Bull. Environ. Contam. Toxicol. (2003) 70:527–532

DOI: 10.1007/s00128-003-0018-0

Bulletin of Environmental Contamination and Toxicology

Nonylphenol Ethoxylates and Their Biodegradation Intermediates in Water and Sludge of a Sewage Treatment Plant

B. Shao,[1,2] J. Hu,[2] M. Yang[1]

[1] State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Post Office Box 2871, Beijing 100085, People's Republic of China
[2] Department of Urban and Environmental Science, Peking University, Beijing, 100871, People's Republic of China

Received: 15 February 2002/Accepted: 6 December 2002

Nonylphenol ethoxylates (NPEOs), usually a mixture with EO chain length ranging from 6 to 20, have been widely applied to industrial, agricultural and domestic uses as important surfactants. It is estimated that approximately 40,000 tons of NPEOs are produced annually in China, which accounts for 10% of the world production (Huang et al. 1998; Nalor et al. 1996). NPEOs discharged into environment can be aerobically degraded to lower ethoxymers and subsequently degraded to NP under anaerobic conditions (Giger et al. 1984).

Recent researches have shown that NPEOs with lower EO Units as well as NP possess the ability to mimic natural hormones of fish by interacting with the estrogenic receptor, and the threshold concentration of NP to elicit vitellogenesis of fish has been found to be as low as 10μg/L (White et al. 1994; Sharpe et al. 1995; Jobling et al. 1996). Therefore, there are increasing concerns on the environmental fate of NPEOs and their biodegradation intermediates. Many related studies have been focused on behaviors of NP and short chain NPEOs (n=1,2,3), or changes of total NPEOs concentration in environment as reviewed by Lee (1999). Studies on variations of all of the homologues of NPEOs as well as biodegradation intermediates, however, have been very limited partly because of analytical reasons (Shang et al. 1999; Maruyama et al. 2000). On the other hand, little information about the occurrence of NPEOs in China is available in spite of the fact that China is a very important country both in production and consumption of NPEOs.

In our previous study, an HPLC-MS method with high resolution and separation performance was developed, and successfully applied to investigation of occurrence of NPEOs in rivers (Shao et al.2002 (a)). In this paper, the behaviour of NPEOs and their biodegradation intermediates during sewage treatment procedure in sewage treatment plant of Beijing were investigated.

Correspondence to: J. Hu

MATERIALS AND METHODS

The pure NP1EO, nonylphenol di- (NP2EO), tri- (NP3EO), tert-(NP4EO), penta-(NP5EO), hexaethoxylates (NP6EO) standards and mixture NP9EO (a mixture of NPEOs with average EO units of 9) and NP15EO (a mixture of NPEOs with average EO units of 15) standards were obtained from Hayashi Pure Chemicals (Tokyo, Japan). Supleclean ENVI-Carb (GCB) solid phase extraction cartridge, a 6x1.4cm I.D. polypropylene tube, was purchased from Supelco (Bellefonte, P. A).

Water samples were collected from an oxidation ditch type sewage treatment plant in Beijing on March 8, 2001. The plant, having three ditches and an average treatment capacity of 150,000m^3, receives part of electronics wastewater, and is operated at an HRT of 18 h and MLSS of 6000 mg/L. The influent, effluent, and mixed liquor in the middle point of one ditch were sampled respectively. And thickened sludge and supernatant from the sludge-thickening tank were also sampled for investigating the distribution of individual NPEOs between sludge and water. The samples were collected into precleaned glass bottles.

For water samples, GCB cartridges were used for concentration according to the method developed by Di Corcia *et al* (1994). Briefly, before extraction, samples were acidified to pH=3 with concentrated HCl. The volumes for extraction were 50ml, 50ml, 250ml, and 50ml for samples of influent, mixed liquor, effluent, and supernatant of sludge-thickening tank, respectively. GCB cartridges were conditioned sequentially with 10ml CH_2Cl_2/CH_3OH (80:20, V/V), 6ml CH_3OH, 6ml water with pH=3. Mixture of DCM and methanol (10ml, 80:20) was used to desorb the analytes. The effluents were then collected and dried under gentle nitrogen, and reconstituted with 5ml acetonitrile.

For sludge sample, a previous method was used (Petrović et al. 2000). Briefly, two grams of air-dried and homogenized sludge samples were Soxhlet extracted with a solvent mixture of $MeOH/CH_2Cl_2$ (1:1) for 14 h. The extract was rotary evaporated to a volume of 1ml and then transfer to a flask with 4×100ml water. The solution was cleaned with a C-18 SPE cartridge containing 500 mg of sorbent (Supelco Co). The cartridge was conditioned by passing 7ml methanol and 3 ml HPLC water at a flow rate of 1 ml/min. Mixture of DCM and hexane (10ml, 80:20), which was used as eluant, was passed through the sorbent bed to desorb NPEOs.

Liquid chromatography was performed on an Alliance 2690 HPLC (Waters

Table 1. Levels of NP and individual NPEOs (ng /L) in influent, aerobic ditch, effluent.

Compounds	Influent (ng/L)	Aerobic ditch (ng/L)	Effluent (ng/L)	Removal (%)
NP	9274.5	806.2	1461.4	84.2
NP1EO	3216.4	164.6	35.5	98.8
NP2EO	27585.5	15986.9	11442.6	58.5
NP3EO	483.5	349.3	193.9	59.8
NP4EO	287.8	288.3	172.3	40.1
NP5EO	1147.2	416.9	313.0	72.7
NP6EO	1209.8	321.9	226.4	81.2
NP7EO	1144.3	494.5	461.8	59.6
NP8EO	1896.7	550.9	556.2	70.6
NP9EO	1965.7	547.5	569.1	71.0
NP10EO	3105.6	419.4	442.4	85.7
NP11EO	2526.3	322.7	298.9	88.1
NP12EO	2016.9	231.9	220.6	89.0
NP13EO	1337.2	128.7	138.8	89.6
NP14EO	820.5	62.8	69.7	91.5
NP15EO	332.7	23.8	28.3	91.5
NP16EO	141.2	9.7	10.1	92.8
NP17EO	49.1	3.0	4.2	91.4
NP18EO	21.3	1.4	1.4	93.4
NP19EO	9.7		0.6	93.8
NP20EO	5.1			100
NP21EO	2.6			100
NP22EO	1.5			100
NP23EO	1.7			100
Total NPEOs	49308.3	20324.2	15185.8	69.2

Company), a C18 precolumn (2.1mm in ID x 50 mm in length, 3μm Capcell Pak, Shiseido Co., Japan), and a silica analytical column (2.0mm in ID x 150 mm in length, 3μm Spherisorb SW3, Waters USA). The details of LC-ESI-MS conditions are described in our previous article (Shao et al, 2002(a)). In view of the low sensitivity for measurement of NP1EO and NP upon using LC-ESI-MS, GC-MS was used to detect these compounds. Operation was performed on a GC-MS (5890SeriesII GC, 5971 MSD, Hewlett-Packed, USA) with a HP-5-MS column (0.25mm ID x 30m in length, 0.25μm). The specific method was described in previous report (Shao et al. 2002 (b)).

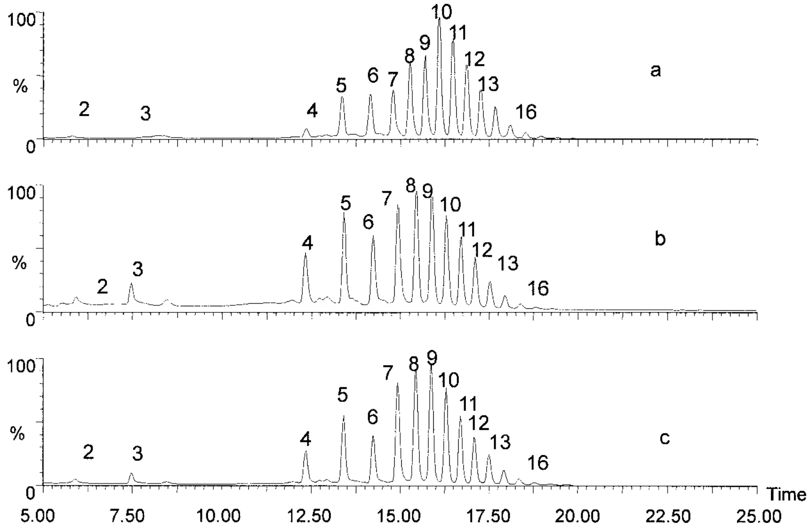

Figure 1. Chromatograms of NPEOs from influent (a), aerobic ditch (b) and effluent(c).

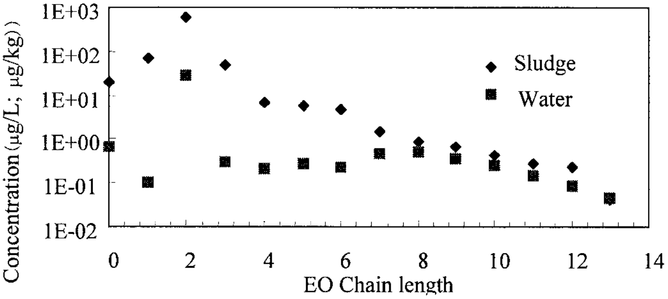

Figure 2. NP and NPEOs homologues in thickened sludge and supernatant.

RESULTS AND DISCUSSION

Table 1 lists the distribution of respective homologues in the influent sample of the sewage treatment plant. The total concentrations of NPEOs were 49.31 μg/L, and NP, NP1EO and NP2EO, which are usually absent in the original surfactants, accounted for about 70% of total concentration (calculated by mass) in the influent, indicating that most of NPEOs had already been degraded before wastewater reached the plant. Similar results were also reported by Ahel *et al.* (Ahel et al, 1994). Compared with concentrations of NP and NP2EO, the concentration of NP1EO was significantly low, suggesting that once NPEOs were degraded into NP1EO, they would be easily transformed into NP.

It is clear from Table 1 that significant reduction of NPEOs and NP occurred once sewage flowed into the ditches. The samples from ditch and effluent, however, did not show large differences. The removal of NPEOs has a tendency to increase with the increase of EO chain length. The removals of NP2EO, NP3EO, and NP4EO were below 60%, significantly low in comparison with those of NPEOs at EO>9. As will be discussed in the later part, the removal of NPEOs during sewage treatment was mainly contributed by two paths: biodegradation of NPEOs from longer ones to shorter ones, and sorption of NPEOs to sludge. For NP, sorption was the primary path. The relatively low removals of NPEOs with short EO chains were perhaps due to the simultaneous occurrence of decomposition and formation of these compounds.

Figure 1a, b, and c show variation of the chromatograms of NPEOs homologue following sewage treatment. It is clear from Figure 1a that, similar to those of the commercial NPEOs homologues, the chromatogram of the influent sample indicates a near Possion distribution although significant biodegradation occurred. The position of the highest peak moved from NP10EO of the influent sample to NP8EO and NP9EO of the other two samples, indicating the possibility of biodegradation of NPEOs. Further studies, however, are required to investigate the contribution of biodegradation during sewage treatment.

The contents of NP and NPEOs homologues in thickened sludge and supernatant liquor of the sludge-thickening tank were also measured (Figure 2). The total concentration of NPEOs and concentration of NP in the thickened sludge were 732.9 and 19.5μg/kg dry weight, respectively. Of NPEOs, NP2EO was the most abundant with a concentration of 595.7 μg/kg. On the other hand, it was found that the concentration of NPEOs ($n \geqslant 7$) is almost equal to that in sludge and the partitioning coefficient was obtained to be near to 1. However, the partitioning coefficient for NPEOs ($n<7$) ranged from 19 to 670L/kg although the concentration in water is similar with that of NPEO with $n \geqslant 7$ except for NP2EO, which would be due to their difference of physico-chemical property.

Acknowledgements The authors acknowledge the financial assistance from National Natural Science Foundation of China (49925103 and 40024101).

REFERENCES

Huang HZ (1998) Handbook of chemical products: industrial surfactants. Chemical Industry Press, Beijing, P. R. China.

Nalor CG, Varineau PT, Field J (1996) Proceeding of the CESIO 4th world surfactant congress; Barcelona, Spain; European Committee on Surfacants and

Detergents: Brussels, Belgium, 378-391

Giger W, Brunner PH (1984) 4-Nonylphenol in sewage-sludge accumulation of toxico metabolites from nonionic surfactants. Science 225: 623-625

White R, Jobling S, Hoare SA, Sumpter JP, Parker MG (1994) Enviromentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135: 175-182

Sharpe RM, Fisher JS, Millar MM, Jobling S, Sumpter JP, (1995) Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. Environ Health Perspect 103: 1136-1143

Jobling S, Sheahan D, Osborn JA, Matthiessen P, Sumpter JP (1996) Inhibition of testicular growth in rainbow trout (Oncorhynchus mykiss) exposed to estrogenic alkylphenolic chemicals. Environ Toxicol Chem 15: 194-202

Lee HB (1999) Review of analytical methods for the determination of nonylphenol and related compounds in environmental samples. Water Qual Res J Canada, 34: 3-35.

Di Corcia A, Sameri R, Marcomini A (1994) Monitoring aromatic surfactants and their biodegradation intermediates in raw and treated sewages by solid-phase extraction and liquid-chromatography. Environ Sci Technol 28: 850-858

Maruyama K, Yuan M, Otsuki A (2000) Seasonal changes in ethylene oxide chain length of poly(oxyethylene) alkylphenyl ether nonionic surfactants in three main rivers in Tokyo. Environ Sci Technol 34: 343-348

Shang DY, Macdonald RW, Ikonomou MG (1999) Persistence of nonylphenol ethoxylate surfactants and their primary degradation products in sediments from near a municipal outfall in the strait of Georgia, British Columbia, Canada. Environ Sci Technol 33: 1366-1372

Shao B, Hu JY, Yang M (2002) (a) Determination of nonylphenol ethoxylates in the aquatic environment by normal phase liquid chromatography electrospray mass spectrometry. J Chroma A 950: 167-174

Petrović M, Barcel ó D (2000) Determination of anionic and nonionic surfactants, their degradation products, and endocrine-disrupting compounds in sewage sludge by liquid chromatography/ mass spectrometry. Anal Chem 72: 4560-4565

Shao B, Hu JY, Yang M (2002) (b). A survey of nonylphnol in equatic Environment of Chongqing Valley. Acta Environ Sci (China) 22:12-16

Ahel, M.; Giger, W.; Schffner, C.; (1994). Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-occurrence and transformation in sewage treatment. Wat Res 28:1143-1152