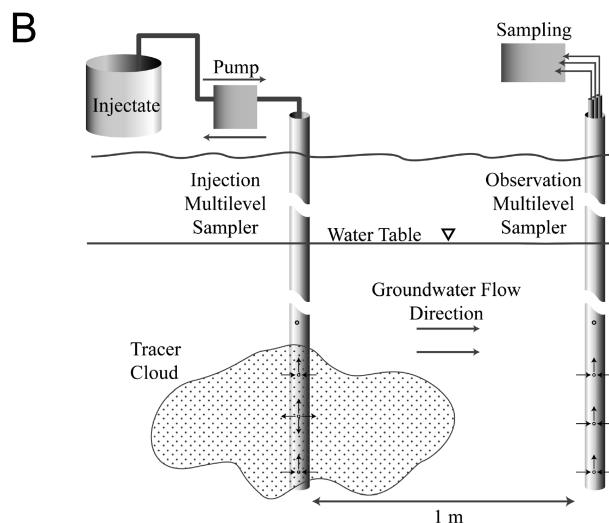
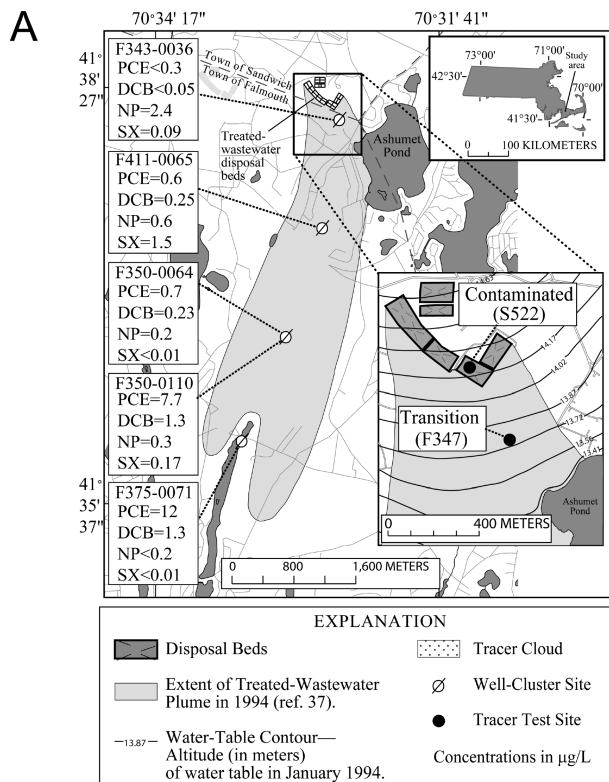


FIGURE 1. (A) Map showing location of wells sampled during September 2005 and the concentrations ($\mu\text{g L}^{-1}$) of tetrachloroethene (PCE), 1,4-dichlorobenzene (DCB), 4-nonylphenol (NP), and sulfamethoxazole (SX). Also shown are the locations of the multilevel sampler arrays used for the transition zone (F347) and contaminated zone (S522) tracer experiments. (B) Schematic of natural gradient in situ tracer experimental procedures. [Monitoring well F343-0036 (value after hyphen is depth in feet below land surface) is 0.19 km downgradient from the infiltration beds; F411-0065 is 1.7 km downgradient; F350-0064 and F350-0110 are 3.1 km downgradient; F375-0071 is 4.5 km downgradient].

pyroxenes, hematite, magnetite, and traces of clay minerals) with varying degrees of metal-oxide grain coatings (34). Sediment organic carbon (SOC) ranges from <0.001 –1% and varies as a function of particle size, mineralogy, and location (35). Dissolved oxygen in the uncontaminated groundwater overlying the plume (coming from surface water recharge to the unconfined aquifer) is near saturation, whereas the

contamination plume has a steep vertical gradient with an anoxic core surrounded by oxic uncontaminated groundwater (36).

Groundwater Sampling and Analytical Procedures.

During September 2005, groundwater was sampled from four wells located along a 4.5 km longitudinal transect of the contaminant plume (Figure 1A) using procedures described elsewhere (37). Water for OWC analysis was filtered through $0.45\text{-}\mu\text{m}$ glass fiber filters and collected in 1-L amber glass bottles either unpreserved or preserved with 1% (v/v) formalin. Unfiltered samples for volatile organic compound analysis were collected without headspace in 40-mL amber glass vials. Samples were stored at 4°C until analysis.

Continuous liquid–liquid extraction with methylene chloride and gas chromatography/mass spectrometry (GC/MS) analysis was used to measure NP and other OWCs (7). Steroid hormones, including E2, were isolated using octadecylsilica solid-phase extraction with methanol elution, the methoxime/trimethylsilyl derivatives were formed (7), and analysis was by gas chromatography/tandem mass spectrometry (GC/MS/MS). Antibiotics, including SX, were analyzed (38) by hydrophilic–lipophilic balance solid-phase extraction with liquid chromatography/tandem mass spectrometry (LC/MS/MS). Volatile organic compounds, including PCE, were analyzed by purge and trap GC/MS (39). Additional OWCs were measured using methods described in the Supporting Information.

Tracer Tests. Natural gradient in situ tracer experiments (31, 40) were conducted during September 2006 at two multilevel sampler (MLS) arrays (Figure 1). Each MLS had 15 sampling ports consisting of different lengths of 0.3 cm inside diameter polyethylene tubing vertically spaced at approximately 0.3-m intervals (37). The arrays have an extraction/injection MLS and downgradient observation MLSs with sampling ports located at the same depths. The 3-dimensional nature of the contamination plume was used to conduct the tracer experiments in two discrete biogeochemical zones. The transition zone site (F347) was located 0.19 km downgradient from the infiltration beds at a depth representing the interface between the overlying oxic uncontaminated groundwater and the anoxic contamination plume (Figure 2A and B). The tracer experiment used an extraction/injection MLS (F347-M10) and an observation MLS (F347-M1) located 1.3 m downgradient. The contaminated zone site (S522) was located in the infiltration beds at a depth corresponding to the historical anoxic core of the plume, which has subsequently become oxic (Figure 2C and D) as the result of natural attenuation following removal of the WWTP source in 1995 (41). This experiment used an extraction/injection MLS (S522-M1) and an observation MLS (S522-M4) located 2.1 m downgradient. The water table altitude at the transition site was 14.5 m above mean sea level and at the contaminated site was 15.0 m above mean sea level.

The tracer experiments involved removing approximately 200 L of oxic groundwater from the extraction/injection MLS using a peristaltic pump, amending the groundwater with sodium bromide (Br^-), SX, NP, and E2 (Table 1), and injecting the groundwater solution back into the aquifer over approximately 3 h at the port from which it was withdrawn (Figure 1B). Samples were collected from the injection MLS at 3-h intervals for 4 days, followed by daily sampling for 5 days. The low pumping rate ($<100\text{ mL min}^{-1}$) from the sampling port had little effect on local groundwater flow, and the protocol minimized removal of tracer cloud volume ($<5\%$ of the injection volume was withdrawn during each experiment). The downgradient observation MLSs were sampled daily for 10 days.

The NP used in the tracer experiments was a branched-chain isomeric mixture (Schenectady International, Schenectady, NY), and the Br^- , SX, and E2 were high purity

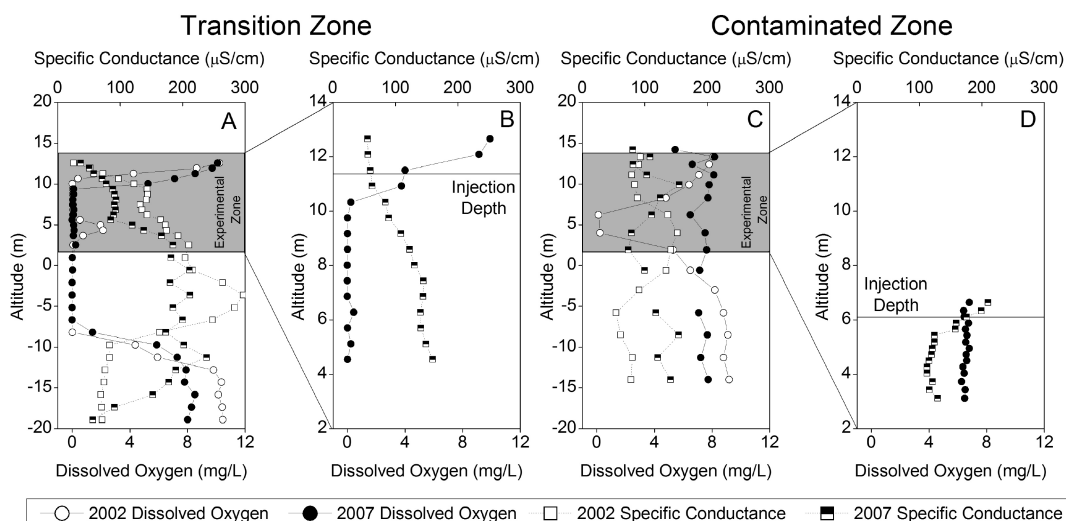


FIGURE 2. Groundwater geochemical conditions in the aquifer where natural gradient in situ tracer experiments were conducted. (A) Dissolved oxygen and specific conductance profiles for the F347 transition zone in July 2002 and June 2007. (B) Dissolved oxygen and specific conductance profiles for the F347 transition zone during the September 2006 tracer experiment. (C) Dissolved oxygen and specific conductance profiles for the S522 contaminated zone in July 2002 and June 2007. (D) Dissolved oxygen and specific conductance profiles for the S522 contaminated zone during the September 2006 tracer experiment. [In 2006 the water table altitude at F347 was 14.5 m above mean sea level and at S522 was 15.0 m above mean sea level].

TABLE 1. Summary of Compounds Used in the Natural Gradient in Situ Groundwater Tracer Experiments^d

Compound	Structure	Formula	Molecular Weight	CASRN	Water Solubility $\mu\text{g L}^{-1}$	Log K_{ow}	Trans- ition C_o $\mu\text{g L}^{-1}$	Contam- inated C_o $\mu\text{g L}^{-1}$
Bromide (Br^-)		NaBr	102.9	7647-15-6	>1000000	--	99200	93600
Sulfamethoxazole (SX)		$\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_3\text{S}$	253.3	723-46-6	610000 ^a	0.9 ^a	440	430
4-Nonylphenol (NP)		$\text{C}_{15}\text{H}_{24}\text{O}$	220.4	84852-15-3	4600 ^b	4.0 ^b	190	170
17 β -Estradiol (E2)		$\text{C}_{18}\text{H}_{24}\text{O}_2$	272.4	50-28-2	13000 ^c	3.9 ^c	0.71	1.9

^a Abbreviations used in text given in parentheses: CASRN, Chemical Abstracts Services Registry Number; K_{ow} , octanol–water partition coefficient; C_o , concentration in the injection port following addition of tracers. ^b Reference (52); ^c Reference (53); ^d Reference (54).

(>99%) single-component standards (Sigma Aldrich, St. Louis, MO). Nominal concentrations (Table 1) were based on the sensitivity of the analytical methods, background concentrations, and a 100-fold factor with respect to detection limits or background concentrations. Bromide was analyzed by ion chromatography (37), and SX, NP, and E2 were analyzed by enzyme linked immunosorbent assays (ELISA), following manufacturers procedures (Abraxis, Warminster, PA). Detection limits were $100 \mu\text{g L}^{-1}$ for Br^- , $2.5 \mu\text{g L}^{-1}$ for NP, $0.015 \mu\text{g L}^{-1}$ for SX, and $0.0015 \mu\text{g L}^{-1}$ for E2. A subset of samples was analyzed for SX and NP using LC/MS/MS and GC/MS (as described above) to confirm the ELISA results.

Breakthrough-Curve Analysis. Groundwater velocity at the injection MLS (v_{inj}) was estimated from the radius of the

spherical tracer cloud (x_r) immediately after injection (t_0) and the time after injection for concentrations to reach $C_t/C_0 = 0.5$ ($t_{0.5}$), where C_t is concentration at time t and C_0 is concentration at t_0 . Based on a 200-L injection volume, an isotropic medium at the small scale of the tracer cloud (32, 33), and a porosity of 0.39, the radius of a spherical tracer cloud symmetrically distributed around the injection port is 0.5 m. At a flow velocity of 0.42 m d^{-1} , a parcel of unperturbed groundwater would move $\sim 0.05 \text{ m}$ during the injection period. Groundwater velocity at the observation MLS (v_{obs}) was estimated using moments analysis (32, 42) to determine time for the Br^- center of mass to reach the sampling point (τ), and the distance between the injection and observation MLS (x_l).

$$v_{inj} = \frac{x_r}{t_{0.5}}; v_{obs} = \frac{x_i}{\tau} \quad (1)$$

where

$$\tau = \left[\int_{t_b}^{t_f} t C_t dt / \int_{t_b}^{t_f} C_t dt \right] \quad (2)$$

and t_b is time at the beginning of breakthrough and t_f is time at the end of breakthrough (assuming an instantaneous pulse source, i.e., center of mass at t_b is at $x_i = 0$). Relative mass recovery (M_{rel}) was determined by (43)

$$M_{rel} = \left[\int_{t_b}^{t_f} \frac{C_t}{C_0} dt / \int_{t_b}^{t_f} \frac{Br_t^-}{Br_0^-} dt \right] \times 100 \quad (3)$$

where Br_0^- is concentration of Br^- at t_b and Br_t^- is concentration of Br^- at time t . Longitudinal dispersivity (α_L) of the conservative tracer Br^- was determined by (44)

$$\alpha_L = \frac{x_i(\Delta t / t_{peak})^2}{16 \ln 2} \quad (4)$$

where Δt is duration of breakthrough when Br^- concentration was greater than 1/2 peak concentration, and t_{peak} is time to peak concentration. Relative retardation factor (R_f) was determined by (45)

$$R_f = \frac{\tau}{\tau_{Br^-}} = 1 + \frac{\rho_b K_d}{\eta} \quad (5)$$

where τ_{Br^-} is time for the center of mass of the Br^- cloud to pass the observation MLS, ρ_b is sediment bulk density, K_d is sediment water distribution coefficient (sediment concentration/water concentration), and η is porosity. Calculation of R_f using eq 5 assumes that initially the conservative and retarded compounds are uniformly distributed throughout the spherical cloud because of the rate-limited mass transfer during the 3-h injection phase when groundwater velocities are relatively fast compared to the ambient groundwater velocity. Sorption of nonpolar organic contaminants is controlled by sediment organic carbon (46) and can be expressed as the organic carbon normalized K_d ($K_{oc} = K_d/f_{oc}$ where f_{oc} = fraction sediment organic carbon), assuming a local equilibrium and linear sorption. The use of eq 5 ignores degradation, which can result in a negative bias to estimated R_f values (42). However, consideration of such effects is beyond the scope of this investigation.

Microcosm Experiments. Laboratory microcosm experiments using aquifer sediments (47) were conducted to evaluate NP and E2 biodegradation potential. Sulfamethoxazole was not investigated because a ^{14}C -labeled standard was not available. Sediment cores were collected adjacent to the MLS arrays at the same depths as the tracer experiments using hollow-stem auger drilling and a wireline piston core barrel. Uniform-ring-labeled ^{14}C 4-normal-NP (U-ring ^{14}C 4-*n*-NP) had an activity of 52 $\mu Ci \mu mole^{-1}$ and 4- ^{14}C E2 had an activity of 54 $\mu Ci \mu mole^{-1}$ (>99% purity, American Radiolabeled Chemicals, St. Louis, MO). The methods were calibrated with ^{14}C HCO_3^- (98% purity, Sigma Biochemicals, St. Louis, MO). Microcosms were prepared in triplicate and consisted of 10 mL serum vials with 5 mL of saturated sediment, 2 mL of sterile-deionized water, and an atmosphere of air. Duplicate sediment controls were prepared by autoclaving 3 times for 1 h, and sediment free controls were prepared in the same manner. Microcosms were amended with approximately 0.02 μCi of the appropriate ^{14}C -substrate to yield initial U-ring ^{14}C 4-*n*-NP concentrations of 24 $\mu g L^{-1}$ and 4- ^{14}C E2 concentrations of 34 $\mu g L^{-1}$. Microcosms were incubated in the dark at 23 °C for up to 54 days, and concentrations of $^{14}CO_2$ in the headspace were determined

at approximately 7-day intervals by isocratic packed column gas chromatography/radiometric detection. Headspace sample volumes were replaced with pure oxygen, and maintenance of oxic conditions was monitored. Dissolved phase concentrations were estimated using Henry's Law partition coefficients.

Results and Discussion

Plume Survey. The 2005 groundwater sampling (Figure 1A) confirmed the presence of contaminants, such as PCE, DCB, and NP, that were reported in the original 1985 sampling (12). Additional OWCs that were not analyzed in the earlier study were detected in the groundwater (15% of the 212 compounds analyzed were detected; Supporting Information, Tables S1 and S2). The distribution of NP was restricted to the area near the infiltration beds, SX was detected along most of the plume, and E2 was not detected. Changes in the distributions of PCE, DCB, and NP between 1985 and 2005 indicate transport in the aquifer (groundwater travel distance during the 20 years between samplings was ~3100 m), consistent with earlier (12) estimated R_f values (based on plume distributions) of 1.0 for PCE and DCB and 2.4 for NP. Despite changes in analytical methods, concentrations for the 1985 and 2005 samplings were similar.

Tracer Tests. Concentrations of Br^- measured in the center of the tracer cloud at t_b were 99% and 94% of the nominal values in the transition and contaminated zones. Concentrations of NP at t_b were less than nominal values, likely, because of incomplete transfer of the neat NP standard and loss by sorption to the mixing containers and injection system. Concentrations of SX and E2 were greater than nominal values indicating potential analytical bias at high concentrations where large dilutions are required. The average relative percent difference for duplicate analyses was 0.7% for Br^- ($n = 12$), 10.2% for SX ($n = 40$), 39.0% for NP ($n = 335$), and 18.4% for E2 ($n = 60$). Results for the LC/MS/MS and GC/MS analyses were correlated with the ELISA data, having r^2 values of 0.95 for SX ($n = 327$) and 0.90 for NP ($n = 33$).

The Br^- results (presented as C_t/C_0 , Figure 3), were used to estimate v_{inj} and v_{obs} (Table 2). In the transition zone experiment, v_{inj} was 0.64 m d $^{-1}$ and v_{obs} was 0.58 m d $^{-1}$, and in the contaminated zone experiment, v_{inj} was 0.72 m d $^{-1}$ and v_{obs} was 0.65 m d $^{-1}$. In addition to differences in the calculation methods, variability between v_{inj} and v_{obs} also reflects local heterogeneities in hydraulic conductivity (33) and transient disturbances during injection of the tracers. At the transition zone, the SX breakthrough curves coincided with the Br^- curves indicating minimal retardation and minor mass loss, which is consistent with the reported behavior of SX in WWTP effluents and receiving waters (9, 48–51). The injection MLS breakthrough curves indicated greater sorption of NP ($R_f = 1.8$) than E2 ($R_f = 0.9$), but after 1.3 m of transport R_f values were similar (1.4 and 1.3, respectively). The M_{rel} values after 1.3 m of transport were 42% and 37% for NP and E2 (Table 2), resulting in estimated in situ attenuation rates of 15% d $^{-1}$ and 18% d $^{-1}$ for NP and E2, respectively. Values of α_L for Br^- reflect aquifer characteristic controlled by the physical structure of the sediments (32, 33) and differed between the transition and contaminated sites. The calculated α_L values for SX, NP, and E2 increased with increasing relative retardation and tailing of the breakthrough curve.

The Br^- and SX breakthrough curves for the contaminated zone injection MLS (Figure 3, Table 2) had similar shapes and C_t/C_0 values as for the transition zone. In contrast, NP sorption was greater in the contaminated zone than in the transition zone and there was substantial tailing of concentrations. In the contaminated zone experiment, E2 was added to the injection solution after 65% of the volume had been injected, which is reflected in faster breakthrough (Figure

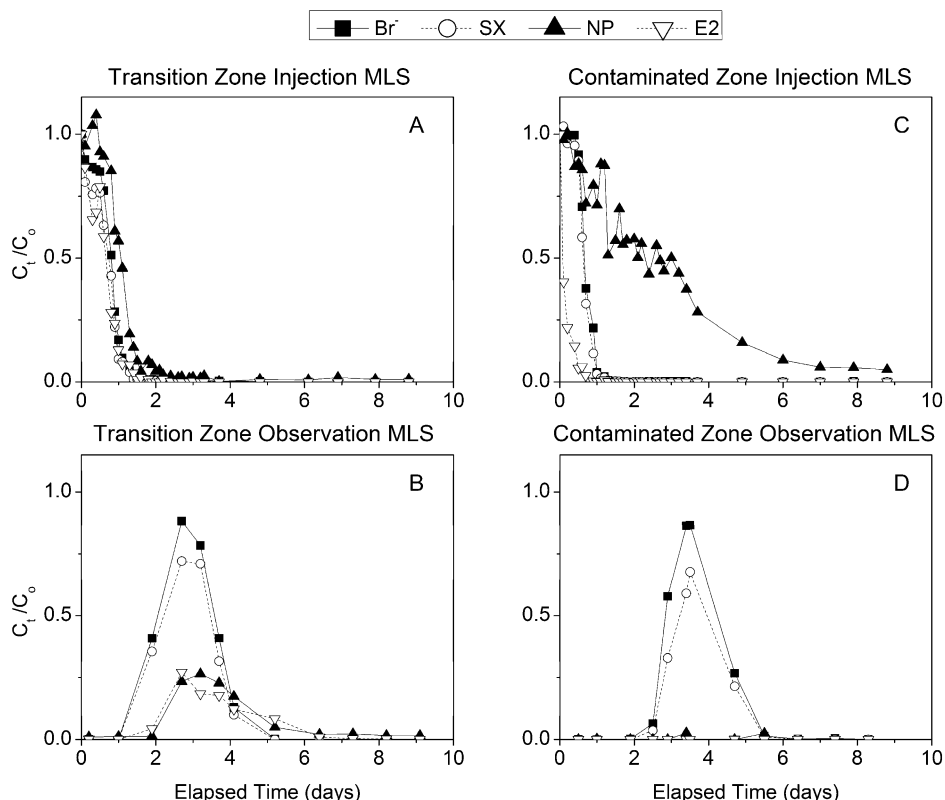


FIGURE 3. Breakthrough curves for bromide (Br^-), sulfamethoxazole (SX), 4-nonylphenol (NP), and 17 β -estradiol (E2) during the natural gradient in situ tracer experiments conducted in September 2006. (A) Transition zone (F347) injection multilevel sampler (MLS). (B) Transition zone observation MLS located 1.3 m downgradient. (C) Contaminated zone (S522) injection MLS. (D) Contaminated zone observation MLS located 2.1 m downgradient. [C_t/C_0 = concentration at time t /concentration at time t_0].

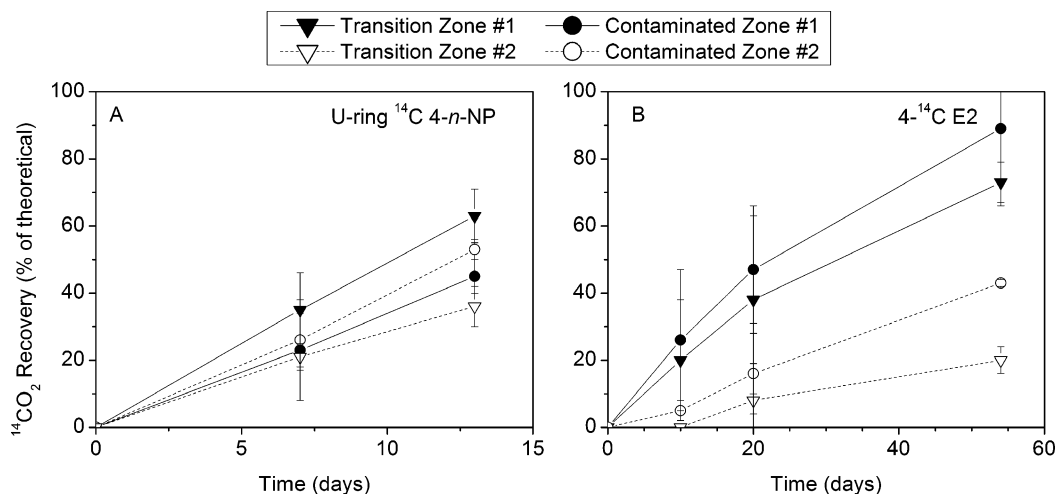


FIGURE 4. Results for oxic microcosm experiments using sediments collected from the transition zone (F347) and contaminated zone (S522). (A) Mineralization of uniformly ring-labeled ^{14}C 4-normal-NP (U-ring ^{14}C 4- n -NP) to $^{14}\text{CO}_2$. (B) Mineralization of 4- ^{14}C 17 β -estradiol (4- ^{14}C E2) to $^{14}\text{CO}_2$. [Transition zone core 1 was collected at an altitude of 11.1–10.5 m above mean sea level (tracer injected at 11.5 m); contaminated zone core 1 was collected at an altitude of 6.6–6.0 m above mean sea level and core 2 was collected at an altitude of 6.0–5.4 m above mean sea level (tracer injected at 6.1 m); data are means \pm standard deviations for triplicate microcosms; no recovery of $^{14}\text{CO}_2$ was observed in autoclaved or sediment free control microcosms.]

3C) owing to the smaller radius of the tracer cloud. In contrast to Br^- and SX, NP and E2 were not detected at the downgradient observation MLS during the course of the experiment. The estimated R_f for NP determined from the injection MLS predicts a travel time of >14 days to the MLS, whereas the experiment was terminated after 10 days and the NP and E2 breakthrough curves were not captured. Greater sorption of NP in the contaminated zone than in the transition zone indicates geochemical het-

erogeneity (34) with the sediments beneath the infiltration beds having enhanced sorption characteristics.

Retardation factors can be estimated from the K_{oc} values of the various compounds. The most water-soluble compound studied was SX (Table 1), which has a log K_{oc} of 1.8 L kg^{-1} (52). Branch-chained NP has a log K_{oc} of 4.0 L kg^{-1} (53) and E2 has a log K_{oc} of 3.3 L kg^{-1} (54). Using the above log K_{oc} values, a bulk density of 1.59 g cm^{-3} , a porosity of 0.39, and a SOC of 0.005% ($f_{oc} = 0.00005$) for the course to fine

TABLE 2. Results from Breakthrough-Curve Analysis of the Natural Gradient in Situ Tracer and Sediment Microcosm Experiments Conducted in the Transition and Contaminated Zones in the Cape Cod Aquifer^a

parameter	transition injection MLS	transition ^b observation MLS	contaminated injection MLS	contaminated ^c observation MLS
bromide (Br ⁻)				
v_{inj} , v_{obs} (m d ⁻¹)	0.64	0.58	0.72	0.65
τ (d)	0.56	2.81	0.48	3.67
α_L (cm)		4.71		3.98
R_f	1.0	1.0	1.0	1.0
M_{rel} (%)		100		100
sulfamethoxazole (SX)				
τ (d)	0.52	2.79	0.40	3.73
α_L (cm)		4.89		3.38
R_f	0.9	1.0	0.8	1.0
M_{rel} (%)		84		72
4-nonylphenol (NP)				
τ (d)	1.00	3.87	2.35	
α_L (cm)		5.28		
R_f	1.8	1.4	4.9	
M_{rel} (%)		42		
mineralization (% d ⁻¹) ^d	3.8		3.9	
17 β -estradiol (E2)				
τ (d)	0.50	3.50		
α_L (cm)		5.14		
R_f	0.9	1.3		
M_{rel} (%)		37		
mineralization (% d ⁻¹) ^d	0.9		1.3	

^a Abbreviations used in text given in parentheses: [MLS, multilevel sampler; m, meters of transport; v_{inj} , average groundwater velocity at the injection MLS; v_{obs} , average groundwater velocity at the observation MLS; τ , hydraulic residence time; α_L , longitudinal dispersivity; R_f , relative retardation factor; M_{rel} , relative mass recovery; mineralization, average initial linear mineralization rate.

^b Tracer pulse was injected into the transition zone at an altitude of 11.5 m above mean sea level over 3.1 h, and the observation MLS was located 1.3 m downgradient from the injection MLS; ^c Tracer pulse was injected into the contaminated zone at an altitude of 6.1 m above mean sea level over 3.3 h, and the observation MLS was located 2.1 m downgradient from the injection MLS; ^d Mineralization determined from the amount of ¹⁴CO₂ recovered (in % of theoretical) and duration of the experiment.

grained sand (35) results in calculated R_f values of 1.0 for SX, 3.0 for NP, and 1.4 for E2, consistent with observed transport (Table 2). Similar natural gradient in situ tracer experiments with LAS at the transition zone site showed chromatographic separation of isomers with varying alkyl-chain lengths with R_f values ranging from 1.0–2.9 (55, 56). Fractionation of NP isomers was not observed (based on GC/MS results), consistent with positional isomers on a single 9 carbon alkyl chain.

Microcosm Experiments. Attenuation of NP and E2 was observed during the natural gradient tracer tests in the transition zone, presumably owing to biotransformation. The sediment microcosm experiments showed that 30–60% of the U-ring ¹⁴C 4-*n*-NP was recovered as ¹⁴CO₂ in 13 days, with initial linear rates of mineralization ranging from 3.8–3.9% d⁻¹ (Figure 4; Table 2). The 4-*n*-NP isomer used in the microcosm experiments is not a component of technical NP formulations, but the linear nature of the side chain makes it more biodegradable than the branched-chain isomers and thus an indicator of maximum biodegradation potential. There was no difference in U-ring ¹⁴C 4-*n*-NP mineralization

between the transition and contaminated zones. Mineralization of 4-¹⁴C E2 also was observed, with 20–90% of the substrate recovered as ¹⁴CO₂ in 54 days. As observed for U-ring ¹⁴C 4-*n*-NP, there was no difference in 4-¹⁴C E2 mineralization between the transition and contaminated zones although there was a difference with depth.

Attenuation rates observed in the transition zone observation MLS was greater than in the laboratory microcosms suggesting that other factors are influencing the estimates. Attenuation in the field does not necessarily indicate mineralization, but could be due to formation of metabolites such as conversion of E2 to estrone (57). Transformation products were not measured as part of this study. Although microcosm and field determined removals are not directly comparable (microcosms were designed to evaluate potential rather than mimic in situ conditions), the data demonstrate that the subsurface microbial community is capable of mineralizing 4-*n*-NP and E2. Similar oxic microcosm experiments conducted with stream sediments reported initial linear rates of mineralization of 7–10% d⁻¹ for U-ring ¹⁴C 4-*n*-NP (47) and 2–6% d⁻¹ for 4-¹⁴C E2 (58). Mineralization of E2 under oxic condition by WWTP solids (57) had an average removal of 74% d⁻¹. Although microcosm experiments were not conducted with SX, the literature on its oxic biodegradation reports mixed results. At low biomass concentrations typical of natural waters, only 4% SX degradation was observed in laboratory experiments over 28 days (48). Under the high biomass conditions of a WWTP, SX biodegradation can exceed 90% (49–51).

Implications. This study documents the subsurface behavior of WWTP effluent derived contaminants at a range of spatial scales from meters to kilometers and temporal scales from hours to decades. The plume distributions indicate long-range transport and persistence of OWCs, whereas the natural gradient in situ tracer experiments were more controlled and provided quantitative data at a localized spatial and temporal scale. Data from both the injection and observation MLSs were used to maximize the information obtained from the resource intensive tracer experiments. For example, at the contaminated site the injection MLS data show that NP transport is retarded by sorption and an R_f can be estimated. Because breakthrough was not observed in the downgradient observation MLS, R_f cannot be estimated from the field results. Although hydraulically different (one is natural gradient and the other is induced gradient), single MLS pulsed experiments (40) are similar to single well push–pull experiments (59) in that they can be completed in relatively short times, only require one well, and do not require a priori knowledge of local flow conditions. Natural gradient pulsed tracer test methods using downgradient MLSs (40, 55) provide more integrated spatial and temporal data for defining hydraulic parameters and are less influenced by disturbances in the injection well. However, they require detailed knowledge of local hydraulic conditions, an appropriately located 3-dimensional sampling grid, and greater logistical resources.

The co-transport of SX and Br⁻ suggests that SX may be a useful tracer of subsurface contamination by WWTP effluent. The occurrence and transport of SX in the contamination plume over kilometers and decades indicates that it is resistant to natural attenuation in the subsurface environment. Previous studies have shown cotransport of free living bacteria and OWCs in the downgradient wells (60) in which SX was detected, indicating long-term exposure of the microbial community to antimicrobial compounds. Although the effect of antibiotics on the biotransformation of co-occurring OWCs is not known, previous in situ experiments at the Cape Cod site showed that high concentrations of antibiotics eliminated hydrogen consumption, a sensitive measure of subsurface microbial activity (61).

Retardation of NP transport by sorption to the aquifer sediments and mass loss by biotransformation under oxic conditions illustrates the complexities of natural attenuation. As suggested by the plume scale distributions, aquifer degradation rates must be slower than those determined in laboratory microcosm and small scale field experiments. This indicates that additional factors such as hydrodynamics, temperature, oxygenation conditions, nutrient limitations, and microbial ecology also control the fate of OWCs. Sorption of NP to the aquifer sediments occurred over the WWTP effluent disposal history at the site, and its persistence under anoxic conditions (47) resulted in an extensive contamination plume near the infiltration beds. Concentrations and distributions of NP determined in the 2005 sampling were relatively unchanged from results determined 20 years earlier. Concentrations of NP measured in the groundwater were similar to values reported for WWTP effluents and impacted surface waters (5, 7, 8, 11), and were near those shown to induce biological effects (19, 20, 62, 63).

The occurrence of OWCs such as SX, NP, and E2 at other impacted groundwater sites (14, 15, 64) and in septic tank effluents (65) indicates their potential as groundwater contaminants, and understanding their behavior under different hydrological, geochemical, and management conditions requires further study. Once introduced into the aquifer, depending on the biogeochemical environment and individual chemical characteristics, contaminants can persist and be transported over long distances.

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Supporting Information Available

Descriptions of the chemical analysis methods and results from the 2005 groundwater sampling. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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