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Dissolved Neutral Nonylphenol Ethoxylates Metabolites in the Haihe River and Bohai Bay, People's Republic of China

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Nonylphenol ethoxylates (NPEO) are the nonionic surfactants widely used in domestic, agriculture and industry (Warhurst, 1995; Naylor, 1996). The degradation products, such as nonylphenol (NP) and the short-ethoxy-chain nonylphenol ethoxylates (mainly NP1EO and NP2EO, the number indicates the ethoxy units) are found able to cause disorder of endocrine system in wildlife (Jobling et al., 1993; Jobling et al., 1996). Due to this reason, NPEO have been banned to use in many countries (Renner, 1997; Jeannot et al., 2002). However in China, the manufacture quantity of NPEO is increasing since 1994 when NPEO was considered as the surfactant developed in priority. It is estimated that approximately 40000 tons/year NPEO are produced in China, accounting for 10% of the world production (Naylor, 1996; Huang, 1998).

Numerous references are available about the environmental occurrence and behavior of nonylphenol and nonylphenol ethoxylates (Ferguson et al., 2001; Isobeet al., 2001; Rice et al., 2003; Li et al., 2004a; Li et al., 2004b). However, only few studies have been performed about these compounds in China (Jin et al., 2004). Knowledge about the contamination levels and possible eco-toxicological risks of these compounds in the aquatic environment in of China is very limited. Until now no regulations have been made in China about NPEO. In this study, we performed a field investigation to know the contamination level and behavior characteristics of NP, NP1EO and NP2EO in the Haihe River and Bohai Bay in the north China. This will be helpful to provide the necessary information for the ecological risk assessment and regulation of these chemicals in China.

MATERIALS AND METHODS

The delta of Haihe Rive and Bohai Bay is one of the most developed regions in China (Figure 1). The master stream of Haihe Rives rises in the cross point of Beiyun, Ziya and Nanyun River in Tianjin City (Sampling site 1 in Figure 1), one of the largest cities in China with a population over 10 million. The river flows through the urban area of Tianjin City (sites 2-4), rural area of Tianjin City and Tanggu City (sites 5-8), and urban area of Tanggu City (sites 9-11), and finally goes into Bohai Bay (sites12-21). Rapid development of industry and agriculture has caused serious pollution problem, which has adversely affected the water

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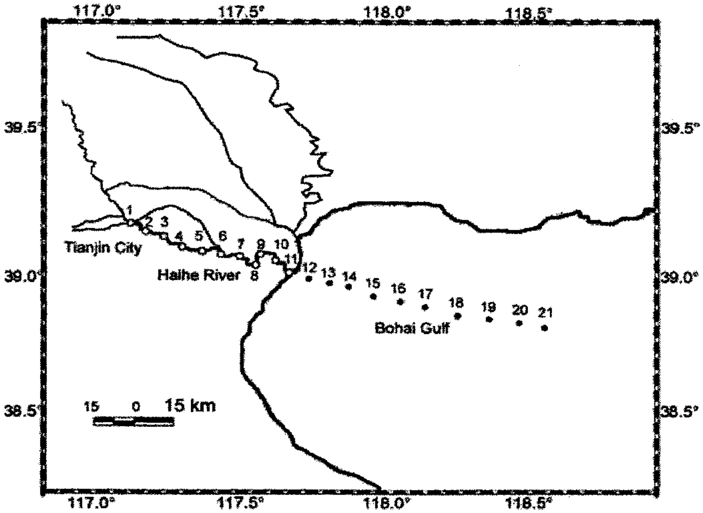

Figure 1. Map of sampling sites.

quality of Haihe River and Bohai Bay (Liu et al., 2003). There are few studies available focusing on alkylphenol ethoxylates in this area. To the best of our knowledge, only NP has been monitored in the Haihe River (Jin et al., 2004). No monitoring data of these endocrine disrupters in the Bohai Bay are available.

4-Nonylphenol (technical mixture with branched isomers) and the NPnEO mixture (n≈2) were purchased from Tokyo Chemical Industry. The NPnEO (n≈2) mixture consists of nonylphenol ethoxylates having 1-3 ethoxy units, and the average number of ethoxy units is 2. The peak composition of the NP1EO and NP2EO was determined by GC-FID analysis and subsequently identified by GC/MS (Isobe et al., 2001). Surrogate internal standard bisphenol A-*d14* and injection internal standard phenanthrene-*d10* as used by Li et al. were from Sigma-Aldrich (Li et al., 2001; Li et al., 2004a; Li et al., 2004b). Silylation reagent BSTFA (N,O-bis(trimethylsilyl)trifuoro-acetamide) with 1% TMCS was from Supelco. All the solvents used were HPLC grade. All the glass equipments were baked at 450℃ for 4h prior to use.

Water samples from the river (sites from 1 to 11) and sea (sites from 12 to 21) were collected under 0.5m of the surface in August 2004. The sampling stations are shown in Figure 1. Throughout the survey a Magellan 315 model global positioning system (GPS) was used to locate the sampling positions. After carried to laboratory as soon as possible, 1 liter of the water was filtered under vacuum through pre-ashed glass fiber filters (0.7 μm, Whatman, GF/F). Appropriate amount of surrogate internal standard (50 μl) was spiked into the filtrates. Then the 1 liter filtrated water was extracted using a solid-phase extraction (SPE) system as described by Spengler et al. (2001). The Supelco SPE Cartridges (EnvI^{tm}-18) were conditioned with 10 ml of acetone firstly, and then with 10 ml of ultra-pure water (Millipore, Watford). Then water samples were passed through the cartridges at a flow rate of about 5ml/min under vacuum. After extraction, the cartridges were freeze-dried (Freezone 4.5, Labcoco company) one night to removal the water. Then the cartridges were eluted with 2×5 ml acetone. After that the volume of the extracts were reduced by gentle nitrogen gas in a water bath. 100 μl of BSTFA (with 1% TMCS) was added to the concentrated extract followed by vigorous shaking for about 60 s at room temperature for derivatization (Li et al., 2001). Then 50μl GC internal standard was added.

A Finnigan Trace DSQ GC-MS with Xcalibur software was used in selected ion mode (SIM). The capillary column used was a DB-5MS (30 m×0.25 mm id × 0.25 mm film thickness). The carrier gas was helium. A split–splitless injector in the splitless mode was used. The inject volume was 1μl. The temperature program was programmed from 40℃ (initial time, 4 min) to 180℃ at a rate of 20℃ min^{-1} holding for 1 min, 180℃ to 245℃ at a rate of 2℃ min^{-1} holding for 2 min, 245℃ to 280℃ at a rate of 20℃ holding for 10min. The temperatures of injector and mass spectrometer were both held at 280℃.

Recoveries were determined by the spiked ultrapure water with the concentration of 1 $\mu g/l$ (n=3). The recoveries of NP, NP1EO and NP2EO were 95.6%, 81.0%, 93.7% and 87.6% respectively, and the RSD were 7.6%, 4.9% and 11.0% respectively. Instrument detection limits (IDLs) of NP, NP1EO and NP2EO were 6, 14.1, and 17.8pg respectively calculated as the amount of analyte giving a peak with a signal-to-noise ratio of 3. Method detection limits (MDLs) were calculated from the IDLs, normalizing to sample amount and the final volume of extract analyzed, which were 3.5, 9.5 and 10.5 ng/l for NP, NP1EO and NP2EO.

RESULTS AND DISCUSSION

Table 1 shows the concentrations of NP, NP1EO and NP2EO along the sampling sites. The concentrations in the river are consistent with recent measurements of these compounds in other similar aquatic systems. Total NPnEO (n=0-3) in Cuyahoga River, Ohio, US reportedly ranged between 130 and 1000 ng/l (Rice et al., 2003). NP and NP1EO in Sumadagawa River in Tokyo, Japan were 80-1080 and 120-810 ng/l; in Tamagawa River were 50-170 ng/l and 60-970 ng/l (Isobe et al., 2001). In the sea water, the level of individual neutral NPEO degradation products was detected at concentration from about 10 ng/L to 100 ng/L. Compared with those similar estuaries, such levels are about one magnitude lower than in Jamaica Bay (220-1050 ng/l of total NPnEO (n=0-3), New York in US) (Ferguson et al., 2001) and Scheldt estuary (35-934 ng/l of NP and 4.6-1029 ng/l of total NPnEO (n=1-2), Netherlands) (Jonkers et al., 2003), and consistent with the concentration in Rhine estuary (31-147 ng/l of NP and 54-471 ng/l of total NPnEO (n=0-2), Netherland) (Jonkers et al., 2003). Generally the level decreased with the gradient to the outer sea, indicating that the source of the NPnEO is mainly from terrestrial input.

Table 1. Concentration of dissolved nonylphenol and nonylphenol ethoylates in Haihe River and Bohai Bay, China.

Site	NP (ng/l)	NP1EO (ng/l)	NP2EO (ng/l)
1	238	165	154
2	348	160	183
3	230	104	139
4	200	88	66
5	238	120	76
6	160	48	55
7	243	97	62
8	249	88	69
9	218	66	61
10	349	102	87
11	429	244	256
12	132	132	118
13	85	41	51
14	62	27	31
15	90	62	113
16	55	34	19
17	72	43	31
18	84	41	48
19	42	16	n.d.
20	34	13	12
21	33	n.d.	n.d.

(1): n.d., not detected (below MDLs)

Since little studies have been performed about alkylphenol in this area, it is difficulty to know the historical flux. To the best of authors' knowledge, there was only one report provided by Jin et al. (2004) concerning the NP levels in the Haihe River sampled in April 2003. Compared with this study, NP detected here sampled in August is a little higher. This may be due to the different water temperature. As shown by the previous studies, the activity of microorganism is high at the warm season, resulting in degrading more long chain alkylphenol ethoxylates into short chain products (Tanghe et al., 1998; Staples et al., 1999; Manzano et al., 1999). The similar phenomenon was also found by Li et al. (2004a) that the NP in the water of Han River, Korea exhibited a higher concentration in August than October and December.

Figure 2 shows the total NPnEO (n=0-2) decreased from site1 to 9 indicating no significant source of NPnEO entering the Haihe River. The level significantly increased in sampling site 11, where a sewage treatment plant outlet was located.

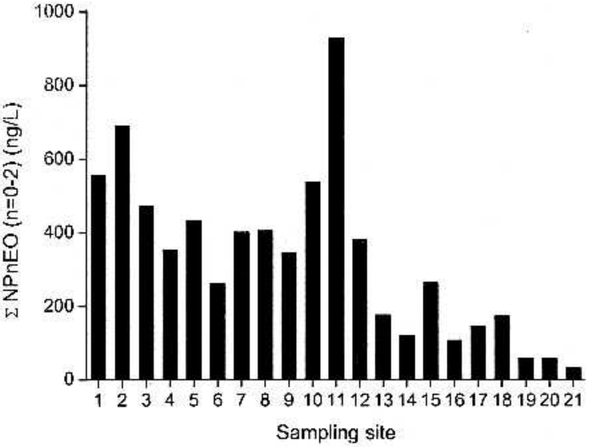

Figure 2. Total NPnEO (n=0-2) with sampling sites (the concentrations of not detected samples are regarded as 1/2 of the MDLs).

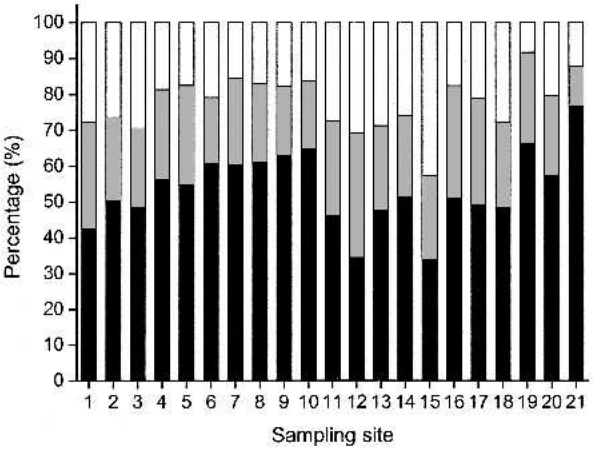

Figure 3. Distribution of the nonylphenolic compounds with sampling sites (the concentrations of not detected samples are regarded as 1/2 of the MDLs; NP:■; NP1EO:▨; NP2EO:□).

The pattern change of NP, NP1EO and NP2EO along the sampling sites was in agreement with the change of the concentration. As can be seen in Figure 3, the percentage of NP increased from site 1 to site 10. This was due to the biodegradation along the river, since it is generally believed that biodegradation will result in shortening or oxidization of the ethoxy chain, leading to an increased ratio of NP/NPnEO (Ahel et al., 1994; Jonkers et al., 2001). Although dilution or evaporation will occur, they will equally or selectively removes nonylphenol relative to the ethoxy-substituted forms, thus will not result in the increased ratio of NP/NPnEO (Ferguson et al., 2001). In the sea water, neither the total concentration nor the distribution pattern had a clear trend as in the river water, probably due to the reason that in the sea water, the chemicals diffused not exactly along the sampling sites but with the salinity gradient. To verify if presence of the higher numbered ethoxylates can be neglected, the highly ethoxlyated NPEO should be analyzed (Ferguson et al., 2001). Hence the extract of the sample of site 11 was analyzed by HPLC/MS as described by Shao et al. (2002) for NP(0-20)EO, and it was found that NP(3-20) only make a significant fraction of about 20%. This is in good agreement with the fact that the wastewater discharge to the Haihe River and Bohai Bay has been secondary biological treated. Since the short chain nonylphenol ethoxylates NP(n=0-2)EO were dominant, their total concentration can be regarded approximately as the total concentration of NP(0-20)EO, and the transformation of the NPEO with long chain can be neglected.

Table 2. Correlations with physical and chemical parameters of surface water $^{(1)}$.

	COD_{Mn}	BOD_5	DOC	TN	TP	DO (river)	DO (bay)
r^2	**0.46**	0	**0.45**	0.03	**0.59**	**0.41**	**0.40**
$P^{(2)}$	**0.0006**	0.99	**0.001**	0.30	**< 0.0001**	**0.032**	**0.048**

$^{(1)}$: only the samples have measurable amount (> n.d.) of analytes were used for linear regression

$^{(2)}$: it is regarded as significantly correlated when $p<0.05$ (bold in the table)

In order to investigate the behavior of the nonylphenolic compounds in Haihe River and Bohai Bay, the correlations with the physical and chemical parameters of the water were studied as table 2 shows (The individual NP, NP1EO and NP2EO had the same correlations with each physical and chemical parameter as the total NPnEO (n=0-2)). From the correlations with COD_{Mn}, it can be inferred that the nonylphenolic compounds may be released into the aquatic environment together with other organic contaminants. It will be convenient to control their contamination simultaneously. The lack of the correlation with BOD_5 suggests they are not the most biodegradable fraction of the organic contaminates in the studied area. Table 2 also shows that they were positively correlated with DOC, indicating bound to the dissolved organic matter is an important fate of these compounds. The high $\log K_{ow}$ of these compounds (NP: 4.48, NP1EO: 4.17, NP2EO: 4.21 (Ying et al., 2002), is an explanation for this. Additionally, it was found that the nonylphenolic compounds in this studied area related with the behavior of phosphorus. Li et al. (2004b) reported the similar phenomenon of NP in Shiwa Lake, Korea. The reason for this correlation should be studied further. No correlations with nitrogenous were found. Besides the collective organic parameters, correlations with the dissolved oxygen were found. (Unlike the other parameters, the correlations with DO were studied in the river and bay separately, because DO is not diluted with the seawater, while all the other collective organic parameters are diluted in the seawater as well as the target chemicals). These negative correlations are perhaps related with the aerobic organisms, which are more active to eliminate the contaminants in the sites with higher DO level. The similar phenomenon occurred in Scheldt estuary, Netherland reported by Jonkers et al. (2003).

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