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Identification of Alkylphenols and Other Estrogenic Phenolic Compounds in Wastewater, Septage, and Groundwater on Cape Cod, Massachusetts

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As part of a larger effort to characterize the impacts to Cape Cod drinking water supplies from on-site wastewater disposal, we developed two analytical methods using HPLC and GC/MS for a range of compounds identified as endocrine-disrupting chemicals (EDCs), including the nonionic surfactants alkylphenol polyethoxylates (APEOs) and their degradation products. We analyzed samples for nonylphenol, octylphenol, and their ethoxylates up to the hexaethoxylate using an HPLC method, with detection limits ranging from 2 to 6 μg/L. A set of phenolic compounds including bisphenol A and nonylphenol were derivatized and analyzed by GC/MS with detection limits from 0.001 to 0.02 μg/L. Total APEOs in untreated wastewater and septage samples ranged from 1350 to 11 000 μg/L by the HPLC method. Nonylphenol was detected in all septage samples at concentrations above 1000 μg/L. Phenylphenol and bisphenol A were detected in septage and wastewater at about 1 μg/L. In groundwater downgradient of an infiltration bed for secondary treated effluent, nonyl/octylphenol and ethoxylates were present at about 30 μg/L. Bisphenol A, nonylphenol monoethoxycarboxylate, and nonyl/octylphenol tetraethoxylate were detected in some drinking water wells at concentrations ranging from below the quantitation limit to 32.9 μg/L. Results suggest that septic systems may be a significant source of APEOs to groundwater.

Introduction

Wastewater is considered a major source of pollution in many areas of the United States, and in some areas, such as Cape Cod, MA, it is an important source of groundwater and drinking water contamination. Drinking water on Cape Cod is supplied entirely from groundwater vulnerable to contamination from local land use, especially wastewater disposal. The high water table of the unconfined sole source aquifer is overlain by highly permeable, sandy soils. In addition to rapid population growth, the reliance on septic systems and cesspools for domestic wastewater disposal and the regional practice of discharging treated wastewater to groundwater provide significant opportunity for contamination of the groundwater (*1, 2*).

Since wastewater is a major drinking water pollutant, it is important to understand its composition. Recent discoveries of hormonally mediated toxic effects in fish downstream of sewage discharge points led to the realization that wastewater contains endocrine-disrupting chemicals (EDCs) (*3*−*5*). The search to identify the specific components of wastewater that act as EDCs led to discoveries that some common household products [for example, heavy-duty laundry powders and liquid detergents, personal care products, and household cleaners (*6*)] contain nonionic surfactants that break down in the environment to form chemicals that can mimic estrogen (*7*). These surfactants are a class of chemicals known as alkylphenol polyethoxylates (APEOs), and they break down in the environment into alkylphenol ethoxycarboxylates and the estrogenic alkylphenols known as nonylphenol and octylphenol (*8, 9*). Other chemicals that are present in consumer products and have been reported to have some potential endocrine-mediated activity include ubiquitous constituents of some plastics, such as phthalates, bisphenol A, hard surface household cleansers like phenylphenol, and some antioxidants, like BHT (*10*−*12*). One recent study involving a random screen of 20 synthetic organic chemicals present in liquid wastewater effluents revealed that half of the compounds were able to interact in some way with the estrogen receptor, and a subset of these produced estrogenic responses in mammalian cells (*13*).

The question of what specific agents in treated wastewater cause the observed reproductive problems in fish downstream of sewage treatment plants is not resolved. Nonylphenol and some related chemicals are suspected and have been shown to induce similar effects in the laboratory when administered as pure compounds (*5, 13*). The observation has also been made that endogenous hormones and pharmaceutical hormones from birth control pills are excreted in women's urine and can also be detected in sewage effluents and that these compounds can also induce reproductive effects in fish (*14*). The activity may also be associated in part with compounds in effluent that have not yet been identified.

The potential health effects of exposure to the alkylphenols and other weakly estrogenic chemicals is the subject of considerable debate (*15*−*19*). Pharmaceutical and endogenous estrogens are considerably more potent than alkylphenols in screening tests (*11*), and so they may be more important sources of exposure to estrogenic chemicals. However, it has been argued that in vitro and short-term in vivo screening tests for estrogenic activity may not provide an adequate basis for predicting safe levels of exposure to the weakly estrogenic synthetic chemicals (*15, 17*). The toxicology of EDCs is an area of intensive research. This paper is focused on characterizing domestic wastewater, especially from septic systems, as a potential source of alkylphenols to groundwater.

There are a number of research articles describing efforts to measure the APEOs and their degradation products in environmental samples (*9, 20*−*23*). Work in this area has been prompted by the aquatic toxicity of the alkylphenols; hence most of the studies have focused on surface water bodies and sediments, particularly downstream of sewage treatment plant and tanning and textile plant discharges. In one study, 30 U.S. rivers receiving industrial or municipal wastewater were tested for nonylphenol and nonylphenol

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ethoxylates. Most sediment samples showed these compounds at concentrations ranging from 170 to 3000 μg/kg; water samples ranged from below the detection limits of 0.1 to 15 μg/L (*9*). Other researchers have reported concentrations of nonylphenol and related compounds typically in the range of 100−500 μg/L in the effluent of sewage treatment plants (*24*, *25*). Clark and co-workers (*20*) identified nonylphenol ethoxylates in New Jersey drinking water (surface water source) at levels of 0.01−0.1 μg/L.

There is much less information available about groundwater contamination with alkylphenols. The only published reports we located were from research conducted on Cape Cod, MA (*22*), and Orange County, CA (*26*). On Cape Cod, an extensive plume of contaminated groundwater has resulted from the continuous disposal of secondary-treated effluent into the groundwater at Otis Air Force Base, now known as the Massachusetts Military Reservation. This treatment facility discharged wastewater to the ground at the same location between 1936 and 1996; the treated sewage has a trace organic composition typical of municipal and domestic effluents. LeBlanc and colleagues at the U.S. Geological Survey (USGS) have been conducting research on the fate and transport of chemicals in this groundwater plume for many years (*27*). These researchers have reported nonylphenol in this plume at concentrations around 1 μg/L (*22*). These researchers observed that the less soluble compounds, like nonylphenol, are sorbed to the aquifer material and do not travel as fast as more soluble compounds, like chloroethanes and chlorobenzenes. However, nonylphenol polyethoxylates and carboxylates are more soluble than nonylphenol itself and will travel faster (*23*). Another important observation made by Barber and colleagues is that for a variety of reasons, including the acidic pH and low organic carbon content of the Cape aquifer, many contaminants that typically degrade in the subsurface have persisted for more than 30 years in the Cape aquifer (*22*). Researchers in Orange County, where drinking water is supplied in part by recharged treated wastewater, have reported nonvolatile alkylphenol ethoxycarboxylates at low microgram per liter levels (*26*).

We were unable to locate any published studies of alkylphenolic compounds discharged to groundwater from septic systems or cesspools. This is an important area for study because on-site wastewater disposal is a significant source of groundwater contamination throughout the United States.

As part of a larger effort to characterize the impacts to Cape Cod drinking water supplies from on-site wastewater disposal and other local land uses, we developed analytical methods for a range of compounds identified as EDCs, including APEOs and their degradation products. We also developed methods for detecting additional phenolic EDCs that have been reported to be estrogenic and were hypothesized to be present in residential wastewater. Nonylphenol, octylphenol, and their ethoxylates up to the hexaethoxylate were analyzed using an HPLC method with detection limits ranging from 2 to 6 μg/L. A set of phenolic compounds including bisphenol A and nonylphenol were derivatized and analyzed by GC/MS with detection limits from 0.001 to 0.02 μg/L. We applied these methods to untreated and treated wastewater and septage, groundwater known to be impacted by these sources, and drinking water wells.

Overview of Study Area. Cape Cod, MA, is a 440 square mile area of sand, gravel, and boulders deposited by glaciers. The year-round population of 200 000 residents grows to about 600 000 during the summer. Most of the population relies on on-site septic systems or cesspools for sewage disposal. Periodically, septic tanks and cesspools are pumped out and the septage is taken to a treatment facility; treated effluent is discharged to groundwater. Until recently, septage was disposed in unlined pits, usually at the local landfill. For the small areas of Cape Cod that are sewered, treated wastewater is discharged to the groundwater.

Groundwater is the primary source of drinking water on Cape Cod. About 80% of the population receives drinking water from 18 municipal water suppliers that draw from about 130 groundwater wells and one surface source. The remainder of the population is served by private drinking water wells. The unconfined aquifer is highly productive, providing public wells with an average of 17.5 Mgal/day in 1978 (*28*), and wells are typically shallow, 60−70 ft below the water table for public wells and approximately 15 ft below the water table for private wells (*29*). Approximately 50% of municipal drinking water supply wells on Cape Cod show nitrate nitrogen concentrations above background levels, an indicator of impact from wastewater or fertilizer; even greater impacts are observed in private wells (*1*). Detectable levels of volatile organic compounds (VOCs), particularly chlorinated solvents, have also been found in about 50% of Cape municipal supply wells.

Our objectives included identifying phenolic EDCs in domestic sewage, investigating whether the same compounds are found in affected groundwater and drinking water wells, and exploring relative concentrations across sample types. We sampled (a) untreated and treated wastewater and septage, (b) groundwater known to be impacted by treated wastewater or untreated septage, and (c) drinking water from wells likely to have had some historical wastewater impact from septic systems. In order to meet our research objectives, we developed two analytical methods, using HPLC and GC/MS, to measure a set of phenolic EDCs in these environmental samples.

Experimental Section

Selection of Target Compounds. Target compounds included phenolic compounds reported to be EDCs based on literature review (*7*, *10*−*13*, *30*) and expected to be present in domestic sewage. Of primary interest were nonylphenol, octylphenol, and their ethoxylates and ethoxycarboxylates, and bisphenol A. Other phenolic compounds were included if they had been reported as EDCs or if they were target compounds of EPA Phenols Method 604. Table 1 lists target compounds for the methods reported in this paper and indicates whether the compound has been identified as a potential EDC (*31*) or specifically as having estrogenic activity (*7*, *10*−*13*, *30*, *32*).

Selection of Sampling Locations. Although most wastewater on Cape Cod is disposed of in on-site septic tanks, we did not have access to monitoring wells near septic tanks. We therefore selected locations that would provide samples that we expected would be similar to samples from septic tanks. Cape Cod has two septage treatment facilities that receive septage by truck from mostly residential septic tanks and three wastewater treatment facilities that treat both wastewater from municipal sewer systems and septage from septic tanks. The Massachusetts Military Reservation operates its own wastewater treatment facility.

A total of five grab samples of septage (untreated) were taken from the two septage treatment facilities on two different days. These samples should be typical of septage found in septic tanks throughout Cape Cod.

Four grab samples of untreated wastewater and three grab samples of treated effluent (treated wastewater or septage) were collected from two of the wastewater treatment facilities on two different days. The source of wastewater on Cape Cod is primarily residential, and we consider the untreated wastewater samples to be generally representative of wastewater as it enters a septic tank. At the treatment facilities, the wastewater and septage are treated (minimum of

TABLE 1. Target Compounds and Method Detection Limits for Phenol and Alkylphenol Ethoxylate Analyses[a]

	IDL	MDL	QL	monitored mass	quant mass
GC/MS Target Compounds					
nonylphenol[b]	0.0084	0.0106	0.0318	221, 263, 207	221
octylphenol[b]	0.0061	0.0049	0.0183	278, 263, 207	278
octylphenol monoethoxylate (OP1EO)[c]	0.01	0.0198	0.0594	251, 322, 252	251
octylphenol diethoxylate (OP2EO)[c]	0.0033	0.0052	0.0156	295, 296, 366	295
nonylphenol monoethoxylate (NP1EO)[c]	0.0144	0.0156	0.0468	251, 265, 336	251
nonylphenol diethoxylate (NP2EO)[c]	0.013	0.0227	0.0681	295, 309, 380	295
nonylphenol ethoxycarboxylate (NP1EC)[c]	0.0867	0.0639	0.26	279, 265, 350	279
bisphenol A[b]	0.0036	0.0054	0.0162	357, 372, 207	357
4,4′-dihydroxybiphenyl (4,4′-biphenyldiol)[b]	0.0029	0.0029	0.0087	330, 331, 315	330
4-*tert*-butylphenol[b]	0.0012	0.0009	0.0036	207, 222, 208	207
2-hydroxybiphenyl (*o*-phenylphenol)[b]	0.0014	0.0008	0.0042	211, 242, 227	211
3-hydroxybiphenyl (*m*-phenylphenol)[b]	0.0015	0.0014	0.0045	227, 242, 211	227
4-hydroxybiphenyl (*p*-phenylphenol)[b]	0.0011	0.0017	0.0051	242, 227, 211	242
4-*tert*-pentylphenol[b]	0.0015	0.0011	0.0045	179, 236, 180	179
butylated hydroxyanisole[b]	0.0017	0.0022	0.0066	237, 252, 238	237
2-*sec*-butylphenol	0.0015	0.0007	0.0045	193, 222, 207	193
4-nitrophenol	0.0022	0.0022	0.0066	196, 211, 150	196
6-bromo-2-naphthol[b]	0.0015	0.0031	0.0093	296, 294, 281	296
2,4-dichlorophenol[b]	0.0014	0.0010	0.0042	219, 221, 234	219
3,4-dichlorophenol	0.0019	0.0009	0.0057	219, 221, 234	219
3,5,6-trichloro-2-pyridinol	0.0041	0.0006	0.0123	256, 254, 258	256
pentachlorophenol[d]	0.0025	0.0032	0.0096	323, 325, 338	323
HPLC Target Compounds					
4-nonyl/octylphenol (NP/OP)[b]	4.19				
4-nonyl/octylphenol monoethoxylate (NP/OP1EO)	5.08				
4-nonyl/octylphenol diethoxylate (NP/OP2EO)	7.90				
4-nonyl/octylphenol triethoxylate (NP/OP3EO)	5.95				
4-nonyl/octylphenol tetraethoxylate (NP/OP4EO)	5.16				
4-nonyl/octylphenol pentaethoxylate (NP/OP5EO)	1.98				
4-nonyl/octylphenol hexaethoxylate (NP/OP6EO)	4.88				

[a] All concentrations in μg/L. MDL, method detection limit. QL, quantitation limit. IDL, instrument detection limit. [b] Reported to be estrogenic (*7*, *10*−*13*, *30*, *32*). [c] Degrades to estrogenic alkylphenols. [d] Reported as a known or potential endocrine-disrupting chemical (EDC) (*31*).

secondary treatment) under aerobic conditions, so we expect the effluent from these facilities to be substantially different from the effluent from septic tanks where anaerobic conditions prevail.

Four samples were collected from single-depth 2 in. diameter groundwater monitoring wells with 2 or 5 ft long screens previously installed by USGS as part of their investigation of a plume of secondary-treated wastewater effluent at Otis Air Force Base. Wells were screened between 38 and 79 ft below ground level and were located approximately 500−900 ft downgradient of the infiltration beds. No wastewater had been discharged to these beds in the previous 6 months. Samples were obtained using a Grundfos Redi-Flow 2 submersible centrifugal pump with all Teflon and stainless steel components and HDPE tubing. These samples are representative of effluent from wastewater treated under aerobic conditions after it is discharged to the subsurface environment.

Five samples were collected with Teflon bailers or Wattera tubing from groundwater monitoring wells in or near three municipal landfills. Wells were screened between 20 and 120 ft below ground level. Where possible, wells near areas of those landfills used historically for disposal of septage were selected for sampling. Because the septage deposited in these landfills is not mixed or treated, there may be minimal aerobic degradation occurring at these facilities, and the leachate may be similar to leachate from septic tanks. However, the proximity of the septage disposal facilities to solid waste facilities makes it very difficult to determine if the source of contaminants is septage or other household wastes.

Twenty-eight samples were taken from the indoor or outdoor taps of residents with private drinking water wells. Sampling locations were selected to provide a range of expected wastewater impact based on historical nitrate nitrogen concentrations or local residential density.

Sample Collection. Two rounds of sampling were conducted: one in the summer of 1996 and the second in early 1997. Samples were collected in accordance with U.S. EPA sampling protocols described in 40 CFR 136. Samples were collected without preservative in 1-L amber glass bottles, packed on ice, and shipped overnight to the laboratory where they were stored at 4 °C, and extracted within 4 weeks. An equipment blank was prepared following the use of monitoring well pumping equipment, and one field blank was prepared for each day of sampling. Blanks were prepared using deionized water filtered through activated carbon.

Standards and Reagents. Standards of 4-nonylphenol, 4-octylphenol, 2,4-dichlorophenol, 3,4-dichlorophenol, 4-*tert*-butylphenol, 2-hydroxybiphenyl, 3,5,6-trichloro-2-pyridinol, 4,4′-biphenyldiol, 4-nitrophenol, pentachlorophenol, butylated hydroxyanisole, 3-hydroxybiphenyl, and 4-hydroxybiphenyl were obtained from Chem Service (West Chester, PA). 4-Nonylphenol ethoxylates (IGEPAL CO-210, IGEPAL C0-520), 4-octylphenol ethoxylates (IGEPAL CA-210), and 6-bromo-2-naphthol were obtained from Aldrich (Milwaukee, WI). 2-*sec*-Butylphenol, 4-*tert*-pentylphenol, and bisphenol A were purchased from Acros (Pittsburgh, PA). Nonylphenol monoethoxycarboxylate (NP1EC) was prepared by Pierre Varineau of Union Carbide and provided by Dr. Jennifer Field of Oregon State University. 2,4,6-Tribromophenol was obtained from Ultra Scientific (North Kingstown, RI). High-purity dichloromethane (DCM), *n*-hexane, and 2-propanol were obtained from Fisher (Pittsburgh, PA). The percentage of mono- and diethoxylates in the IGEPAL CO-210 and CA-210 was determined by normal phase HPLC (*21*). The derivatization reagent *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 10% trimethylchlorosilane (BSTFA + 10%

TMCS) was obtained from Regis Technologies (Morton Grove, IL).

Extraction and Derivatization. The extraction procedures for the HPLC and GC/MS method were the same except as noted below. A 1000-mL aliquot of sample was measured and placed into a 2-L separation funnel. The sample was then extracted with three portions of 60 mL of DCM. The extracts were collected and combined into a 250-mL Kuderna−Danish evaporator. Previous extraction tests showed that the application of anhydrous sodium sulfate as a drying agent can reduce the recovery of some phenolic compounds. Therefore, no anhydrous sodium sulfate was used. For drinking water samples, the extract was heated to 80 °C and evaporated to a volume of approximately 5 mL. After cooldown, the extract was concentrated to a final volume of 1.0 mL using dry nitrogen at room temperature. Wastewater samples were taken to a final volume of 10 mL.

For the GC/MS analysis, a 50-μL volume of 20 ng/mL internal standard (2,4,6-tribromophenol) was added to the sample, and the pH was adjusted to less than 2 using 1:1 sulfuric acid prior to the extraction. In order to meet target detection limits in the parts per trillion range, the extracts were derivatized by adding 25 μL of BSTFA + 10% TMCS reagent into 500 μL of the extract. This derivatization procedure was selected on the basis of previous experience with similar compounds. In the first round of sampling, the extract was heated at 70 °C for 2 h. Under these conditions, the recovery for nonylphenol and octylphenol was poor. As a result, these target compounds were analyzed by HPLC in the first round. For the second round of sampling, the extracts were heated at 90 °C for 5 h, resulting in more complete derivatization and acceptable recoveries, greater than 65%, for nonylphenol (NP), octylphenol (OP), their mono- and diethoxylates (NP1EO, NP2EO), and nonylphenol ethoxycarboxylate (NP1EC). Target APEOs for the GC/MS method were limited to the lower molecular weight compounds.

Gas Chromatography/Mass Spectroscopy. The derivatized sample extract was analyzed on a VG Fisons MD800 GC/MS equipped with an A200F autosampler operating in electron impact ionization mode at 70 eV. Separation was performed using a 30 m × 0.25 mm i.d. DB-5.625 column with a 0.25-μm film thickness (J&W Scientific, Folsom, CA). The injector was operated in splitless mode at a temperature of 240 °C with a 3-μL injection volume. The GC oven temperature was held at 40 °C for 4 min and then increased to 300 °C at a rate of 10 °C/min. It was then held at 300 °C for 2 min. Selective-ion monitoring mode (SIM) was used for GC/MS analysis. Three ions of each derivatized analyte and internal standard (2,4,6-tribromophenol) were monitored for determination. These are listed in Table 1. The base peak ion (or the second most intense peak ion if there was interference with the base peak) was used as quantification ion of each compound.

Prior to sample analysis, the GC/MS was calibrated by using five working standards at a calibration range of a factor of 200. Since 4-nonylphenol consists of a mixture of para-substituted monoalkylphenols with various isomeric nonyl groups, its chromatogram is a peak cluster instead of a single peak. All isomers of 4-nonylphenol have the same diagnostic ions. Similar peak clusters are also observed in the chromatograms of nonylphenol ethoxylates and nonylphenol ethoxycarboxylate, which are both synthesized from 4-nonylphenol. A multipeak integration approach, which integrates the total areas of the peak cluster of the quantification ion, was used in quantification of nonylphenol, nonylphenol ethoxylates, and nonylphenol ethoxycarboxylate. Matrix spikes and duplicates were performed for approximately every 20 samples.

High-Performance Liquid Chromatography. The HPLC method targeted nonylphenol, octylphenol, and mono-, di-, tri-, tetra-, penta-, and hexaethoxylates of nonyl- and octylphenols (degradation products of larger chain ethoxylates present in detergents). The method was modified from the normal phase HPLC procedure developed by Ahel and Giger (*21*). HPLC separation of the DCM extracts was performed using a 4.6 mm × 25 cm Lichrosorb-NH_2 normal phase HPLC column connected to a Waters 600E solvent delivery system. A gradient elution was used from 99:1 to 25:75 hexane:2-propanol in 30 min and then held for an additional 15 min before returning to the initial conditions. Detection was accomplished using UV absorption at 277 nm.

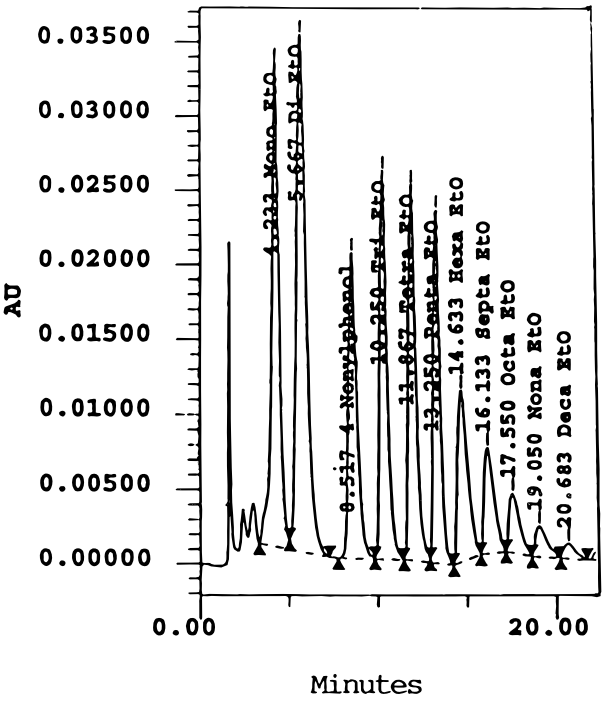

FIGURE 1. HPLC chromatogram of a standard mixture of 4-nonylphenol, IGEPAL CO-210, and IGEPAL CO-520.

A major limitation in the methods development was the lack of availability of pure standards of the target compounds. Although compounds with different degrees of ethoxylation were easily distinguished as shown in Figure 1, the method cannot distinguish between 4-nonylphenol and octylphenol or between their corresponding ethoxylates. Consequently, results are reported as "nonyl/octylphenol" to indicate that they represent the total concentration of 4-nonyl- and octylphenol and as "nonyl/octylphenol monoethoxylate" (total of 4-NP and OP monoethoxylates) through "nonyl/octylphenol hexaethoxylate" (total of 4-NP and OP hexaethoxylates). The concentrations of the polyethoxylates were calculated using the molar response factor for 4-nonylphenol. This approach was validated by analyzing a 265 μg/L IGEPAL CO-520 standard. The total calculated concentration of ethoxylates, based on the molar response factor for 4-nonylphenol, was 278 μg/L, which represents a 4.9% deviation from the true prepared concentration. The method also does not provide for a means of confirmation when peaks are present within the retention time window. Because of these limitations, the identification of target compounds should be regarded as tentative.

The HPLC was calibrated by using five calibration standards over a range of concentrations (10−300 μg/L). The calibration curve for each of the analytes was very linear. Matrix spikes and duplicates were performed for approximately every 20 samples.

Recovery, Precision, and Detection Limits. The method detection limit for the GC/MS method was evaluated by

spiking seven replicates of reagent water with all target analyses at a concentration five times the estimated method detection limit. After extraction, derivatization, GC/MS-SIM analysis, and quantification, the standard deviation S of the seven replicates for each analyte compound was calculated. The method detection limit (MDL) was obtained by

$$\mathrm{MDL} = t_{(n-1,1-\alpha=0.99)}S$$

where $t_{(n-1,1-\alpha=0.99)}$ is the Students' value appropriate for a 99% confidence level and S is the standard deviation estimate with $n - 1$ degrees of freedom. The instrument detection limit (IDL) was determined by analyzing 7−10 replicates of the lowest working standard and following the same calculation procedures used for the MDL. The quantitation limit was calculated to be three times the higher value between the MDL and IDL. In this paper we have reported results below the quantitation limit with the qualifier "TR" for trace. A list of detection limits for all target analyses is given in Table 1. Results show that method detection limits ranged from 0.01 to 0.02 μg/L for 4-nonylphenol, NP1EO, NP2EO; 0.064 μg/L for NP1EC; from 0.005 to 0.02 μg/L for 4-octylphenol, OP1EO, OP2EO, and from 0.001 to 0.003 μg/L for 15 other phenolic compounds.

A spike and recovery study was performed to determine the efficiency and reproducibility of both methods for each of the target analytes. Three replicates of reagent water were spiked with all target analytes. The spiked samples were then extracted and analyzed. The spiked samples for GC/MS analysis were derivatized prior to analysis. The experimental results show that among 22 target analytes, 17 analytes have average recoveries falling between 80 and 110%. For 4-nonylphenol, 4-octylphenol, NP1EO, NP2EO, NP1EC, OP1EO, and OP2EO, recoveries of 66−107% were obtained. The relative standard deviation (RSD) for replicate recovery analyses ranged from 0 to 17%.

The instrument detection limits (IDLs) for the HPLC method were calculated from the mean and standard deviation of nine replicates of the lowest working standard (5−20 μg/L) by

$$\mathrm{IDL} = t_{(n-1,1-\alpha=0.99)}S$$

where $t_{(n-1,1-\alpha=0.99)}$ is the Students' value appropriate for a 99% confidence level and a standard deviation estimate with $n - 1$ degrees of freedom. IDLs for this method ranged from 2 to 6 μg/L (Table 1), which were too high to be especially useful for groundwater and drinking water samples.

To determine sample recovery for the HPLC method, the peak area of spiked sample extracts was compared with peak areas of a recovery standard prepared by diluting 100 μL of the spiking solution (0.1 mg of 4-nonylphenol, 1.5 mg of IGEPAL CO-210, and 3.5 mg of IGEPAL CO-520/mL of DCM) to a volume of 1.0 mL of DCM. Percent recoveries ranged from 101 to 114%.

Inorganic Analyses. Standard U.S. EPA methods were used to analyze samples for inorganic parameters that might be associated with wastewater contamination. Samples were analyzed for nitrate nitrogen (Method 4500 for drinking water, Method 353.2 for wastewater and contaminated groundwater); ammonia nitrogen (Method 350.1); boron, potassium, and sodium (Method 200.7); alkalinity (Method 310.1), chloride (Method 120.1); and specific conductance (Method 120.1).

Results and Discussion

Wastewater and Septage Samples. Many of the target compounds were detected in the untreated wastewater and septage samples, with highest concentrations in septage. Concentrations in treated effluent were considerably lower than in either untreated wastewater or septage. Table 2 summarizes results for these samples.

Total nonyl/octylphenol and ethoxylates in untreated wastewater and septage samples ranged from 1350 to 11 000 μg/L by the HPLC method, which includes up to the hexaethoxylated compounds. Despite the limitations of the HPLC method described above (e.g., high detection limits; tentative compound identification), the results from this method provide useful information about the distribution of APEOs that can be expected in wastewater and septage. Concentrations of nonyl/octylphenol were typically about 25% of the total, and the di-, tri-, and pentaethoxylates were the other most prevalent forms of APEOs. Total APEOs in the treated effluent sample were less than 30 μg/L.

The GC/MS method allowed us to confirm the identity of the compounds detected, but this method is limited to the smaller molecular weight ($n < 3$) APEOs. Using the GC/MS method, nonylphenol was detected in all septage samples at concentrations above 1000 μg/L. Concentrations in untreated wastewater were much lower, ranging from 25 to 33 μg/L. Nonylphenol was detected at 15.9 μg/L in the one treated effluent sample analyzed by GC/MS. Nonylphenol monoethoxycarboxylate (NP1EC) was a relatively minor component of the untreated samples, but its concentration of 41.5 μg/L in the treated sample was the highest of all APEOs analyzed.

Phenylphenol, a hard surface cleanser with broad commercial and residential use (*33*), was present in untreated wastewater and septage samples at 1−3 μg/L and at lower concentrations in treated effluent. Bisphenol A, which has uses in epoxy resins and polycarbonate plastic and is also identified as a fungicide (*33*), was detected in septage at about 1 μg/L and in other samples at lower concentrations.

Groundwater Impacted by Wastewater and Septage. All groundwater samples were taken during the first round of sampling and were analyzed for APEOs by the HPLC method only. Groundwater samples taken from the plume of secondary-treated wastewater effluent had similar levels of APEOs, using this method, as samples of effluent taken directly from a wastewater treatment facility discussed above (about 30 μg/L). Concentrations of bisphenol A and other target compounds in the plume were also similar or slightly lower than levels in treated wastewater (Table 3). This finding suggests that the treated wastewater that was the source of the groundwater plume contained higher concentrations of APEOs and other target compounds than the treated effluent samples we collected or that concentrations in groundwater have not been attenuated by time or distance from the infiltration beds. Of course, interpretation of these data is limited by the small number of samples.

In groundwater samples taken from under septage lagoons of municipal landfills, concentrations of bisphenol A and 4-*tert*-butylphenol were most similar to levels in septage samples and were higher than levels in untreated or treated wastewater. This is consistent with the hypothesis that degradation of compounds in untreated septage at landfills occurs very slowly. It is also possible that the household waste in the landfills is a source of these compounds.

These results suggest that septic systems may be a significant source of APEOs to groundwater. Results provide preliminary information about the types of compounds and concentrations that can be expected in wastewater entering a septic tank and in septage. Because septic tanks are anaerobic and some compounds may be preferentially adsorbed to the septage that remains in the septic tank, the leachate from septic tanks may be somewhat different from the wastewater or groundwater samples we analyzed. In order to study the fate and transport of compounds discharged from septic tanks, it will be necessary to install a

TABLE 2. Concentrations of Target Compounds in Untreated and Treated Wastewater and Septage Samples[a]

	max field blank/DL	untreated septage			untreated wastewater			treated septage/wastewater		
		no. detects/ no. analyzed	range	av of detects	no. detects/ no. analyzed	range	av of detects	no. detects/ no. analyzed	range	av of detects
GC/MS Target Compounds										
nonylphenol	0.061	2/2	1000−1500	1200	2/2	25−33	29	1/1		16
octylphenol	0.023	2/2	35−42	39	2/2	0.20−0.74	0.47	1/1		0.15
octylphenol monoethoxylate (OP1EO)	0.029	2/2	8.0−9.8	8.9	1/2		0.21	0/1		
octylphenol diethoxylate (OP2EO)	<0.016	0/2			1/2		0.067	0/1		
nonylphenol monoethoxylate (NP1EO)	0.039TR	2/2	440−580	510	2/2	15−21	18	1/1		5.5
nonylphenol diethoxylate (NP2EO)	0.027TR	2/2	79−100	90	2/2	6.4−8.0	7.2	1/1		0.80
nonylphenol ethoxycarboxylate (NP1EC)	<0.26	2/2	37−57	47	2/2	1.3−1.7	1.5	1/1		42
bisphenol A	0.003TR	4/5	0.11−1.7	0.82	3/4	0.094−0.15	0.11	3/3	0.02−0.055	0.038
4,4′-dihydroxybiphenyl (4,4′-biphenyldiol)	0.003TR	5/5	0.04−1.4	0.77	4/4	0.1−0.38	0.24	1/3		0.02
4-*tert*-butylphenol	<0.0036	5/5	0.16−3.2	0.85	4/4	0.042−0.1	0.070	2/3	0.02−0.032	0.026
phenylphenol (total)	0.005	5/5	0.16−3.9	1.7	3/4	2.2−3.5	2.7	2/3	0.017−0.05	0.034
4-*tert*-pentylphenol	<0.0045	2/5	0.03−0.03	0.03	2/4	0.029−0.19	0.20	0/3		
butylated hydroxyanisole	<0.0066	5/5	0.13−0.53	0.30	2/4	0.025−0.05	0.038	0/3		
2-*sec*-butylphenol	<0.0045	2/5	0.03−0.08	0.055	1/4		0.020	0/3		
4-nitrophenol	0.008	1/5		0.16	2/4	0.091−0.21	0.15	2/3	0.034−0.12	0.077
6-bromo-2-naphthol	<0.0093	1/5		0.05	0/4			0/3		
2,4-dichlorophenol	0.003TR	2/5	0.07−0.10	0.085	4/4	0.042−0.22	0.27	2/3	0.061−0.16	0.11
3,4-dichlorophenol	0.002TR	1/5		0.02	0/4			0/3		
3,5,6-trichloro-2-pyridinol	0.005TR	2/5	0.16−0.19	0.18	1/4		0.05	0/3		
pentachlorophenol	0.005TR	2/5	0.04−0.05	0.045	1/4		0.02	0/3		
HPLC Target Compounds										
4-nonyl/octylphenol (NP/OP)	<4.2	1/1		3700	2/2	46−2400	1200	0/2		
4-nonyl/octylphenol monoethoxylate (NP/OP1EO)	<5.1	0/1			2/2	38−210	120	0/2		
4-nonyl/octylphenol diethoxylate (NP/OP2EO)	<7.9	1/1		4000	2/2	180−2000	1100	0/2		
4-nonyl/octylphenol triethoxylate (NP/OP3EO)	<6.0	1/1		2300	2/2	890−1000	950	0/2		
4-nonyl/octylphenol tetraethoxylate (NP/OP4EO)	<5.2	1/1		77	0/2			1/2		18
4-nonyl/octylphenol pentaethoxylate (NP/OP5EO)	<2.0	1/1		900	1/2		2000	1/2		12
4-nonyl/octylphenol hexaethoxylate (NP/OP6EO)	<4.9	1/1		58	1/2		81	1/2	9.4−86	48
sum HPLC alkylphenol ethoxylates				11 000		1400−7500	5400		27−98	78

[a] All concentrations in μg/L. DL, detection limit. TR, below quantitation limit.

TABLE 3. Concentrations of Target Compounds in Contaminated Groundwater[a]

	max field blank/DL	plume from wastewater treatment plant			plume from landfill/ septage lagoon		
		no. detects/ no. analyzed	range	av of detects	no. detects/ no. analyzed	range	av of detects
GC/MS Target Compounds							
nonylphenol	0.061	NA			NA		
octylphenol	0.023	NA			NA		
octylphenol monoethoxylate (OP1EO)	0.029	NA			NA		
octylphenol diethoxylate (OP2EO)	<0.016	NA			NA		
nonylphenol monoethoxylate (NP1EO)	0.039TR	NA			NA		
nonylphenol diethoxylate (NP2EO)	0.027TR	NA			NA		
nonylphenol ethoxycarboxylate (NP1EC)	<0.26	NA			NA		
bisphenol A	0.003TR	4/4	0.003TR−0.029	0.016	5/5	0.004TR−1.41	0.32
4,4′-dihydroxybiphenyl (4,4′-biphenyldiol)	0.003TR	3/4	0.011−0.021	0.014	4/5	0.004TR−0.014	0.01
4-*tert*-butylphenol	<0.0036	4/4	0.007−0.051	0.028	5/5	0.002TR−2.70	0.56
phenylphenol (total)	0.005	3/4	0.002TR−0.043	0.026	2/5	0.004TR−0.033	0.02
4-*tert*-pentylphenol	<0.0045	0/4			0/5		
butylated hydroxyanisole	<0.0066	0/4			1/5		0.002TR
2-*sec*-butylphenol	<0.0045	0/4			2/5	0.004TR−0.005	0.005
4-nitrophenol	0.008	2/4	0.002TR−0.014	0.008	3/5	0.002TR−0.003TR	0.003TR
6-bromo-2-naphthol	<0.0093	0/4			2/5	0.008TR−0.077	0.043
2,4-dichlorophenol	0.003TR	3/4	0.002TR−0.052	0.027	1/5		0.035
3,4-dichlorophenol	0.002TR	1/4		0.003TR	3/5	0.004TR−0.005TR	0.004TR
3,5,6-trichloro-2-pyridinol	0.005TR	2/4	0.008TR−0.014	0.011TR	2/5	0.005TR−0.007TR	0.006TR
pentachlorophenol	0.005TR	3/4	0.003TR−0.009TR	0.005TR	2/5	0.010−0.026	0.018
HPLC Target Compounds							
4-nonyl/octylphenol (NP/OP)	<4.2	1/4		1.5TR	NA		
4-nonyl/octylphenol monoethexylate (NP/OP1EO)	<5.1	0/4			NA		
4-nonyl/octylphenol diethoxylate (NP/OP2EO)	<7.9	4/4	14−38	28	NA		
4-nonyl/octylphenol triethoxylate (NP/OP3EO)	<6.0	1/4		4.4TR	NA		
4-nonyl/octylphenol tetraethoxylate (NP/OP4EO)	<5.2	2/4	4.6TR−5.0TR	4.8TR	NA		
4-nonyl/octylphenol pentaethoxylate (NP/OPSEO)	<2.0	0/4			NA		
4-nonyl/octylphenol hexaethoxylate (NP/OP6EO)	<4.9	0/4			NA		
sum HPLC alkylphenol ethoxylates			14−48	32			

[a] All concentrations in μg/L. DL, detection limit. TR, below quantitation limit. NA, not analyzed.

series of groundwater monitoring wells located in plumes of septic system leachate.

Drinking Water Samples. Bisphenol A, nonylphenol monoethoxycarboxylate (NP1EC), and nonyl/octylphenol tetraethoxylate were the only target compounds detected in samples from private drinking water wells at concentrations above the levels detected in field blank samples. We consistently detected nonylphenol, octylphenol, and their mono- and diethoxylates in field blanks (0.02−0.06 μg/L), and we occasionally detected bisphenol A, phenylphenol, and 4,4′-dihydroxybiphenyl at or near the instrument detection limits (approximately 0.002 μg/L) in blank samples. Blank contamination may be due to residues of detergents used to prepare sample containers, leaching from the Teflon-lined plastic lids of the sample bottles, or some other unidentified source.

Bisphenol A was detected in six of 28 private drinking water well samples at concentrations above the maximum detected in any blank. Two of these samples had concentrations above the quantitation limit (0.044 and 0.020 μg/L). Bisphenol A was detected in two of 12 field blank samples at concentrations near the instrument detection limit of 0.0036 μg/L. In addition, we detected NP1EC at a concentration below the quantitation limit (<0.26 μg/L) in one of the well samples that also contained bisphenol A. NP1EC is a mobile degradation product of APEOs (*23*). We also detected nonyl/octylphenol tetraethoxylate (NP/OP4EO) by HPLC in one drinking water well at 32.9 μg/L.

It is difficult to identify the source(s) of bisphenol A, NP1EC, and NP/OP4EO in the drinking water wells based on these limited data. NP1EC and NP/OP4EO might be expected in shallow wells based on their mobility and the elevated concentrations of parent APEOs in wastewater and septage (>10 000 μg/L). Bisphenol A, however, was detected in wastewater and septage samples at lower levels (<5 μg/L), so it is unclear whether levels in the wells are associated with wastewater impacts to the aquifer.

On the basis of local land use or historical measurements of nitrate nitrogen, we anticipated a range of wastewater impacts in the drinking water wells we sampled. Nitrate nitrogen measurements are an indicator of potential impact from wastewater or fertilizer, both of which are nitrogen rich. The wells that contained trace amounts of bisphenol A had nitrate levels elevated above background levels, which could indicate a wastewater or fertilizer source. In addition, the one well in which both NP1EC and bisphenol A were detected had elevated levels of both nitrate nitrogen (7.2 mg/L) and boron (3 mg/L); boron is considered an indicator of wastewater impact (*22*).

The goal of the work described in this paper was to identify what phenolic EDCs to expect in a range of sewage-impacted environmental samples and to test new analytical methods. After our first round of sampling, we concluded that the detection limits for the HPLC method for detecting APEOs were not adequate to detect these compounds in groundwater impacted by wastewater. We then turned our focus to developing a GC/MS method to detect some of these compounds. The GC/MS method we used had low detection limits but was limited to the lower molecular weight APEOs. More work is needed to develop an efficient method for detecting APEOs at low concentrations in environmental samples. In addition, recent reports of endogenous and pharmaceutical estrogens in wastewater (*14*) indicate that analytical methods should be modified to also target these compounds.

An investigation of the local hydrogeology and the subsurface fate and transport of compounds from wastewater was beyond the scope of this preliminary study. However, the results from this study can be used to design a more detailed study investigating the fate and transport of APEOs and other EDCs in groundwater discharged from specific wastewater sources, such as residential septic tanks. Our findings of high concentrations of estrogenic alkylphenols in wastewater and septage, as well as some indication of impacts on shallow drinking water wells, suggest that further study of the impacts of septic systems on groundwater should be a priority in order to ensure that drinking water supplies are adequately protected by existing regulations and waste disposal practices.

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