Supporting Information for

Analysis of estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA.

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Detailed analytical methodology of organic wastewater contaminants (OWC)

Analysis of Organic Wastewater Contaminants

Samples for analysis of OWCs were filtered through 47 mm Gelman GF/C filters within 24 hours after collection. Where available, isotopically labeled surrogate standards (^{13}C-labeled caffeine, NP, NP1-3EO, and deuterated EDTA, E1, E2, E1-3-glucuronide and sulfate, and E2-3-glucuronide and sulfate) were then added prior to SPE extraction that was performed within 72 hours of sampling. The analyses by HPLC-MS or GC-MS (EDTA) then relied upon the method of isotope dilution (*1-5*) which accounts for incomplete recoveries or matrix induced suppression of ionization during electrospray-MS.

Steroid estrogens and their conjugates. E1 and E2 were extracted and purified from groundwater (500 - 900 mL) and septic tank (100 mL) samples (for July 2003 and November 2003 samplings) by reverse phase SPE followed by purification with immuno-affinity SPE as described elsewhere (*2*). Instrumental analysis was performed by HPLC-MS analysis using a Waters (Milford, MA) Alliance 2690 HPLC system, electrospray ionization and a Micromass (Waters Corp, Milford, MA) LCT time-of-flight (ToF) mass analyzer equipped with a 4.6 GHz time-to-digital converter (*5*). The estrogen conjugates were determined according to the method of Reddy et al. (*4*) which included extraction from acidified water by Oasis HLB SPE cartridges, purification of extracts by selective washing of the Oasis HLB cartridge followed by weak anion-exchange HPLC, and detection by tandem quadrupole MS (Quattro LC, Micromass, Manchester, UK). The analysis of conjugates was performed only during the August 2004 sampling, and the effect of extracting acidified groundwater was to co-extract much more dissolved organic matter matrix. When the less polar fraction containing E1 and E2 was eluted from the Oasis SPE cartridges prior to conjugate elution (*5*), the greater abundance of co-eluted matrix led to very low recoveries (presumed to be due to nonspecific competition on the immuno-affinity column) of deuterated E1 and E2 in the most septic leachate affected station 1 samples; hence, E1 and E2 are not reported for every sample analyzed for the estrogen conjugates in August 2004. Method detection limits for E1, E2, and the glucuronide and sulfate conjugates were approximately 0.1 -

0.2 ng/L for groundwater samples analyzed in this study (*2,5*). Levels of E1, E2, and their conjugates were undetectable in both acidified and unacidified field blank samples.

NPEO metabolites. The methods used to determine NPEO metabolites primarily followed those reported elsewhere (*1,6*). Groundwater (1 L) and septic tank (200 mL) samples were extracted using Bondesil C-18 SPE cartridges (Varian, Sugarland, TX) and, following elution with acetone, were solvent exchanged into mobile phase solvent. Extracts were analyzed without further purification. NP1-3EO were analyzed by electrospray-MS in positive ionization mode as sodium adducts using isochratic chromatographic methods reported elsewhere (*1*), except with a different HPLC-MS system: HPLC-ToF-MS with capillary and cone voltages of 2.8 KV and 25 V, respectively. The HPLC-MS analysis of NP, NP1EC, and NP2EC utilized electrospray-MS operated in negative ionization mode ($M\text{-}H^+$ ions) with a method modified from that followed elsewhere (*1,6*); HPLC separation of the NPECs (which fragmented in part to deprotonated NP during ESI) from NP was accomplished utilizing a water:methanol mobile phase gradient (from an initial 45:55 volume fraction mixture with linear gradient to a 10:90 mixture at 18 minutes held for an additional 8 minutes). Capillary and cone voltages were set at -2.5 KV and -45 V, respectively. NP, NP1EC, and NP2EC were each quantified relative to a surrogate ^{13}C-NP standard. Groundwater concentrations were typically orders of magnitude greater than field blank levels of NPEO metabolites (e.g., greater than 10,000 ng/L for most NP detections compared to blank values of 0-68 ng/L, with lower but similar (relative to sample detections) blank levels for other NPEO neutral metabolites. Possible blank contamination may have played a role in relatively low level detections (not blank-corrected) for samples collected below the plume (wells 1-5 and 2-3), for which the sum of neutral NPEO metabolites detected were 20 and 196 ng/L, respectively. No detectable blank contamination was determined for NP1EC or NP2EC.

Caffeine and paraxanthine. Analysis of caffeine and paraxanthine in samples followed the methods of extraction and HPLC-MS detection reported elsewhere (*7*), except that ^{13}C-caffeine was used as surrogate standard, and the HPLC-MS was conducted with the HPLC-ToF-MS

system described above. Briefly, 1-L samples were extracted using HLB Oasis SPE cartridges, eluted with methanol containing trifluoroacetic acid, and caffeine and paraxanthine were analyzed by HPLC-ESI-MS as M+1 ions with capillary and cone voltages set at 2.6 KV and 30 V, respectively. Caffeine was quantified by isotope dilution and paraxanthine by comparison with ^{13}C-labeled caffeine. The blank levels of caffeine and paraxanthine were below method detection limits of approximately 5 and 10 ng/L, respectively.

Fluorescent whitening agents DAS and DSBP. The fluorescent whitening agents 4,4-bis [(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2, 2-disulfonate (DAS) and 4,4-bis(2-sulfostyryl)biphenyl (DSBP) were analyzed by a modification of the method of Poiger et al. (*8*). Samples (120 mL volumes for the septic tank to 650 mL for groundwater samples) were extracted by C-18 SPE cartridges (200 mg; Varian) following preconditioning with methanol and deionized water. Following elution with 10 mL 0.01 M tetrabutylammonium chloride in methanol, samples were concentrated and transferred to 1 mL clear glass vials, and aliquots were analyzed by HPLC with fluorescence detection as described elsewhere (*8*). A significant modification in the method included the addition of a UV irradiation step, in which glass vials were exposed for 40 minutes at 2 cm distance to a 4 watt UV lamp (254 nm; Spectroline model EF-140C) prior to HPLC separation. This modification produced a stable ratio between fluorescent E (DSBP) and E,E (DAS) isomers relative to non-fluorescent Z and E,Z isomers. The calibration was to external standards that had been photoisomerized under the same conditions as the samples. As compared to the method of Poiger et al. (*8*), which employs post column photochemical transformation and provides isomer compositional analysis, this method only yields measurements of total DAS and DSBP. The reported groundwater concentrations of DAS are substantially above the MDL (2.4 ng/L in sample matrix, with no detectable signal in the blanks). The levels of DSBP reported in groundwater (1-15 ng/L) are higher than MDL levels (0.4 ng/L) but may be affected by low and variable (0-0.52 ng/L) levels in the field blanks.

EDTA. The chelating agent ethylenediaminetetraacetic acid (EDTA) was measured using a modification (*3,9*) of the method of Schaffner and Giger (10). Samples (100 mL) were evaporated to dryness, acidified with 50 percent (v/v) formic acid/distilled water, and evaporated to dryness. Acetyl chloride/propanol (10 percent v/v) was added, and the sample was heated at 90^{o}C for 1 hour to form the propyl-esters that were solvent-extracted and measured using GC/MS in both the full-scan and selected ion monitoring (SIM) modes under conditions detailed elsewhere (*3,9*).

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