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Nonylphenol Polyethoxy Carboxylate Metabolites of Nonionic Surfactants in U.S. Paper Mill Effluents, Municipal Sewage Treatment Plant Effluents, and River Waters

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Because of their weakly estrogenic properties, interest in nonylphenol polyethoxylates and their carboxylated metabolites is resurging. To assess their occurrence in surface waters impacted by municipal and industrial effluents, an analytical method was developed for the quantitative determination of nonylphenol polyethoxy carboxylate (NPEC) metabolites of nonylphenol polyethoxylate surfactants in paper mill effluents, municipal sewage treatment plant effluents, and river waters. Strong anion exchange solid phase extraction disks were used for NPEC isolation from aqueous samples, and NPEC elution from the disk is simultaneously combined with derivatization. Extracts were analyzed by gas chromatography and positive mode chemical ionization mass spectrometry. The detection limit of the method ranged from 0.2 μg/L for NP1EC to 2 μg/L for NP4EC. The total concentration of NPECs in a group of paper mill and municipal sewage effluents ranged from below detection to 1300 μg/L. The total concentration of NPECs in several river waters ranged from below detection to 13.8 μg/L.

Introduction

Alkylphenol polyethoxylate (APEO) surfactants are used worldwide in various industry, institutional, and household applications. U.S. production exceeded 242 million kg in 1990 (*1*). Industrial uses (55% of total volume) include the manufacture of plastics, textiles, paper, and agricultural chemical products (*2*). Institutional applications (30% of total volume) include vehicle cleaning, commercial laundry products, and hard surface cleaners. Personal care products, contraceptives, cosmetics, and household laundry products account for the majority of household applications (15% of total volume) (*2*).

The biodegradation pathway of APEOs to short-chain ethoxylate and ethoxy carboxylate metabolites (APECs) is well-established (*3*, *4*). A thorough literature review of APEOs and their biodegradation pathway is presented by Talmage (*2*). The occurrence of nonylphenol polyethoxylates (NPEO) and their metabolites has been documented in sewage sludge, sewage effluents, and in river water in Europe (*3*, *5*−*9*). However, few reports document the occurrence of NPEOs in U.S. sewage effluents and river water (*10*−*12*) and the occurrence of octylphenol polyethoxy carboxylates (OPECs) in U.S. sewage effluents (*13*, *14*). To the best of our knowledge, no reports have established the distribution or concentration of APECs in U.S. river waters nor in industrial effluents, and only a single report documents NPECs and OPECs in drinking water (*15*). Because the concentration of NPECs is greater in sewage effluents than in sewage influents due to the biodegradation of NPEOs during sewage treatment (*5*−*8*) and because the toxicological characteristics of NPECs may vary among the oligomers, it is important to understand the occurrence and composition of NPECs in effluents and river waters. Moreover, NPECs are relatively water soluble so that the concentrations of NPECs in river water are typically higher than that of the short-chain ethoxylated NPEO metabolites or nonylphenol, which have lower water solubilities (*9*).

Concerns over the toxicity of APEOs and their biodegradation products led to a voluntary ban in Europe on the use of APEOs in household cleaning products. However, industrial use continues in Europe, and no such ban has been enacted in the United States even though the U.S. EPA issued a Chemical Hazard Information profile on nonylphenol in 1986 (*16*, *17*). While acute toxicity data exist for selected APECs (*18*, *19*), no information is currently available on the chronic toxicities of APECs.

More recently, nonylphenol (NP), octylphenol, nonylphenol diethoxylate, and nonylphenol monoethoxy carboxylate (NP1EC) were shown in to be weakly estrogenic to fish, bird, and mammal cells in vitro (*20*, *21*). In vivo studies with rainbow trout indicated that low microgram per liter concentrations of NP, NPEOs, and NPECs inhibit testicular growth (*22*, *23*). In addition, NP recently was shown to adversely affect testicular size and spermatogenesis in rats (*23*).

Previous analytical approaches to the determination of NPECs in aqueous samples consisted of acidifying the water sample to pH 2 followed by liquid−liquid extraction and derivatizaton with diazomethane (*13*, *14*, *24*) or boron trifluoride in methanol or HCl in methanol (*8*). Solid phase extraction employing C18 bonded-phase silica or graphitized carbon black is an alternative extraction method for concentrating NPECs from water (*7*, *25*). Continuous liquid−liquid extraction was reported for the detection of NPECs and OPECs in extremely large volume samples of drinking water (500 L) (*15*). For separating and quantitating NPECs, normal and reverse phase high-performance liquid chromatography was used with either UV or fluorometric detection (*7*, *8*, *25*). Liquid chromatography/mass spectrometry was used in two reports for the detection of NPECs in sewage (*7*) and drinking water (*15*). Fewer methods exist for the quantitative determination of NPECs by gas chromatography/mass spectrometry (GC/MS), perhaps due to the need to derivatize NPECs to their volatile esters prior to analysis (*8*, *14*, *24*).

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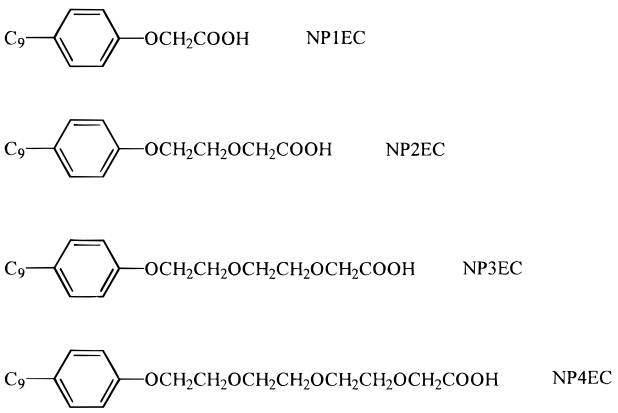

FIGURE 1. NPEC structure and nomenclature.

In order to overcome the tedious steps involved in derivatizing organic acids, Field et al. used strong anion exchange (SAX) Empore disks first to isolate the carboxylated metabolites of the pesticide Dacthal (*26*) and chlorophenoxy acid herbicides (*27*) from water. Subsequently, the ion-exchanged analytes are simultaneously eluted from the SAX disk and derivatized to their methyl esters using methyl iodide in a single step. The technique is a modification of the procedure presented by Chatfield et al. (*28*), in which bulk strong anion exchange resin acted as the catalyst in the alkylation reaction between the ion-exchanged chlorophenoxy acid herbicides and methyl iodide.

As part of a larger effort to better understand the fate and effects of alkylphenol polyethoxylates, the purpose of this study was to determine the occurrence of NPECs in the lower Fox River, a river that flows into Green Bay, WI (*29*). The Fox River Vally has a high concentration of paper manufacturing plants, and the river also receives effluent from several municipal wastewater treatment plants. The Fox River was selected for this study because of the concern over the potential impacts of the NPEOs and NPECs associated with paper production and sewage disposal.

In this paper, we describe an alternative analytical method for the determination of NPECs in municipal sewage treatment plants (STPs) and paper mill effluent and in river water by GC/MS. This work is part of a larger effort to better understand the fate and effects of nonylphenol polyethoxylates. Isolation from water is performed using SAX disks followed by simultaneous elution from the disk and NPEC derivatization for analysis by GC/MS. The developed method is applied to a survey of paper mill effluents, STP effluents, and Fox River water collected from the region near Green Bay, WI.

Experimental Section

Standards and Reagents. Standards of 4-nonylphenoxyacetic acid (NP1EC, 90% purity), 4-nonylphenol ethoxyacetic acid (NP2EC, 45% purity), 4-nonylphenol diethoxyacetic acid (NP3EC, 80% purity), and 4-nonylphenol triethoxyacetic acid (NP4EC, 60% purity) (see Figure 1 for structures) were prepared as their free acids by Pierre Varineau of Union Carbide by reacting chloroacetic acid in the presence of sodium hydroxide with nonylphenol and with nonylphenol monoethoxylate, diethoxylate, and triethoxylate according to the methods of Marcomini et al. (*25*). Although the purity of the standards was not >99%, they were analyzed both by HPLC and GC/MS, and all constituents were identified and quantified. Trace levels (<3%) of nonylphenol, NPEO precursors, and NPECs were identified in each standard except for the NP4EC standard, which contained 16% NP3EO. The identified impurities did not interfere with the quantitation or in calculating percent recoveries.

For use as surrogate and internal standards, 4-bromophenylacetic acid (98%) and 2-chlorolepidine (99%) were purchased from Aldrich Chemical (Milwaukee, WI). Methyl iodide (neat) was used as purchased from Aldrich Chemical. All solvents were Burdick and Jackson GC grade (Baxter, Muskegon, MI). The surrogate standard, 4-bromophenylacetic acid, was added to the water samples just prior to extraction and was used for the quantitation of NPECs. The internal standard was added just prior to derivatization and was used to determine the absolute recovery of the 4-bromophenylacetic acid surrogate standard.

Sample Collection and Preservation. Composite (24-h) samples of effluent were collected by Integrated Paper Services in July 1995 from paper mill and STPs that discharge into the lower Fox River near Green Bay, WI. Composite samples of Fox River water were collected in September 1995 and were composed of grab samples obtained across a transect (1/4, 1/2, and 3/4 across). The Fox River samples were obtained from locations above and below the section of the river receiving paper mill and municipal STP effluent. The downstream sample of Fox River water was sampled at the Tower Drive Bridge located upstream from the Green Bay municipal STP, 700 m from the point at which the river enters Green Bay. Composite (transect) samples were also collected from rivers in the eastern portion of the United States that were previously sampled for NPEOs (*16*).

All samples were collected in baked, brown glass bottles with Teflon-lined lids. River water samples were preserved with 1% (v/v) formalin. The industrial effluents were not preserved; however, unpreserved samples of effluent sampled over a period of 4 weeks showed no loss of NPECs. All samples were shipped on ice immediately after collection, stored at 4 °C, and extracted within 4 weeks of receipt. The pH was measured for each sample using a model 45A pH meter (Chemtrix Inc., Hillsboro, OR), and the ionic strength, as indicated by measurements of specific conductance, was measured using a model 850002 specific conductance meter with a CVP-101P cell (SPER Scientific Ltd., Tempe, AZ). Both the pH meter and specific conductance meter were calibrated prior to use. Samples of paper mill and STP effluents (75 mL) and 250-mL samples of river water were analyzed for the survey.

Solid Phase Extraction and Derivatization. SAX disks (25 mm diameter) were obtained from Varian (Sugarland, TX) and soaked in acetonitrile overnight to remove disk impurities. The 25-mm disks were placed in 25-mm screw-together polypropylene filter assemblies (Micro Filtration Systems, Dublin, CA) and attached to a vacuum manifold that was donated by Supelco (Bellefonte, PA). A 75-mL polypropylene reservoir was attached to the top of each disk assembly (Figure 2). The disk was first rinsed with 5 mL of acetonitrile followed by 5 mL of deionized water. Water samples (up to 500 mL) were spiked with 1 μg of the 4-bromophenylacetic acid surrogate standard and allowed to sit overnight. Samples were centrifuged in 250-mL baked glass tubes for 30 min at 2000 rpm. The supernatant was applied to the disk under full vacuum (25 mmHg). Any particulate matter in the bottom of the centrifuge tube was added to the disk as a slurry once the supernatant has nearly passed through the disk. The sample bottle and reservoir

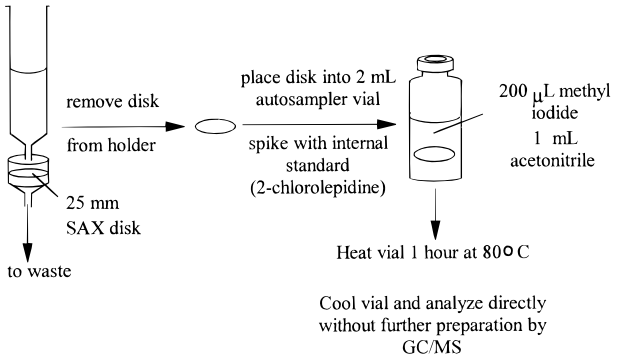

FIGURE 2. Solid phase extraction flow diagram.

are then rinsed with distilled water (5 mL) and extracted through the disk. Once the sample passes through the disk, the disk is dried under full vacuum (25 mmHg). Up to 1 h may be required to dry disks that are used to extract samples with particulate matter. Once dry, the disks are removed and placed directly into a 2-mL GC autosampler vial (Figure 2). Acetonitrile (1 mL) was added just to cover the disk along with 200 μL of methyl iodide and 1 μg of the 2-chlorolepidine internal standard. The autosampler vial was then tightly capped and heated at 80 °C for 1 h (Figure 2). Once cooled, the autosampler vials were then analyzed directly without further manipulation. Caution: Derivatization reactions with methyl iodide should be carried out in a fume hood and caution should be taken when piercing the vial septum if manual injections are performed since the vial contents are under pressure.

Recovery and Precision. To determine the accuracy and precision of the solid phase extraction method, spike and recovery experiments were first performed using deionized water. Duplicate samples of deionized water (50, 250, and 450 mL) were each spiked to give a total concentration of 40 μg/L of each NPEC standard and 1 μg/L of the 4-bromophenylacetic acid surrogate standard. A second set of spike and recovery experiments was performed using a blank surface water collected from Oak Creek near Corvallis, OR, that was determined to contain no NPECs above detection. Sets of four to five replicate 250-mL samples of blank surface water were spiked to give final concentrations of 4, 10, 20, and 40 μg/L for each NPEC standard and 1 μg/L of the 4-bromophenylacetic acid surrogate standard.

Standard addition experiments were performed with samples of STP effluent, a composite sample of paper mill effluent, and Fox River water. Prior to adding known amounts of NPEC standards, the background concentrations of NPECs were determined by analyzing four replicate 75-mL samples of effluents and river water (250 mL). Once the background concentrations were determined, duplicate samples of each sample type were spiked with an additional 2−10 μg of each NPEC standard and extracted as described above.

Gas Chromatography/Mass Spectroscopy. Gas chromatography with chemical ionization mass spectrometry (CI-GC/MS) was used to identify and quantitate NPECs in all effluents and river water samples. A Finnigan Model 4023 mass spectrometer equipped with a Varian 3400 gas chromatograph was used for the CI acquisition. The gas chromatograph was equipped with a 10 m × 0.25 mm × 0.25 μm Econocap SE-54 column (Alltech Assoc., Deerfield, IL). The injector was operated in splitless mode at a temperature of 320 °C with a 2-μL injection volume. The initial GC oven temperature of 70 °C was ramped at 20 °C/min to 300 °C with a 1-min initial hold time. The mass spectrometer was operated in the positive ion mode with a source temperature of 140 °C and ammonia as reagent gas (0.6 Torr). All NPECs produced molecular ion adducts with ammonia to give base peaks corresponding to $[M + NH_4]^+$. Although positive CI-GC/MS with methane as reagent gas was reported for the detection of NPEO and NPEC (*24*, *30*, *31*), ammonia as reagent gas gave intense ammonia−molecular ion adducts for each NPEC with little or no secondary fragmentation. Therefore, the mass spectrometer was operated in multiple ion detection mode with *m/z* 246, 310, 354, 398, and 442 to detect the $[M + NH_4]^+$ ions of the methyl esters of 4-bromophenylacetic acid surrogate standard, NP1EC, NP2EC, NP3EC, and NP4EC, respectively.

Because calibration curves for the NPECs were not linear, an external calibration curve was prepared for each group of samples prior to analysis. Calibration curves were constructed from the methyl esters of NPEC standards (0.05−20 ng/μL) and 1 μg of the methyl ester of 4-bromophenylacetic acid surrogate standard; all standards were derivatized to their methyl esters using diazomethane. Quadratic equations were used to fit each NPEC quantitation curve. To quantitate the recovery of the 4-bromophenylacetic acid surrogate standard, a quantitation curve was constructed from 0.5 to 10 ng/μL of the methyl ester of the 4-bromophenylacetic acid surrogate standard and 1 ng/μL of the 2-chlorolepidine internal standard.

Results and Discussion

Solid Phase Extraction. The first task in method development was to establish that 4-bromophenylacetic acid could be used reliably as a surrogate standard for NPEC. The *absolute* recovery of 4-bromophenylacetic acid and NPECs spiked into deionized water samples (50−500 mL) was determined from calibration curves constructed from standards and the 2-chlorolepidine internal standard. Typical selected ion chromatograms for standard NPECs are given in Figure 3 where each NPEC is depicted as a cluster of peaks within a defined retention time interval. Nearly equivalent recoveries for 4-bromophenylacetic acid (91−100%) and standard NPECs (98−112%) indicate high recoveries of both the 4-bromophenylacetic acid and NPECs and, hence, the suitability of 4-bromophenylacetic acid as a surrogate standard for NPECs.

Using the same sample extracts, the *relative* recovery of NPECs was calculated from calibration curves constructed using the 4-bromophenylacetic acid surrogate standard. High recoveries (97−110%) for the NPECs were obtained and were in the same range as that of the absolute NPEC recovery. Nearly equivalent relative and absolute NPEC recoveries indicated that NPEC isolation from water and subsequent derivatization is efficient and that the presence of the disk did not compromise recovery and confirmed the use of 4-bromophenylacetic acid as a suitable surrogate standard. In addition, high recoveries for NPECs in samples up to 500 mL indicate that no breakthrough occurred while using a single 25-mm SAX disk. Moreover, because quantitative recovery was obtained for 4-bromophenylacetic acid surrogate and NPECs standards, the disk elution and derivatization conditions including 200 μL of methyl iodide and a reaction temperature of 80 °C for 1 h proved

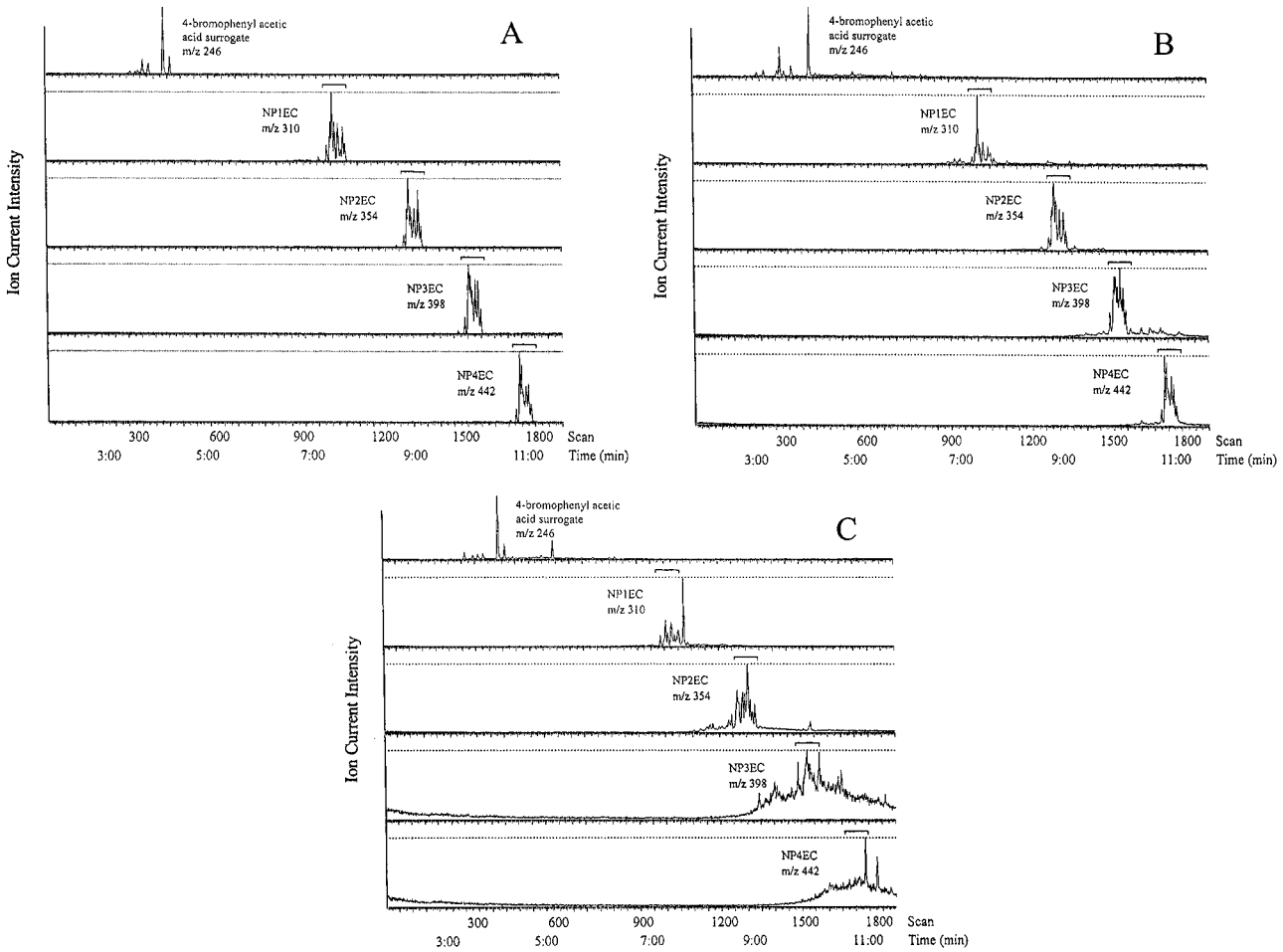

FIGURE 3. Typical CI-GC/MS chromatograms of (A) NPEC standard, (B) paper mill effluent, and (C) river water.

TABLE 1

Average Percent Recoveries for NPECs at Four Concentrations in 250-mL Samples of Blank Surface Water[a]

	recovery (%)			
concn (μg/L)	NP1EC	NP2EC	NP3EC	NP4EC
40[b]	108 ± 1 (1%)	115 ± 3 (3%)	105 ± 5 (5%)	89 ± 8 (9%)
20[c]	106 ± 1 (1%)	109 ± 3 (3%)	90 ± 9 (10%)	73 ± 9 (12%)
10[d]	89 ± 4 (4.4%)	92 ± 4 (5%)	87 ± 3 (4%)	100 ± 3 (3%)
4[c]	118 ± 1 (0.8%)	148 ± 5 (3.4%)	140 ± 6 (4.5%)	<DL[e] (–)

[a] The relative standard deviation is given in parentheses. [b] Four replicate samples analyzed. [c] Five replicate samples analyzed. [d] Seven replicate samples analyzed. [e] <DL, below detection limit.

satisfactory for use with a 25-mm SAX disk. Chromatograms for blank extractions (performed by processing 500-mL deionized water samples that contained neither the 4-bromophenylacetic acid nor NPEC standards) did not contain peaks that interfered with either the surrogate standard or NPECs.

To determine if NPEC recovery was acceptable over a range of concentrations, a blank surface water was spiked to give 4–40 μg/L of each NPEC standard. Recoveries ranged from 87 to 108% for NPEC concentrations in the range of 10–40 μg/L. At 4 μg/L, recoveries were higher for all NPECs except NP4EC, which was below detection at that concentration (Table 1). Recoveries significantly greater than 100% at low NPEC concentrations were attributed to the nonlinear NPEC calibration curves that were sensitive to small differences in NPEC/surrogate standard peak area ratios at low NPEC concentrations.

The recovery of the 4-bromophenylacetic acid surrogate standard averaged 114 ± 2.3%, which indicated that the method performed satisfactorily despite the presumably variable composition of the industrial and sewage effluents. The pH of effluent samples ranged from 6 to 8.2, and the specific conductance ranged from 470 to 5040 μS/cm. River water samples ranged from a pH of 7.1 to 8.5 and from 140 to 380 μS/cm in specific conductance. For reference, solutions 0.01 M and 0.1 M in KCl give specific conductance values of 1000 and 12 860 μS/cm, respectively. Therefore, no pH adjustment of the effluent or river water samples was necessary prior to extraction.

Accuracy and Precision. The recovery of NPECs from samples of a composite paper mill effluent and Fox River water was evaluated by means of standard addition experiments. Once it was established that the effluent samples contained no peaks that interfered with either

TABLE 2

Background Concentrations and Recovery of NPECs Spiked into a Composite Paper Mill Effluent Sample and River Water[a]

sample	NP1EC	NP2EC	NP3EC	NP4EC
paper mill effluent (background) (μg/L)[b]	10.6 ± 0.9 (8.2%)	56.7 ± 4.93 (8.7%)	24.0 ± 1.0 (4.1%)	22.6 ± 0.4 (1.8%)
spike recovery (%)[c]	124	138	116	146
river water background (μg/L)[d]	0.6 ± 0.03 (6.2%)	1.6 ± 0.05 (3.0%)	nd[f]	nd
spike recovery (%)[e]	113	119	130	170

[a] The relative standard deviation is given in parentheses. [b] Four replicate samples (75 mL). [c] Duplicate samples (75 mL) each spiked with 5 μg of each NPEC. [d] Four replicate samples (250 ML). [e] Duplicate samples (250 mL) each spiked with 2 μg of each NPEC. [f] nd, not detected.

TABLE 3

Detection and Quantitation Limits for NP1EC−4EC in Surface Water

	NP1EC	NP2EC	NP3EC	NP4EC
detection limit (S/N > 3)	0.04	0.2	0.4	0.4
quantitation limit (S/N > 10)	0.2	0.4	2.0	2.0

NPECs or the surrogate standard, the background NPEC concentrations were determined for replicate effluent and river water samples. Recoveries of 106−138% were obtained for NP1EC-3EC, and recoveries of 73−170% were obtained for NP4EC (Table 2). The relative standard deviation for replicate sample analyses ranged from 1 to 12%.

Detection and Quantitation. Single samples of a surface water that were determined to contain no NPECs above detection were spiked to give a range of NPEC concentrations. The detection and quantitation limit of the method was defined as those concentrations of individual NPECs needed to produce a signal to noise (S/N) of 3:1 and 10:1, respectively (Table 3). Quantitation limits for NP1EC and NP2EC were lower at 0.2 and 0.4 μg/L, respectively, while 2.0 μg/L each of NP3EC and NP4EC were required to achieve sufficient signal for quantitation.

Survey of River Water and Effluents. Fifteen paper mills and six STPs that discharge into the Fox River near Green Bay, WI, were sampled and analyzed for this study (Table 4). Typical selected ion chromatograms for NPECs in paper mill and STP effluent are given in Figure 3. All but one effluent sample contained concentrations of NPECs above detection. Some changes in the relative peak intensities were evident in the selected ion chromatograms for NPECs in paper mill and STP effluents as compared to NPEC standard (Figure 3) and may be the result of selective biodegradation of the NPEC alkyl chain isomers. In addition to analyzing the effluent samples for NPECs, we examined a subset of effluent samples for the presence of halogenated NPEC derivatives previously reported for wastewaters (*13*, *24*), namely, the brominated and chlorinated derivatives; however, none were detected.

The total concentration of NPECs in paper mill effluents ranged from below detection to 1300 μg/L. With the exception of two mills, paper mill effluents typically contained less than 100 μg/L NPECs. The precision of replicate paper mill sample analyses for individual NPECs, indicated by the relative standard deviation, ranged from 4 to 15% (Table 4).

The NPEC concentrations in STP effluents ranged from 140 to 270 μg/L (Table 4). The NPEC concentrations for municipal STPs are of a similar range reported by others for municipal STP effluent (*6*, *8*). Relative standard deviations for replicate municipal STP effluent ranged from 1 to 6% for the individual NPECs. The values determined in this study were higher than that reported by Di Corcia et al. (*7*) even though they considered a greater number of NPEC oligomers (up to NP10EC).

Over half of the paper mill effluents contained only NP1EC and NP2EC. In all cases, NP2EC was the dominant oligomer (Table 4). The average proportions of NPECs in paper mill effluents were NP1EC (16%), NP2EC (72%), NP3EC (10%), and NP4EC (2%). All NPEC oligomers were detected in all the municipal STP effluents tested with the average proportion of NPECs as follows: NP1EC (7%), NP2EC (54%), NP3EC (31%), and NP4EC (8%), indicating that municipal STPs on average have a higher percentage of NP3EC and NP4EC relative to NP1EC. Others have reported a greater abundance of NP2EC and OP2EC relative to the one ethoxy carboxylate and higher oligomers in laboratory and field studies (*4*, *7*, *8*). Our findings are in agreement with those of Di Corcia et al. (*7*), who reported that NPECs with three or fewer ethoxylate groups were typically the most abundant oligomers in municipal STP effluents.

The samples were collected by Integrated Paper Services of Appleton, WI, under contract to the Chemical Manucture's Association's Panel on Alkylphenols and Ethoxylates, with assistance from the Wisconsin Paper Council and Wisconsin Department of Natural Resources. The samples were collected by the contractor from paper mills and STPs only under the agreement that the contractor maintain the anonymity of the sample's source. Therefore, specific information on the type of wastewater treatment employed by each paper mill was not available to us. For this reason, it is difficult to assess why the paper mill effluents varied in their NPEC concentrations and oligomer composition. Most paper mills that discharge to the Fox River have activated sludge treatment while some have only primary treatment.

The only information available to us was whether a sample was a pulp mill effluent or an STP effluent (Table 4). Typically, industrial wastewater treatment is characterized by higher temperatures, increased hydraulic residence times, and greater degrees of acclimatization than that of municipal STPs (*32*). Because municipal STPs operate at ambient temperatures, more seasonal variation in effluent composition could be expected from municipal STPs than from industrial effluents.

The concentration of NPECs in the upstream Fox River sample obtained at the outlet of Lake Winnebago was below detection (Table 5). The downstream sample of Fox River water contained a total concentration of NPECs of 13.5 μg/L and was composed of NP2EC (87.4%) and NP1EC (12.6%). Because the Fox River samples were

TABLE 4

Concentrations of NP1−4EC in Paper Mill and STP Effluents Discharged to Fox River Located near Green Bay, WI[a]

	concentration (μg/L)				
	NP1EC	NP2EC	NP3EC	NP4EC	total NPEC
			Paper Mill		
1[b]	3.7 ± 0.2 (5.4%)	14.4 ± 0.6 (4.2%)	nd[c]	nd	18.1
2[b]	2.7 ± 0.4 (14.8%)	13.9 ± 1.9 (13.7%)	8.1 ± 0.6 (7.4%)	nd	24.7
3	5.2	14.1	nd	nd	19.3
4	4.2	22.0	11.8	10.4	48.4
5	6.9	45.9	12.0	7.8	72.6
6	3.7	31.2	18.9	nd	53.8
7	140.0	931.0	172.0	26.7	1,269.7
8	2.5	16.9	nd	nd	19.4
9	8.5	12.0	nd	nd	20.5
10	28.8	59.8	22.4	nd	111.0
11	9.4	47.1	nd	nd	56.5
12	2.1	14.7	nd	nd	16.8
13	2.3	14.7	nd	nd	17.0
14	nd	18.6	nd	nd	18.6
15	nd	nd	nd	nd	nd
			Sewage Treatment Plant		
1[b]	29.4 ± 0.3 (1.0%)	106.7 ± 1.2 (1.1%)	24.8 ± 1.5 (6.0%)	14.8 ± 0.9 (6.1%)	175.7
2	11.8	128.0	60.6	13.0	213.4
3	7.6	80.7	42.4	11.8	142.5
4	8.3	112.0	91.0	29.2	240.5
5	7.8	64.1	54.6	17.3	143.8
6	13.7	144.0	105.0	9.7	272.4

[a] Average of duplicate samples except where noted. [b] Average of four replicate samples; the relative standard deviation is given in parentheses. [c] nd, not detected.

TABLE 5

Concentrations of NP1−4EC in Fox River near Green Bay, WI, and in Other Rivers in the Eastern United States[a]

	concentration (μg/L)					
sample	NP1EC	NP2EC	NP3EC	NP4EC	NPEC total	NPEC mass flow (kg/d)
Fox River upstream	nd[c]	nd	nd	nd	nd	0.0
Fox River downstream[b]	1.7 ± 0.2 (10.4%)	11.8 ± 1.1 (9.3%)	nd	nd	13.5	49.2
Kanawha River (Nitro, WV)	0.6	2.3	nd	nd	2.9	*d*
Kanawha River (Charleston, WV)	nd	nd	nd	nd	nd	*d*
Brandywine Creek	0.6	5.2	nd	nd	5.8	*d*
Great Egg Harbor River	nd	nd	nd	nd	nd	*d*
Delaware River	2.0	4.3	nd	nd	6.3	*d*
Fish Creek	nd	1.4	nd	nd	1.4	*d*
Mohawk River	nd	nd	nd	nd	nd	*d*
Cuyahoga River	nd	nd	nd	nd	nd	*d*

[a] Average of duplicate analyses. [b] Average of four replicate samples; the relative standard deviation is given in parentheses. [c] nd, not detected. [d] River flow not available.

obtained in September and not July, correlations between the mass flows of NPECs from paper mills and municipal STPs with that of the Fox River could not be made. However, given the flow of the Fox River at the time of sampling (41.32 m^3/s), the mass flow of NPECs in the Fox River in September was 49.2 kg/d. The total mass flow of NPECs to Green Bay was likely higher since the Green Bay municipal STP, which is the largest plant located on the Fox River, was not sampled in this study as the outfall of this municipal STP is located in the mixing zone between the Fox River and Green Bay.

A set of eight additional river waters was sampled for this study, and the rivers sampled were included in a larger study aimed at measuring the concentrations of NP and NPEOs in 30 U.S. rivers (*16*). Of the eight samples, five gave concentrations above detection in a range of 1.4−6.3 μg/L (Table 5). Only NP1EC and NP2EC were detected in river water, and NP2EC was the dominant oligomer at 2−7 times the concentrations of NP1EC. The concentrations of NPECs in the Fox River and other U.S. rivers are approximately a factor of 10 lower than that reported for the Glatt River in Switzerland, presumably due to dilution (*9*). The Glatt is a smaller river with a discharge of 3−9 m^3/s (*9*) as compared to the Fox River with a discharge of 41.3 m^3/s.

Limited information is available on the toxicity and potential for bioaccumulation of NPECs in aquatic organisms. For this reason, it is difficult to assess the impact of NPECs detected in effluents and river water on aquatic organisms. The NPEC concentrations measured in effluents and river water in this study are below those associated with acute and chronic fish toxicity (*2*, *18*, *19*). Further research is required to determine if environmental levels of NPECs detected in this study are sufficiently

high to induce estrogenic effects in fish and other aquatic organisms.

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