Trace Analysis of Alkylphenol Ethoxylates[1]

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A method for quantitative determination of trace amounts of alkylphenol ethoxylates (APE) in environmental water is described. Levels of 1 to 3 μg/L can be detected and resolved into their complete oligomer distribution (1EO to 18EO) while maintaining integrity of the oligomer distribution. This is a major improvement over previous methods; for the first time distortion of oligomer distribution due to work-up conditions of earlier methods has been prevented.

Isolation of the APE from water is achieved using a simple and rapid dual-column procedure. The first column removes interfering ionic materials, the second traps the APE on alkyl-bonded silica. Assay of the extract employs HPLC with a fluorescence detector.

The method was used for analyzing treated wastewater and river water. A much better picture of the biodegradation behavior of APE in the environment has emerged as a result of keeping APE oligomer distribution intact during sample extraction. There is no accumulation of alkylphenol and the low EO oligomers during wastewater treatment, although the oligomer distribution may become skewed toward these species. Concentrations in the receiving waters examined were very low, in the range of 1–2 μg/L total APE species (OEO to 18EO).

For decades the biodegradation and environmental fate of alkylphenol ethoxylate (APE) nonionic surfactants have been subjects of intense debate and research (1). Since APE, in particular nonylphenol ethoxylates (NPE), are widely used in industrial and household detergents—about 450MM lb in the U.S. in 1988 (2)—their environmental levels need to be measured reliably in order to assess any risk to the environment.

Our goal was to simplify the extraction of environmental water samples so that NPE could be measured quantitatively by HPLC at ppb to sub-ppb levels, both cheaply and rapidly. A crucial requirement was maintaining the integrity of the NPE oligomer distribution as it moved from dilute aqueous solution through extraction into concentrated organic solution and injected onto the chromatographic column.

This goal has been accomplished through streamlining earlier extraction methods and attention to the details of sample protection during extraction. The method as now described may be used routinely; analysis time from water sample extraction and chromatography is as little as three hours, compared with up to 12 hr for the earlier procedures. Detection limit is about 0.1 μg/L for each oligomer.

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Analytical methodology has been evolving rapidly in recent years for detecting APE at low levels. The first reported efforts to quantify APE in sewage by chromatography were cumbersome, slow and incomplete (3,4); gas chromatography and mass spectrometry could detect and quantitate the lowest molecular weight species NP (nonylphenol), NPE_1, and NPE_2 (detection limit 10 micrograms per liter or parts per billion [μg/L or ppb] for each species), only after laborious clean-up procedures involving solvent extraction and column chromatography through alumina. Ease of analysis and detection limits were greatly improved when high pressure liquid chromatography was applied to these species. No clean-up was needed after extractive steam distillation through cyclohexane (5); the extract, containing NP and the lower ethoxylates, could be injected directly into the HPLC. Detection limit was 0.5 μg/L. Higher ethoxylates were now accessible (6), since NPE have UV absorption, but only with the use of complex extractions and clean-up prior to HPLC injection; detection limit was 1–3 μg/L for each oligomer. Sensitivity was increased enormously with the use of HPLC fluorescence detection (7,8). This new method was used to measure die-away of NPE by biodegradation in river water (9). A lengthy extraction procedure was still required with sewage samples, but detection limit for individual NPE oligomer was lowered to 2 nanograms (8,10). Two German sewage treatment plants were studied for efficiency of NPE removal using the extraction and HPLC fluorescence detection procedures (10).

A big step toward replacing the solvent extraction procedures was percolation of water solutions through octadecylsilica (11). Alkylphenol ethoxylates and alkylbenzene sulfonates could be analyzed together in raw sewage using this technique. This method, however, lacked sensitivity (detection limit 4μg/L total APE) and precision.

Methods specific for nonylphenoxyether carboxylates, intermediates in the degradation of NPE, were also developed by Giger (12). This report described the performance of several treatment plants for removing nonylphenol-based species in the Zurich, Switzerland, vicinity. Assays using the cumbersome extraction procedures (6) provided the first data on treatment plant efficiency.

Prior work indicated that degraded NPE was largely composed of NP and the lower EO oligomers NPE_1 and NPE_2, the species least water-soluble and most toxic to aquatic fauna (6). Our improved sample handling procedures allow us to define NPE oligomer distribution in environmental samples with much greater accuracy and precision and to demonstrate that there is no substantial skewing of the oligomer distribution toward the low EO end.

It should be noted that the present method does not distinguish among the various alkylphenols and their ethoxylates. Nonylphenol is by far the major al-

kylphenol in common use, so its ethoxylates were used as the reference standards during method development.

EXPERIMENTAL

Water sample collection. Raw and treated wastewater samples from remote locations were collected as 24-hr composites and preserved with 1% formalin. The filled glass containers, 1 quart to 1 gallon, were shipped by public carrier to the laboratory, and refrigerated when received.

Samples of river water were collected from the Colorado River downstream from Austin, Texas, as 1 gal grab samples. A boat was used to reach midstream. Preservatives, if any, were added to the jugs onshore. Samples were refrigerated to 4°C within three hours after collection.

Extraction of NP and NPE from water—NP and low oligomers. Steam distillation was performed on 1 L water samples in a modified Nielsen-Kryger apparatus (Ace Glass 6555-13). The sample was heated to reflux with 2 mL iso-octane for 1 hr. The resulting iso-octane solution could be injected directly into the HPLC without further handling.

NPE. Water samples up to 1 L were passed through a dual column apparatus consisting of four pieces of glassware mounted vertically in series. On top was a 1 L solvent reservoir charged with the sample with a valve in the bottom. The water flowed into a 10-inch × 3/4-inch glass column containing 40 cc mixed-bed ion-exchange resin (Biorad 501 × 8 (D), 20–50 mesh), which removed all ionic species. (Adding 150 mL methanol to the water sample improved recoveries.) The now-deionized water then passed through a second glass column, 4-inch by 1/2-inch, containing 0.7 g of octadecylsilica (Baker, 7031-0), which adsorbed organic material. The assembly was mounted atop a 1 L vacuum flask under aspirator vacuum for more rapid flow. After passage of the water was complete, warm methanol (55°) was used to flush the organics off the adsorbent; methanol was removed at 45° under a stream of nitrogen just to dryness. Residue was taken up into a dichloromethane/hexane (25/75) mixture for HPLC analysis.

At all times the sample was protected from air and dissolved oxygen.

Loss of NPE spike (3 μg) in methanol occurred during simple solvent blow-down, apparently because of adsorption to the vial glass. The same effect was observed during methanol removal from NPE extracts when a new lot of octadecylsilica was used; the original lot (used for the entire present study) gave high (>90%) recoveries. The losses were minimized (>80% recoveries) when a small amount of alcohol ethoxylate (5 μL) was spiked into the methanol prior to blow-down. The analyte remained dissolved in the alcohol ethoxylate, which did not interfere with subsequent HPLC analysis. The preferred ethoxylate was C_{1214} alcohol-3EO (SURFONIC® L24-3 or equivalent).

Standard NPE blend. A mixture of NPE was used for method and instrument calibration (Table 1). From HPLC analysis of the NPE_4 and NPE_9 the blend was calculated to contain 3.0 wt% each of NP, NPE_1 and NPE_2, and 6.0% NPE_3. HPLC analysis of the blend came close to these values, but was not exact because these low EO oligomers were incompletely resolved. Stock solutions were unstable so they were made fresh daily.

TABLE 1

Standard Blend of NPE

Component	Wt%	NPE EO no.		Wt % by HPLC	
		0	8	2.5	9.7
Nonylphenol	3.0	1	9	1.4	9.7
		2	10	3.0	9.0
Distilled NPE_1	2.5	3	11	6.3	7.7
		4	12	7.4	6.1
NPE_4 (SURFONIC® N-40)	24.0	5	13	8.4	4.5
		6	14	9.1	3.1
NPE_9 (SURFONIC N-95)	70.5	7	15	9.2	2.0
			16		0.5
			17		0.4

TABLE 2

Elution gradient*

Time (min)	Flow, cc/min	%A	%B
Initial	0.75	99	1
1.4	1.00	99	1
1.5	1.00	97	3
20	1.00	58	42
21	2.00	99	1
25	0.75	99	1
35	Next injection		

*Detector settings: 229 nm exitation, 310 nm emission, PMT gain @ 11, rise time @3, lamp @1.

HPLC procedures. Apparatus included a Waters Associates liquid chromatograph with two Model 510 high pressure pumps, Model 680 solvent programmer, Rheodyne 7125 injector with 100 microliter loop, Hewlett Packard HP1046A fluorescence detector, and Waters 840 data system. The column used was Rainin Microsorb 250 mm × 4.6 mm 5 micrometer CN.

Solvents were the purest grades available. Elution solvent A (20/80 tetrahydrofuran/hexane, v/v) was passed through a 4.6 mm × 150 mm column, dry packed with alumina (activity 1), inserted between the A and B pumps to trap traces of peroxides. The trap was changed every 2 to 4 days. Elution Solvent B was 10/90 (v/v) water/isopropyl alcohol.

Methanol used for sample extraction was checked for purity by blowing down 30 mL to dryness, taking up the residue in 25/75 dichloroethane/hexane and examining by HPLC. Some lots of methanol showed high background levels of NPE. Likewise water was a source of background NPE. Deionized water was further purified in a Milli-Q system of adsorbent cartridges. Frequent blank extractions using only reagent water were necessary. Sodium sulfite (100 ppm) was added to the degassed water to scavenge dissolved oxygen.

The elution gradient is presented in Table 2.

Figure 1 illustrates the resulting chromatography using the NPE standard blend (Table 1) both unextracted (3 μg/mL stock solution) and extracted (3 μg/L spike).

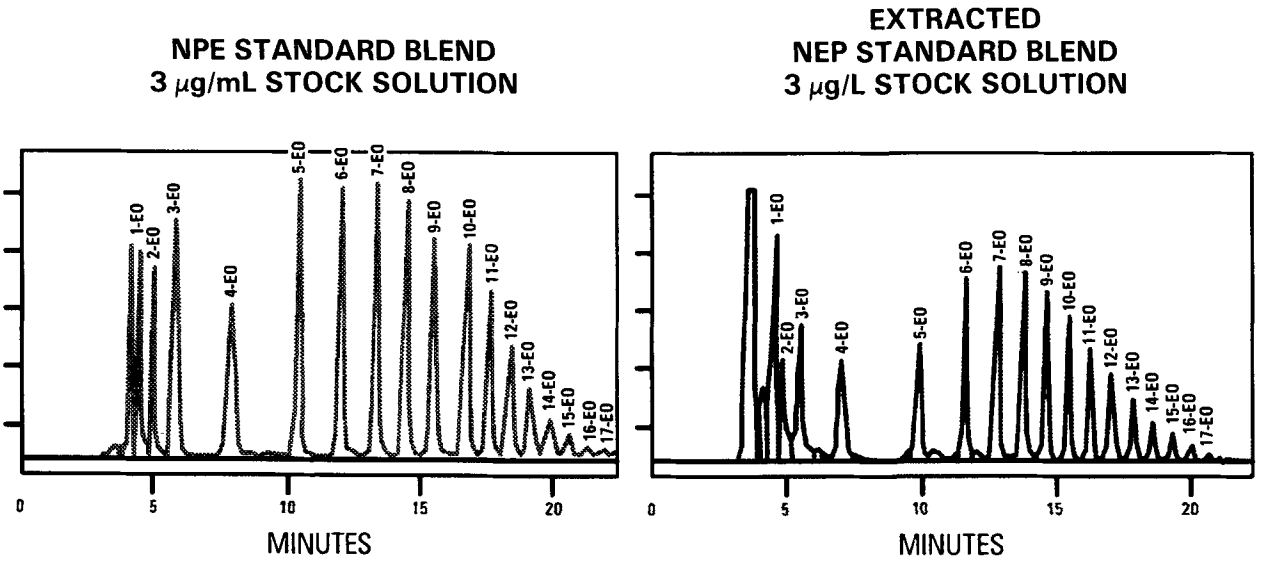

FIG. 1. HPLC chromatograms of NPE standard blend.

RESULTS AND DISCUSSION

Integrity of oligomer distribution. A very sensitive measure of the handling procedures for NPE-containing samples, both spiked laboratory water and environmental water, is their effect on NPE oligomer distribution. Nonylphenol in the NPE blends could not be reliably quantitated using the dual column extraction. Probably it was partially adsorbed onto the basic ion exchange resin; its volatility during final solvent removal could have also led to material losses. NP was therefore assayed by the steam distillation method.

The effect of oxygen on NPE during extraction was found to be very destructive. Figure 2 illustrates how oxygen exposure can grossly distort oligomer distribution and deplete the total concentration. Nitrogen used for blanketing the system was contaminated with air during a second work-up of unspiked Colorado River water ("bad N_2") after a satisfactory ("good N_2") extraction. (See NPE in River Water, below.) There was a striking increase in NPE_1 and NPE_2 levels and a corresponding loss of NPE_5 to NPE_{15}. Overall loss was 26 wt%.

Other sample work-ups showing this effect could be traced to improperly de-oxygenated solvents or incomplete nitrogen padding as well as sample storage in polyethylene bottles.

We conclude that reports of abnormally high levels of low EO oligomers and loss of high oligomers in environmental samples were probably due to improper work-up procedures (6,7), rather than a biodegradation effect.

Recovery of spiked doses of NPE (3.0 μg/L) from river water (see Preservation Study, below) are shown in Figures 3 and 4. The first gives (a) the total of spike + background NPE oligomer distribution, (b) the background and (c) the difference between the two (net spike); the second displays net-spike oligomer distribution compared to that of the standard NPE blend. The distributions compare very well from NPE_4 to NPE_{15}. But the low EO oligomers have quite different patterns. NPE_1 in the net spike is much higher while NPE_2 and NPE_3 are lower than those in the standard. Possibly traces of oxygen in the extraction system caused this distortion, or there may have been interfering substances in the extract.

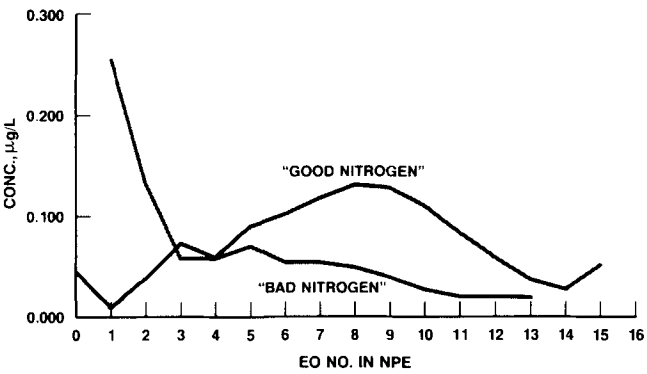

FIG. 2. Effect of oxygen on NPE oligomer distribution during river water extraction.

Preservation study. The dual column extraction method was used to determine how best to preserve river water samples in the laboratory. Four chemical preservatives were tested by analyzing preserved river water over a period of four weeks. The samples were collected one-quarter mile downstream from the Govalle Treatment Plant on the Colorado River in Austin, Texas,

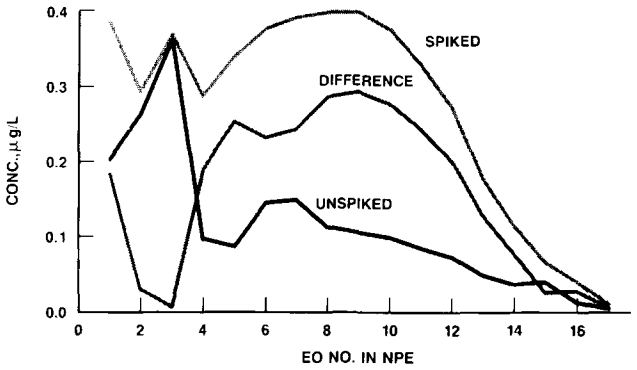

FIG. 3. River water spiked with NPE standard blend NPE oligomer distribution.

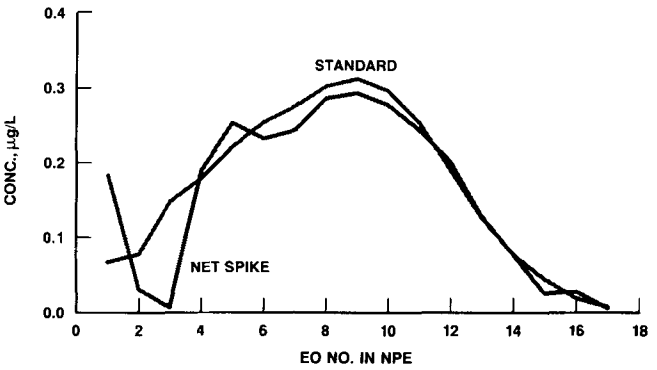

FIG. 4. Net NPE spike in river water and NPE standard blend.

in September 1988. They were spiked with 3.0 µg/L of standard NPE blend and stored at 2-4°C. Figure 5 displays the results graphically.

Sulfite and bisulfite were employed (100 ppm) because of their ability to scavenge oxygen. In spite of the 100% excess sulfite loading, all samples became re-aerated within one to two weeks, apparently because of poor bottle lid seals. The sulfite and bisulfite preserved samples deteriorated drastically beginning after the first week, dropping 60 to 80% in NPE concentration after four weeks.

Formalin (1% dose) was more satisfactory, maintaining NPE concentration within 10% of the original through at least two weeks. Formalin/sulfite showed no loss of NPE through the second week and 25% loss after four weeks. The same combination maintained full NPE level in the unspiked water sample. The unpreserved sample showed good stability as well, declining only 10% in four weeks.

We conclude that refrigeration alone is adequate for preserving water samples over a period of four weeks, but formalin should be used if there is any chance of sample warming during shipment or storage. Sulfite or bisulfite alone must be avoided.

Method precision and accuracy. The above preservation study allowed us to determine the reproducibility and accuracy of the dual column extraction method and the HPLC instrument. Eleven assays of the five spiked water samples, before NPE concentration losses became evident, gave high precision, as did four assays of the unspiked water. The difference between the two averages (i.e., the net spike) agrees very well with the average of five standard NPE spike extraction assays (Table 3). Only a slight loss (~4%) occurred during extraction of lab spikes, while recovery from the river water spikes was 84%.

NPE in river water. The Colorado River flows through Austin, Texas receiving urban run-off and treated wastewater from the city. Because NPE are widely used in industrial and household cleaning products, we assume that they find their way by both routes into the river. We analyzed the river to determine their concentration and oligomer distribution. Figure 6 illustrates the patterns found during the autumn of 1988.

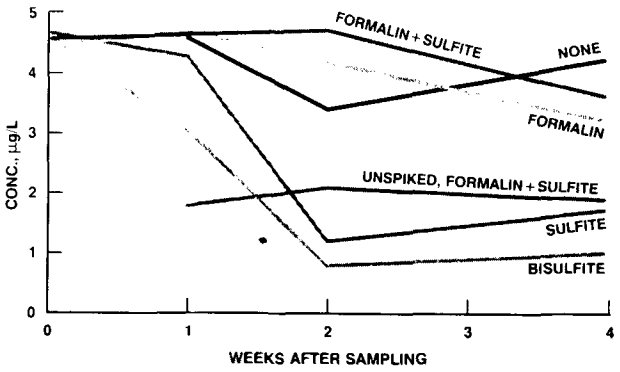

FIG. 5. River water preservation study.

The sample collected upstream of any treated wastewater outfall provides a measure of NPE due to untreated sources. Total NPE concentration, including NP, was 1.1 µg/L on Nov. 5. Distribution was close to that of NPE_9, the most commonly used NPE in cleaning products.

The first downstream sample was collected at the same location, just downstream from the Govalle Treatment Plant outfall, as the preservation study samples (above) indicate. Total NPE level was more than 50% higher than the upstream sample, 1.9 µg/L, but most of the increase was due to the low EO oligomers (NP to NPE_5). NPE_2 and NPE_3 were especially high. The second downstream sampling site was 10 to 15 miles downstream of Govalle at Webberville, also downstream from two other large treatment plants. Concentration and oligomer distribution were nearly identical to those

TABLE 3

Precision and Accuracy of Dual Column Extraction Method

		+ 95% Confidence limits
Average of 5 laboratory spikes (2.91 μg/L NPE blend in deionized water)	2.80	0.09
Accuracy of HPLC assay	96%	
Average of 11 analyses of spiked river water samples	4.52	0.14
Average of 4 analyses of unspiked river water samples	2.08	0.46
Difference = the spiked dose	2.44	
Recovery (basis 2.91 μg/L calculated spike)	84%	

at Govalle. Distributions match that of the treated wastewater (Fig. 7); the possibility of interferences in the NPE_2-NPE_3 region was not ruled out.

Comparison of the unspiked curve (Fig. 3, sampled 9-22-88) with Figure 5 shows that the NPE oligomer pattern is consistent. More examples are needed before any conclusion can be drawn whether this is a characteristic pattern.

Wastewater treatment plants. Samples of raw and treated wastewater were obtained from High Point, North Carolina, in May 1988. They were 24-hr composites collected on two consecutive days from two treatment plants. The Eastside Plant serves primarily industrial customers, including numerous textile processing mills and furniture manufacturers. The Westside Plant receives primarily domestic wastewater. Table 4 summarizes the analyses.

Influent concentrations ranged from 1600 to 2500 μg/L NPE; effluent levels were reduced to about 50 to 100 μg/L. Therefore removal of NPE ranged from 93 to 98%. No accumulation of NP or low EO oligomers occurred; NPE_1 removal was 89 to 95%. NP effluent concentration was 1 to 2.5 μg/L; influent NP levels were not measured. NPE removal was the total of biodegradation and adsorption onto sludge (13), so a complete NPE material balance was not obtained.

Figure 8 shows that all NPE_{1-18} oligomers were removed to about the same extent (≥90%). Oligomer

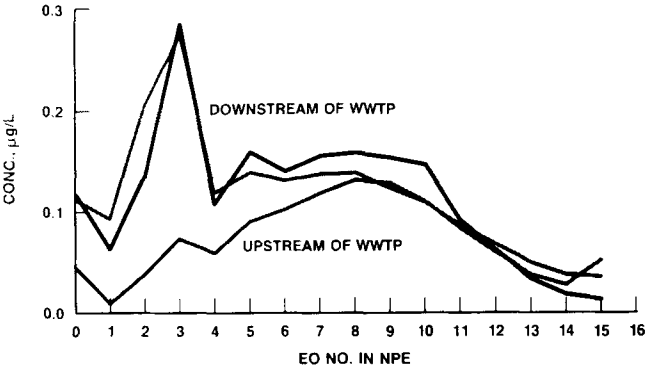

FIG. 6. Colorado River water NPE oligomer distribution.

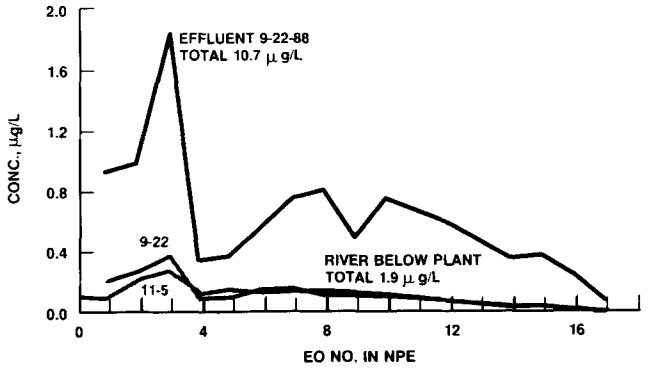

FIG. 7. Govalle Plant, Austin, Texas, effluent NPE oligomer distribution.

TABLE 4

High Point, North Carolina, Wastewater Analysis for Nonylphenol and Its Ethoxylates

Treatment plant	Date (1988)	Source[a]	NP,[b] ppb	NPE_{1-18},[c] ppb	NPE_{1-18} removal[d] (%)	NPE_1,[b] ppb	NPE_1,[c]	NPE_1 removal (%)
Eastside	5-24	Influent		1600		—	69	
		Effluent	0.8, 1.0[e]	104	93	0.5, 0.7	3.4	95
	5-25	Influent		1940, 1980[e]		—	55	
		Effluent	2.5	102	95	3.4	3.8	93
Westside	5-24	Influent		2520		—	49	
		Effluent	1.8, 1.3[e]	56, 51[e]	98	2.0, 1.6	3.1	93
	5-25	Influent		2270		—	36	
		Effluent	2.0	88	96	4.0	2.6	89

[a]The 24-hr composite samples, preserved with 1% formalin.
[b]Steam distillation workup method.
[c]Dual column extraction method.
[d]Apparent removal, by weight.
[e]Duplicate workups and analyses.

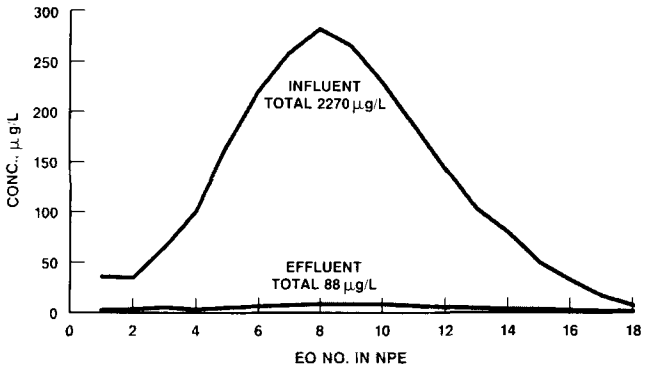

FIG. 8. High Point, North Carolina, West Plant 5-88 degradation of NPE oligomers.

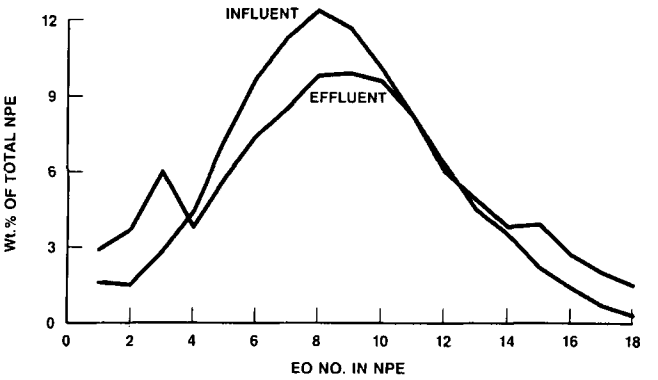

FIG. 9. High Point, North Carolina, West Plant 5-88 NPE oligomer distribution.

distribution changed only slightly, becoming relatively higher in the low EO and highest EO oligomers (Fig. 9). Thus there is no significant dependence of biodegradation rate on EO number. Since nonylphenol itself is not accumulating, this provides strong evidence that it is degrading to the same high extent.

Performance of the High Point treatment plants was excellent as measured by NPE removal. Giger and co-workers (12) have reported that performance of the treatment plants which they sampled varied widely from about 90% NPE removal down to 1%. NPE elimination at those plants correlated with ammonia elimination (nitrification).

Effluent from the Govalle Wastewater Treatment Plant in Austin, Texas, was analyzed (Fig. 7). The sample was obtained at the same time as the preservation study river-waster samples (above). NPE level was much lower than those in the High Point effluents, but the oligomer distribution was more skewed toward the low EO side. NPE_{1-3} account for 35% of the total NPE concentration; the remaining 65% consisted of oligomers up to NPE_{17}. This pattern was reflected in the downstream river NPE oligomer distribution measured at two different times (Fig. 6).

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