Trophodynamic Behavior of 4-Nonylphenol and Nonylphenol Polyethoxylate in a Marine Aquatic Food Web from Bohai Bay, North China: Comparison to DDTs

JIANYING HU,^{*,†} FEN JIN,^{†,‡} YI WAN,[†] MIN YANG,[‡] LIHUI AN,^{†,‡} WEI AN,[†] AND SHU TAO[†]

College of Environmental Science, Peking University, Beijing, 100871 China, and State Key Lab of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

4-Nonylphenol (4-NP) is of particular concern because of its ubiquity in aquatic environment and its endocrine-disrupting effects in aquatic organisms. On the basis of its octanol–water partition coefficient ($10^{4.6}$), it has a potential to bioaccumulate in aquatic food webs. However, there are no reported field studies on the trophodynamics of 4-NP and its precursor, nonylphenol polyethoxylate (NPEOs) surfactants, in aquatic food webs. This study reports the trophodynamics of 4-NP and NPEOs ($4 < s < 16$) in a marine aquatic food web from Bohai Bay, North China. 4-NP and NPEOs ($4 < s < 16$) were determined in 14 marine species including plankton, benthic invertebrates, fish, and marine birds. This paper provides the first report on the occurrence of NPEOs with $s > 5$ in marine biota. Co-analysis of DDTs in all samples allowed a direct comparison of the bioaccumulation behavior of DDTs with that of NP and NPEOs. The lipid equivalent concentration of DDE and 2,2-bis(chlorophenyl)-1-chloroethylene (DDMU) increased with increasing trophic level, and the trophic level was determined by stable isotope ratios. The trophic magnification factors (TMFs) of DDE and DDMU were 3.26 and 3.7, respectively. Lipid equivalent concentrations of 4-NP and of all NPEOs did not exhibit a statistically significant correlation with trophic levels in the food web, and the TMF of NP was 0.83, which was similar to those of all NPEOs (0.45–1.22). These results show that in the studied aquatic food web, there was no trophic magnification for 4-NP and NPEOs, whereas DDE and DDMU biomagnified.

Introduction

4-Nonylphenol (4-NP) is the biodegradation intermediate of nonylphenol polyethoxylate (NPEOs) surfactants in aquatic environments, and it is of particular concern because of its endocrine-disrupting effects in aquatic organisms, mammals, and birds (1–4). NPEOs are commonly used worldwide as an emulsifying, dispersing, wetting, and foaming agent in various industrial, institutional, agricultural, and household

applications (5). China currently produces approximately 50 thousand tons of NPEOs surfactants per year (6), and the global production level is approximately 500 thousand tons per year (7). Residues of 4-NP and NPEOs have been reported to be ubiquitous in river water, groundwater adjacent to contaminated rivers, seawater, and tap water (8–11) as well as in sediment and fish tissues (12–16). It appears that 4-NP and NPEOs are rather persistent in the environment, although both microbial and photochemical degradation has been reported (17–18).

In addition to their toxicity, prevalence, and environmental persistence, their bioaccumulation in aquatic organisms is another vital criterion for assessing the ecological risk of 4-NP and NPEOs. Upper trophic level aquatic organisms are primarily exposed to chemicals with a log octanol–water partition coefficient (Kow) of approximately 4–5 and higher through dietary accumulation (19). 4-NP and NPEOs such as NP1EO and NP2EO exhibit moderate octanol–water partition coefficients, with logarithms of 4.0–4.6 (20–22). Because of this hydrophobicity, 4-NP is often assumed to have the potential to bioaccumulate in biological organisms, and Ekelund et al. reported the bioconcentration factor (BCF) of 4-NP in mussels to be 3400 (23), which is 2.5 times higher than the theoretical value of 1280 (24) predicted on the basis of the Kow value. However, several laboratory studies that investigated the bioconcentration of 4-NP in various aquatic species including algae, plants, invertebrates, and fish suggested a wide range of BCF (0.9–741) and bioaccumulation factor (BAF) (6–487) (25), a value less than the expected theoretical value on the basis of the Kow value. In terms of these data, it has been suggested that the ability of 4-NP to bioaccumulate in aquatic biota in the environment is low to moderate. Additionally, the bioaccumulation of NP1EO and NP2EO was reported to be similar to that of 4-NP (26), and there is relatively little data available for NPEOs with long ethoxylate (EO) chains (27). While several reports have been published concerning the bioaccumulation of 4-NP and NPEOs in the field and in laboratory aquatic organisms, there is insufficient information about the trophodynamics of 4-NP and NPEOs in aquatic food webs.

In this paper, we present a field study investigating the trophodynamics of 4-NP and NPEOs in a marine food web (including plankton, benthic invertebrates, six fish species, and marine birds) from Bohai Bay in north China. The trophodynamics of DDTs in the food web were also determined to enable a direct comparison of the unknown trophodynamic behavior of 4-NP and NPEOs to that of the recognized bioaccumulation behavior of DDTs.

Materials and Methods

Sampling Methodology and Samples. Aquatic food web components were collected in May, June, and September 2002 in the Bohai Bay (39°12'N, 117°59'E, Figure 1). Seabirds were collected in November 2002 on the coast of Bohai Bay (39°07'N, 117°44'E). The part of the marine food web investigated in this study included primary producers (including phytoplankton and zooplankton), five invertebrate species (crab (*Portunus trituberculatus*), burrowing shrimp (*Upogebia* sp.), short-necked clam (*Ruditapes philippinarum*), veined rapa whelk (*Rapana venosa*), and bay scallop (*Argopecten irradians*)), six fish species (weever (*Lateolabrus japonicus*), catfish (*Chaeturichthys stigmatias*), bartail flat-head (*Platycephalus indicus*), white flower croaker (*Nibea albiflora*), wolffish (*Obontamblyopus rubicundus*), and mullet (*Liza so-iuy*)), and one seabird species (herring gull, *Larus argentatus*). The primary producers were obtained from

* Corresponding author phone: 86-10-62765520; fax: 86-10-62765520; e-mail: hujy@urban.pku.edu.cn.

[†] Peking University.

[‡] Chinese Academy of Sciences.

FIGURE 1. Location of sampling sites, ●: sampling locations of planktons; ▲: sampling locations of fishes and invertebrates; ■: sampling locations of seabirds.

vertical tows (bottom to surface) using 77- μ m-mesh nets 31.6 cm in i.d. $\times$ 140 m in length for phytoplankton and 160- μ m-mesh nets 37 cm in i.d. $\times$ 140 m in length for zooplankton. Isotope and chemical analyses for the plankton were made on a pooled sample taken from six locations (39°00'N, 117°53'E; 39°00'N, 118°00'E; 38°45'N, 117°53'E; 38°45'N, 118°00'E; 38°30'N, 117°53'E; and 38°30'N, 118°00'E). Invertebrates and fish were caught with a bottom trawl. The isotope and chemical analyses for invertebrates were conducted on a pooled sample of more than six samples taken from six locations. Six-month-old seabirds were captured before their winter migration commenced. All samples were stored at -20°C prior to analysis.

Chemicals and Standards. All dichloromethane, methanol, 2-propanol, acetonitrile, and hexane were HPLC grade or pesticide grade obtained from Fisher Scientific (New Jersey). *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, and *p,p'*-DDE were all purchased from Chemservice (Chester, England), and *p,p'*-DDMU was from Sigma (St. Louis, MO). 4-NP (technical grade), a mixture of compounds with branched side chains, was purchased from Kanto Chemicals (Tokyo, Japan). Authentic standard nonylphenol mono- (NP1EO), di- (NP2EO), tri- (NP3EO), tetra- (NP4EO), penta- (NP5EO), and hexaethoxylates (NP6EO) and mixture standard of NP9EO (a mixture of NPEOs with an average of 9 EO units) and NP15EO (a mixture of NPEOs with an average of 15 EO units) were purchased from Hayashi Pure Chemicals (Tokyo, Japan). PCB 121 and PCB 189 (IUPAC) were purchased from Accu Standard (Connecticut). Standard stock solutions were prepared in acetonitrile. BSTFA reagent was obtained from Supelco (Bellefonte, United States).

Sample Preparation. Frozen biota samples were thawed for 12 h and homogenized using an analytical mill. All equipment rinses were done without detergent to avoid sample contamination. Freeze-dried phytoplankton and zooplankton, soft tissues of invertebrates, and the muscles of fish and birds (5–30 g wet weight) were mixed with 20 g Na_2SO_4 and spiked with recovery surrogate (PCB 121 and PCB 189, 4-*n*-nonylphenol). Then, the spiked samples were Soxhlet extracted for 24 h using 200 mL dichloromethane/methanol (7:3 v/v) mixture solution. The extracts were rotoevaporated and reconstituted by 50 mL dichloromethane/methanol (7:3 v/v) mixture solution. The 50-mL extract was divided into four portions. The first 20-mL extract was rotoevaporated and reconstituted by 1 mL hexane and then was passed through 12 g 5% H_2O deactivated neutral Al_2O_3 (200- μ m-mesh size, Shanghai Ludu Chemicals, China) packed in a glass column (10 mm i.d.) for cleanup and fractionation. DDTs were eluted with 30 mL high-purity hexane and 30 mL hexane/dichloromethane (3:1 v/v). The eluant was dried and dissolved in 0.5 mL of hexane for gas chromatograph-mass spectrometer (GC-MS) analysis.

For 4-NP, NP1EO, and NP2EO analysis, the second portion of the extract (10 mL) was rotoevaporated and reconstituted

by 1 mL hexane and then was loaded onto the aminopropyl silica cartridges (500 mg, 6 mL, Waters) conditioned with 9 mL acetone, 3 mL methylene chloride, and 9 mL hexane. After the extract of the biota sample was passed through the cartridge, a 1-mL rinse of hexane was added. The cartridge was eluted with 8 mL hexane/[2-propanol] (90:10 v/v), and the eluant was nitrogen-evaporated to dryness and reconstituted by 0.5 mL hexane for gas chromatograph-mass spectrometer (GC-MS) analysis. When analyzing the muscles of seabird, a reversed phase cleanup based on the C18 procedure was implemented to purify the extracts after the aminopropyl cleanup step (28) and was derivatized by adding 100 μ L BSTFA reagent into 100 μ L of the extract (29). During the analysis of these samples, laboratory blanks were incorporated in the analytical procedure as described in Supporting Information.

For NPEO analysis, the third portion (10 mL of extract) was dried to 1 mL under a gentle nitrogen stream and then was transferred to a flask with 4 $\times$ 100 mL water. The solution was rinsed with a GCB SPE cartridge using the same procedure based on Di Corcia's extraction method (30). The residual water was removed by passing a gentle nitrogen stream through the cartridges for about 10 min, and then the NPEOs were desorbed from the cartridges by 10 mL methylene chloride/methanol (80:20 V/V). Finally, the residues were dried under a gentle nitrogen stream and were reconstituted with 2 mL ion reagent, an acetonitrile/water (95:5 V/V) solution containing 1 mmol/L sodium acetate for HPLC-ESI-MS analysis.

The final 10 mL extract was separated to determine the lipid percentage. The extracts were then rotated to dryness and heated at 65°C for about 30 min, and lipid amounts were determined gravimetrically. The lipid content was calculated on a wet weight basis.

The procedure described above was validated for recovery (ranging from 99% to 113% for DDTs and from 79% to 84% for 4-NP). Percentages for the recovery of surrogates determined in the biota samples were $90.6 \pm 8.4\%$ for PCB 121, $97.8 \pm 8.3\%$ for PCB 189, and $95\% \pm 12\%$ for 4-*n*-NP, and concentrations reported here have not been corrected for recovery. Precision was estimated at better than 20% on the basis of analysis of three replicate samples.

Chemical Analysis. The instrumental conditions for analyzing 4-NP, NPEOs, 4-*n*-NP, DDTs, PCB 121, PCB 189, and the quantitation and quality assurance quality control (QA/QC) are all provided in the Supporting Information.

Food Web Characterization. The concentration ratio of $^{15}\text{N}/^{14}\text{N}$, expressed relative to a standard (i.e., $\delta^{15}\text{N}$), has been shown to increase with increasing trophic level because of the preferential excretion of lighter nitrogen isotopes (31–32). The ratio has been suggested to be a useful empirical measure of trophic status and has been used in several trophodynamic studies of persistent organic pollutants (33–34). The concentration ratio of $^{13}\text{C}/^{12}\text{C}$ (i.e., $\delta^{13}\text{C}$) generally remains relatively constant with increasing trophic level (35), although a small degree of enrichment of $\delta^{13}\text{C}$ from producers to consumers may occur in coastal marine systems (36). To analyze for nitrogen and carbon stable isotopes, samples of phytoplankton, zooplankton, crabs, fish, and seabirds were first homogenized by an analytical mill. The samples were then freeze-dried, and lipids were removed from all samples by methanol extraction for about 12 h to reduce variability due to isotopically lighter lipids. Before isotope analysis, the samples were dried at 80°C for about 4 h. Subsequently, about 0.5-mg samples were set in 8 $\times$ 5 mm Sn capsules and combusted at 1000–1050 $^{\circ}\text{C}$. Nitrogen and carbon dioxide gas was then transported through the interface (ConFlo III, Finnigan MAT) and analyzed using a mass spectrometer (THERMO Delta plus, Finnigan MAT). Replicate measurements of internal laboratory standards (glycine) for 10 times

indicate measurement errors of $\pm 0.3\%$ for stable nitrogen isotope measurements. Stable isotope values were expressed as

$$\delta^{15}\text{N} = ((^{15}\text{N}/^{14}\text{N}_{\text{sample}}/^{15}\text{N}/^{14}\text{N}_{\text{standard}}) - 1) \times 1000 (\text{‰}) \quad (1)$$

The $^{15}\text{N}/^{14}\text{N}_{\text{standard}}$ values were based on atmospheric N_2 (air).

Trophic Level Calculation. Trophic levels of each aquatic organism can be calculated using the relationship by Fisk et al. (37) and Muir et al. (38):

$$\text{TL}_{\text{consumer}} = 2 + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{zooplankton}})/3.8 \quad (2)$$

where $\text{TL}_{\text{consumer}}$ is the trophic level, and the trophic level (TL) of zooplankton was assumed to be 2. For the calculation of trophic levels in birds, the diet-tissue isotope fractionation factor is $+2.4\%$, and the equation should be modified to (37)

$$\text{TL}_{\text{bird}} = 3 + (\delta^{15}\text{N}_{\text{bird}} - 2.4 - \delta^{15}\text{N}_{\text{zooplankton}})/3.8 \quad (3)$$

where TL_{bird} is the trophic level of bird, and $\delta^{15}\text{N}_{\text{bird}}$ is the stable nitrogen isotope value of birds.

Trophic Magnification and Biomagnification Factor Calculations. The trophic magnification factors (TMFs) are based on the relationships between the trophic level and the NP or NPEO concentration using simple linear regression:

$$\log \text{NP or NPEO concentration (lipid corrected)} = a + b \times \text{TL} \quad (4)$$

The concentrations below the detection limit were treated as half of the detection limit. The b in eq 4 was used to calculate TMF by the following equation:

$$\text{TMF} = 10^b \quad (5)$$

Correlations between concentrations and trophic levels were examined by Spearman's rank correlation test, and when the value of p was below 0.05, the linear regression between concentration and trophic level was regarded as significant. Biomagnification factors were calculated by the following equation:

$$\text{BMF} = ([\text{predator}]/[\text{prey}]) / (\text{TL}_{\text{predator}}/\text{TL}_{\text{prey}}) \quad (6)$$

where $[\text{predator}]$ and $[\text{prey}]$ are the lipid corrected concentrations in the predator and prey species, respectively.

Lipid Equivalent Concentrations. To present the concentrations of DDTs and NP in the various species on a common basis, observed wet weight concentrations were expressed in terms of lipid equivalent concentrations to remove the effect of differences in lipid content or sorbing matrixes between organisms. Concentrations in the benthic invertebrates, fish, and birds were normalized on a sample-specific basis according to

$$C_{\text{lipid}} = \frac{C_{\text{wet}}}{L}$$

where L is the lipid fraction of the sampled tissue.

Results and Discussion

Trophic Levels of Organisms in the Marine Food Web from Bohai Bay. The $\delta^{15}\text{N}$ value in this part of the marine food web ranged from 4.08 to 13.28‰, as shown in Table 1. $\delta^{15}\text{N}$ values can be divided into four groups: phytoplankton and zooplankton (4.08–6.36‰); invertebrates including scallops, crabs, shrimp, clam, and whelk (5.81–11.05‰); fishes (8.22–

TABLE 1. Mean Biological Parameters (Lipid Content (%), $\delta^{15}\text{N}$ ‰, $\delta^{13}\text{C}$ ‰, and Trophic Level) and Concentrations (ng/g Equivalent Lipid) of NP, Total NPEOs, and DDTs in 14 Marine Organisms Collected from Bohai Bay, North China

species	length (cm)	weight (g)	tissue lipid % ± SD (n) ^c	$\delta^{15}\text{N}$ ± SD	$\delta^{13}\text{C}$ ± SD	trophic level	NP	NPEOs	DDMU	μ,p' -DDE	ΣDDT
phytoplankton			2.25 ^a	4.08 ± 0.12	21.48 ± 0.64	1.70 ± 0.12	440.0 ± 88	290 ± 58.2	ND	7.9 ± 1.6	7.93 ± 1.6
zooplankton			1.84 ^a	6.36 ± 0.19	20.47 ± 0.61	2.00 ± 0.05	677.8 ± 136	780 ± 156	ND	27.7 ± 5.5	27.7 ± 5.5
bay scallop			6.43(8) ^b	5.81 ± 0.17	18.87 ± 0.57	1.86 ± 0.05	155.5 ± 31.1	371.8 ± 212.0	2.0 ± 0.4	19.4 ± 3.9	32.2 ± 6.4
crab			5.85(6) ^b	11.05 ± 0.33	16.79 ± 0.50	3.23 ± 0.09	154.2 ± 64.6	81.6 ± 25.7	2.2 ± 0.5	30.8 ± 5.4	131.8 ± 26.4
burrowing shrimp			13.62(6) ^b	10.18 ± 0.31	15.35 ± 0.46	3.01 ± 0.08	173.8 ± 64.4	86.7 ± 28.1	2.8 ± 0.6	50.4 ± 10.1	109.9 ± 22.0
short-necked clam			4.05(17) ^b	7.64 ± 0.23	15.70 ± 0.47	2.34 ± 0.07	170.3 ± 34.1	183.9 ± 36.8	4.3 ± 0.9	33.7 ± 6.7	77.5 ± 15.5
veined rapa whelk			6.23(10) ^b	8.77 ± 0.26	17.55 ± 0.53	2.64 ± 0.07	142.6 ± 23.4	512.0 ± 334.2	0.4 ± 0.1	19.1 ± 3.8	33.0 ± 6.6
catfish	20(17–24)	83(56–120)	4.16 ± 1.00(12)	12.48 ± 0.14	15.36 ± 0.51	3.61 ± 0.04	151.4 ± 52	678.2 ± 388.9	15.1 ± 5.7	38.2 ± 10.3	69.8 ± 19.0
weever	35(22.5–48)	728(180–1276)	4.64 ± 0.58(2)	13.28 ± 1.87	14.28 ± 2.07	3.82 ± 0.49	277.9 ± 94.8	251.6 ± 140.8	3.1 ± 0.6	67.7 ± 13.6	83.7 ± 16.7
bartail flathead	36(23.5–61)	357(95–1119)	2.54 ± 0.77(6)	12.41 ± 0.90	17.20 ± 0.86	3.59 ± 0.24	196.6 ± 99.6	187.8 ± 130.7	9.1 ± 1.1	110.1 ± 54.4	292.7 ± 132.7
wolfish	27(20–30)	36(18–56)	4.43 ± 2.04(10)	12.13 ± 0.43	13.99 ± 1.26	3.52 ± 0.11	309.3 ± 99.1	485.3 ± 390.2	2.18 ± 3.3	59.0 ± 25.6	136.5 ± 10.4
mullet	20(15.5–24.5)	93(44–158)	4.36 ± 1.24(15)	9.97 ± 1.68	11.59 ± 0.82	2.95 ± 0.44	324.9 ± 119.0	49.2 ± 9.1	10.3 ± 4.3	64.3 ± 29.7	286.9 ± 124.5
white flower croaker	21(10.5–34)	195(10–538)	3.14 ± 1.00(6)	12.39 ± 1.48	17.60 ± 1.35	3.59 ± 0.39	177.1 ± 35.4	342.3 ± 68.5	20.3 ± 9.4	149.1 ± 100	392.0 ± 176.1
herring gull	35(31–41)	211(142–321)	10.14 ± 4.4(6)	11.74 ± 1.63	22.19 ± 1.86	3.79 ± 0.43	239.6 ± 99.9	139.0 ± 67.5	98.2 ± 77.4	1894 ± 1417	1992 ± 1490

^a Pooled sample collected from six locations. ^b Pooled sample. ^c SD for pooled sample was calculated as 20% of result.

14.6 ‰); and herring gull (9.71–13.42‰). Finally, these $\delta^{15}\text{N}$ values were converted to trophic levels, and the trophic levels calculated for the organisms sampled in this study ranged from 1.70 to 3.82 (Table 1) with a trophic level of 2.0 set for zooplankton.

DDTs, 4-NP, and NPEOs Concentrations in Marine Biota from Bohai Bay. All DDT-related compounds analyzed in this study included *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, and *p,p'*-DDMU, and the analytical results are provided in Table 1 and Supporting Information Table 2. Only *p,p'*-DDE was detected in all kinds of biota samples, and *o,p'*-DDE and DDMU were detected in all of the samples except for the crab samples. The *p,p'*-DDE concentration increases with TL, starting with phytoplankton and zooplankton (11.31–43.75 ng/g lipid), and progressing to invertebrates (19–50.5 ng/g lipid) and then to fish (38–149 ng/g lipid); *p,p'*-DDE in bird reached 1894 ng/g lipid. A similar trend was also found in DDMU concentration variation in the marine food web from Bohai Bay.

4-NP was detected in all samples, and the concentration ranged from 142.6 to 677.8 ng/g lipid. 4-NP concentration levels have been reported previously in mollusks such as clams, cuttlefish, squid, and mussels from marine ecosystems. Wenzel et al. reported the retrospective monitoring of 4-NP in common mussels (*Mytilus edulis*) from the North Sea and Baltic Sea (39). Wenzel's study clarified the fact that 4-NP was detected in all mussel samples from 1985 until 1997, with the highest value of 9.7 ng/g wet weight at Eckwarderhorne in 1985, while the 4-NP concentrations were lower than limit of quantification (2 ng/g wet weight) after 1997. The concentrations detected in clams and mussels from the Adriatic Sea (15) were 243–265 ng/g lipid. The 4-NP concentration in short-necked clams detected in this study was 170 ng/g lipid which is slightly lower than for short-necked clams from the Adriatic Sea. The concentrations in bay scallops, crabs, burrowing shrimp, and veined rapa Whelk were also determined and were similar to the 4-NP concentration in short-necked clams. Of six fish species, the 4-NP concentration in catfish was the lowest (151.4 ng/g lipid), and the highest concentration (more than 300 ng/g lipid) was found in mullet and wolfish. Although little reports were found for 4-NP concentration level in marine fish, the 4-NP concentrations in flounder from a United Kingdom estuary were reported in the range of 5–55 ng/g wet weight, which was slightly higher those (5.0–24.3 ng/g wet weight) detected in the present study. This study also presents the 4-NP concentration in herring gull and phytoplankton, and the concentration for the former is 239.6 ± 99.9 ng/g lipid ($n = 4$) and that for the latter is 440 ng/g lipid (composite sample from six sampling sites) which was slightly lower than the 4-NP concentration in zooplankton, 677.8 ng/g lipid (composite sample from six sampling sites).

While several studies have examined the residues of 4-NP and NPEOs with short EO units ($s < 6$) in fish (3, 10, 13, 14, 16), there is little documenting the existence of NPEOs with more than five units in biota, and there are no reports of the occurrence of NPEOs in marine biota. As shown in the Supporting Information, each NPEO with $3 < s < 16$ was detected in all biota samples, which provided the first report on the occurrence of NPEOs with $s > 5$ in marine biota. Also, the total NPEOs concentration ranged from 183.9 ng/g lipid in short-necked clam to 678.2 ng/g lipid in catfish, which is similar with the 4-NP concentration in the same biota samples (Table 1). NPEOs with EO chains of 6–13 were the most abundant, and their profiles showed some difference from that of a standard NPEOs sample. This result may be associated with the low sewage treatment ratio in China; a similar profile was also found in fish from Changjiang River (11).

Trophodynamics of DDTs. Not surprisingly, strong positive relationships were found between trophic levels and DDE

TABLE 2. Statistical Results of Regression Analysis between log Concentration and Trophic Level (Slope, *p*-Value of Slope) and Trophic Magnification Factors (TMF) for 4-NP, NPEOs, and DDTs

	NP	NPEOs	NP5EO	NP6EO	NP7EO	NP8EO	NP9EO	NP10EO	NP11EO	NP12EO	NP13EO	NP14EO	NP15EO	DDE	DDMU	ΣDDT
slope	-0.08	-0.01	-0.35	-0.29	-0.12	-0.11	-0.09	-0.05	-0.03	0.03	0.09	0.05	0.06	0.51	0.58	0.6
TMFs	0.83	0.80	0.45	0.51	0.76	0.78	0.81	0.90	0.93	1.06	1.22	1.12	1.15	3.26	3.83	3.98
<i>p</i> -value	0.15	0.43	0.10	0.12	0.24	0.32	0.42	0.93	0.89	0.70	0.53	0.56	0.49	0.007	0.03	0.002
r^2	0.09	0.04	0.25	0.12	0.04	0.04	0.02	0.01	0.005	0.004	0.03	0.02	0.02	0.47	0.46	0.57

TABLE 3. Biomagnification Factors (BMFs) for NP, Total NPEOs, and DDTs on the Basis of Herring Gull/Fish Comparison

predator/prey	NP	NPEOs	DDMU	<i>p,p'</i> -DDE	ΣDDT
herring gull/catfish	1.51	0.20	6.19	47.22	27.20
herring gull/weever	0.87	0.56	32.25	28.19	23.99
herring gull/bartail flathead	1.15	0.70	10.22	16.30	6.45
herring gull/wolffish	0.72	0.27	4.19	29.82	13.56
herring gull/mullet	0.57	2.20	7.40	23.11	5.81
herring gull/white flower croaker	1.28	0.38	4.59	12.03	4.80

FIGURE 2. DDE and DDMU concentrations (ng/g, lipid-corrected) and trophic level relationships for the marine food web of Bohai Bay. The lines represent linear regression. (a) DDE, $p = 0.007$, $r^2 = 0.47$; (b) DDMU, $p = 0.03$, $r^2 = 0.46$. Trophic levels are based on $\delta^{15}\text{N}$. See text for details regarding sample types analyzed and species codes.

and DDMU concentrations (lipid-corrected) in biota, showing the high biomagnification potential of the two chemicals in the marine food web from the Bohai Bay (Figure 2a and b). The increase of concentrations of DDE and DDMU with trophic level was statistically significant for these two target DDTs ($p = 0.007$ for DDE and 0.025 for DDMU). The concentrations below the detection limit for DDMU were treated as half of the detection limit. The slopes determined by linear regression analysis of logarithmic DDE and DDMU concentrations and trophic levels were 0.51 and 0.58 , respectively. The TMFs of DDE and DDMU determined from the slope of concentration–trophic level relationships in this work were 3.26 and 3.83 , respectively. While no food web magnification of DDMU was reported, that of DDE was in good agreement with TMFs of 3.7 and 4.5 from the Barents Sea (40) and in the Arctic freshwater food web (41), respectively. The observed biomagnifications of DDE and DDMU in the food web at Bohai Bay proves that the food web studied is appropriate for testing the trophodynamics of 4-NP and NPEOs.

Trophodynamics of 4-NP and NPEOs. Figure 3a shows the relationship between trophic levels and the 4-NP concentration in biota. Lipid equivalent concentrations of 4-NP in biota appear to decrease slightly with increasing trophic level in the food web. However, regression analysis indicates that this correlation is not statistically significant ($p = 0.15$). The TMF of 4-NP was 0.83 . Linear regression analysis was also carried out for relationships between the trophic level and the lipid equivalent concentrations of each NPEO (Figure 3b and Supporting Information Figure 3). Because the concentrations of NPEOs with $s = 1–3$ were not

FIGURE 3. Concentrations (ng/g, lipid-corrected) of 4-NP and total NPEOs–trophic level relationships for the marine food web from Bohai Bay. The lines represent linear regression. (a) 4-NP, $p = 0.15$, $r^2 = 0.09$; (b) NPEOs, $p = 0.43$, $r^2 = 0.04$.

detected in all biota samples, their TMFs could not be obtained in this study. Also, the concentration of NP4EO was under the detection limit in the bird samples, which decreased the power to detect statistically significant trends in the food web, and so the result of regression analysis of bird samples is not listed in Table 2. The lipid equivalent concentration of each NPEO with an $s > 9$ in biota appeared to increase slightly with increasing trophic levels in the food web; however, this correlation was not statistically significant ($p > 0.05$). The TMFs of all NPEOs ranged from 0.45 to 1.22 , and the TMFs for NPEOs were slightly increased with the increase of ethoxylate (EO) chain length, which could possibly be related to the fact that NPEOs with an average ethoxylate chain length of 4 has a Kow of 3.24 , while NPEOs with an average ethoxylate chain length of 9 has a Kow of 3.59 (42).

These results indicate that the TMF of 4-NP was similar to that of the total NPEOs, and a small degree of trophic dilution may take place for 4-NP and NPEOs, although this did not appear to be statistically significant. This trend of lipid equivalent concentration varying with trophic level is in agreement with that of phthalate esters with a comparable Kow such as di-iso-butyl (DiBP, $\log \text{Kow} = 4.27$, $\text{TMF} = 0.81$), di-*n*-butyl (DnBP, $\log \text{Kow} = 4.27$, $\text{TMF} = 0.70$), benzylbutyl phthalate (BBP, $\log \text{Kow} = 4.70$, $\text{TMF} = 0.77$), which also show a small degree of trophic dilution and are less statistically significant ($p: 0.2–0.35$) (43). Although the detailed mechanism causing biomagnification is not yet fully understood, the trophic biomagnification was proposed to be dependent on the log Kow and metabolism of a chemical. The above results suggest that the metabolism rates of 4-NP and NPEOs would be comparable to those of phthalates because of their similarity of Kow.

The above results demonstrate that 4-NP and NPEOs were not obviously magnified in the studied aquatic food web, whereas DDE and DDMU were obviously magnified. However, the concentrations of 4-NP and NPEOs in organisms except for birds were higher than concentrations of DDE, which results from the ubiquity of NP and NPEOs in this marine environment. The endocrine-disrupting effects of 4-NP and NPEOs on the marine organisms require further investigation.

Predator/Prey Biomagnification Factors (BMFs). Although seabirds often feed on a range of diet items, fish were commonly found in the stomach of herring gull. BMFs based on herring gull/fish (single-prey diets) comparison and corrected for trophic level differences are summarized in Table 3. For the herring gull to catfish, bartail flathead, white flower croaker predator/prey relationship, the BMFs for 4-NP were 1.15–1.51 and the average BMF was 1.02, suggesting no biomagnification between trophic levels. The average BMF for NPEOs was 0.72, and the BMFs except for herring gull to mullet predator/prey relationship were smaller than 1 suggesting a slight dilution between trophic levels. On the other hand, the average BMFs of *p,p'*-DDE, DDMU, and Σ DDTs were 26.1, 10.8, and 13.6, respectively. When comparing BMFs for *p,p'*-DDE, DDMU, and Σ DDTs to 4-NP and NPEOs, the large discrepancy was observed for all herring gull to fish predator/prey.

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Supporting Information Available

A figure showing the concentrations of NPEOs. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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