Occurrence of nonylphenol, nonylphenol ethoxylate surfactants and nonylphenol carboxylic acids in wastewater in Japan

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Abstract Nonylphenol (NP) is known to be a byproduct of nonylphenol ethoxylates (NPnEO) which are used as detergents in industry. It is important that not only NP but also NPnEO and their related substances are analysed when behaviour of NP in the wastewater treatment process is surveyed. NPnEO are biodegraded to shorter ethoxylate (EO) chain NPnEO or nonylphenol carboxylates (NPnEC) under aerobic conditions, and then biodegraded to NP under anaerobic conditions. NP is one of the suspected endocrine disruptors (ED). Moreover, shorter EO chain NPnEO has greater toxicity than longer EO chain NPnEO. We conducted a field survey of NP and its related substances in 20 wastewater treatment plants (WWTP). The concentrations (median) of NP and its related substances in the WWTPs' influent ranged from 0.1 to 8.3 μg/L, showing NP concentration as the same level as those previously reported. The reduction of the long EO chain NPnEO in the WWTPs was almost complete, while the removal efficiency for the short EO chain NPnEO was less significant than the long EO chain NPnEO, suggesting that the degradation rate of the short EO chain NPnEO was lower than that of the long EO chain NPnEO in the wastewater treatment processes.

Keywords Nonylphenol; nonylphenol carboxylic acids; nonylphenol ethoxylate surfactants

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Introduction

In recent years, a new problem has emerged in our water environment, namely, endocrine disruptors (ED) that may adversely affect the reproductive functions of human beings and wildlife. In Japan the EDs issue has arisen since the book *Our Stolen Future* (Colborn *et al.*, 1996) was introduced in 1997. Contamination of water with EDs poses new and potential environmental (and social) problems. The Japan Environmental Agency (JEA) published strategic programmes on environmental endocrine disruptors (SPEED'98), in which basic policies and specific approaches to the problem are documented (JEA, 1998). In this document, the JEA listed 65 chemicals that are suspected of causing abnormalities in animals at extremely low levels. The Ministry of Land, Infrastructure and Transport (MLIT) of Japan decided to clarify the status of EDs' concentration in the water environment, conducting extensive studies of the major rivers and wastewater treatment plants (WWTP) (MLIT, 2001a). Among 65 suspected substances, the MLIT selected 25 compounds for the WWTP studies, based on the annual production of the chemicals and the levels detected in the environment. Nonylphenol (NP) was one of the selected substances in the WWTP studies. The study by the MLIT, thus far, has found NP in wastewater at significant levels (MLIT, 2001a; Tanaka *et al.*, 2003). NP exhibits toxicity to aquatic microorganisms (Comber *et al.*, 1993). In addition, NP tends to have higher oestrogenic potentials than do synthetic, industrial chemicals (Yakou *et al.*, 1999; Tanaka *et al.*,

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2001). Alkylphenol (AP) is known to be a byproduct of alkylphenol polyethoxylate (APnEO) which is used as a detergent in industry (Ahel *et al.*, 1994). Nonylphenol polyethoxylate (NPnEO) is one of the APnEOs. The degradation pathway of NPnEO is considered the same as APnEO. It is important that not only NP but also NPnEO and their related substances are analysed when the behaviour of NP in a wastewater treatment process is surveyed. NPnEO is said to be biodegraded to the shorter ethoxylate (EO) chain NPnEO or nonylphenol carboxylates (NPnEC) under aerobic conditions, and then biodegraded to NP under anaerobic conditions (Ahel *et al.*, 1994). NP is one of the suspected EDs. Moreover, the shorter EO chain NPnEO exhibits toxicity to aquatic microorganisms (Ahel *et al.*, 1994). This paper describes the analytical methods and results of the field study on NP and its related substances in wastewater treatment processes in Japan.

Materials and methods

Analytical method for NP and NPnEO

In this study, NP and NPnEO were analysed based on the HPLC method (JSWA, 2002). Sample preparation for this method consists of solid-phase extraction with a Sep-Pak Plus PS-2 cartridge (for the filtrate), supersonic liquid extraction by acetone (for suspended matter) and cleaning with Sep-Pak Silica. The pretreated (cleaned-up) sample was analysed using a HPLC. A summary of the overall analytical scheme for WWTP influent and effluent is illustrated in Figure 1.

First, a 1,000-mL wastewater sample was filtered through a 1-μm pore size glass fibre filter. Residue on the filter was extracted by supersonic extraction with 10–30 mL of acetone. The acetone extract was concentrated to 5 mL then added to the filtrate. The solution was passed through a Sep-Pak Plus PS-2 cartridge. Flow rate was maintained at 10 mL/min. The Sep-Pak Plus PS-2 cartridge was conditioned with 5 mL of methanol and 10 mL of purified water prior to extraction. The Sep-Pak Plus PS-2 cartridge was dehydrated using a Sep-Pak extraction manifold under vacuum condition for approximately 1 h. Then, NP and NPnEO were eluted from the Sep-Pak Plus PS-2 cartridge with 10 mL

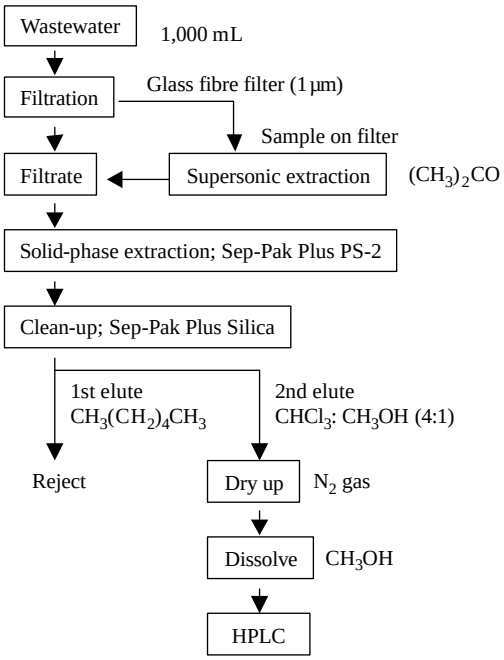

Figure 1 Flow diagram of analytical method for NP and NPnEO

Table 1 Analytical conditions of HPLC

HPLC	Type of HPLC	Waters Alliance 2690
	Column	Inertsil Ph, 4.6 mm ϕ × 150 mm, 40 °C
	Eluent	CH_3OH : H_2O (7:3), 1.0 mL/min
	Detection	Fluorescence detector Excitation 225 nm lambda Emission 300 nm lambda
	Injection volume	20 μL

of methanol. The eluent was blown dry with a gentle stream of nitrogen gas. The dry residue was dissolved in 1 mL of hexane with supersonic extraction and cleaned-up with a Sep-Pak Plus Silica cartridge, which was conditioned with 10 mL of chloroform/methanol (4:1, v/v) and 30 mL of hexane prior to extraction. The cartridge was washed with 10 mL of hexane (1st elute). NP and NPEC were eluted from a Sep-Pak Plus Silica cartridge with 5 mL of chloroform/methanol (4:1, v/v) (2nd elute). The 2nd eluent was collected and concentrated until just dry under a gentle stream of nitrogen gas. The dry residue was dissolved in 1 mL of methanol, which was then analysed by HPLC. Operating conditions of the HPLC are presented in Table 1. Detection limit of the method was 0.1 μg/L. The detection limit was defined as three times the standard deviation (3 s) of the measurements divided by the concentration of the standard solution.

Analytical method for NPnEC

In this study, NPnEC was analysed based on the LC/MS/MS method (Yasojima *et al.*, 2002). Sample preparation for this method consists of solid-phase extraction with a GL-Pak Carbograph cartridge (for the filtrate), and supersonic liquid extraction by acetone (for suspended matter). The pretreated sample was analysed using LC/MS/MS. A summary of the overall analytical scheme for the WWTP influent and effluent is illustrated in Figure 2.

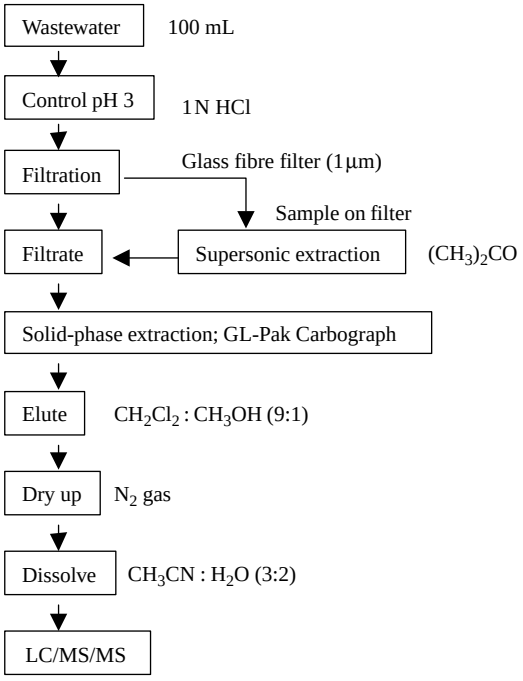

Figure 2 Flow diagram of analytical method for NPnEC

Table 2 Analytical conditions of LC/MS/MS

HPLC	Type of HPLC	Agilent 1100			
	Column	Agilent Zorbax Eclipse XDB-C18, 2.1 mm ϕ × 150 mm, 40 °C			
	Eluent	$CH_3CN{\cdot}H_2O$ (32), 0.3 mL/min			
	Injection volume	10 μL			
MS/MS	Type of MS/MS	TSQ			
	Ionisation	ESI, Negative			
	Collision gas	Argon			
	Measurement ion	Substances	Parent ion	Product ion	Collision energy
		NP1EC	227.2	219.1	20 eV
		NP2EC	321.3	219.1	20 eV
		NP3EC	365.4	219.1	30 eV
		NP4EC	409.6	219.1	30 eV
		NP5EC	453.5	219.1	30 eV
		NP6EC	497.6	219.1	30 eV
		NP7EC	541.7	219.1	40 eV
		NP8EC	585.7	219.1	40 eV
		NP9EC	629.8	219.1	40 eV
		NP10EC	673.8	219.1	40 eV

First, a 100-mL wastewater sample was controlled to pH 3, and filtered through a 1-μm pore size glass fibre filter. Residue on the filter was extracted by supersonic extraction with 10–20 mL of acetone. The acetone extract was concentrated to 1 mL then added to the filtrate. The solution was passed through a GL-Pak Carbograph cartridge. Flow rate was maintained at 10 mL/min. The GL-Pak Carbograph cartridge was cleaned-up with 10 mL of acetonitrile/purified water (3:2, v/v). The GL-Pak Carbograph cartridge was dehydrated using a Sep-Pak extraction manifold under vacuum condition for approximately 1 h. Then, NPnEC was eluted from the GL-Pak Carbograph cartridge with 7 mL of dichloromethane/methanol (9:1, v/v), which contained 25 mM of formic acid. The eluent was blown dry with a gentle stream of nitrogen gas. The dry residue was dissolved in 1 mL of acetonitrile/purified water (3:2, v/v), which was then analysed by LC/MS/MS. Operating conditions of the LC/MS/MS are presented in Table 2. Detection limit of the method was 0.01 μg/L. The detection limit was defined as three times the standard deviation (3 s) of the measurements divided by the concentration of the standard solution.

Field survey of NP, NPnEO and NPnEC in wastewater

The field surveys were conducted at 20 WWTPs where treated and untreated wastewater samples were collected for the analysis of NP, NPnEO and NPnEC. The capacities of these WWTPs range from 12,000 to 680,000 m^3/d. Thirteen of them apply a conventional-activated sludge process. Three WWTPs employ an anaerobic-oxic-activated sludge process (A/O process). Other WWTPs adopted various combined processes: *i.e.*, a conventional-activated sludge process with rapid filtration; a conventional-activated sludge process with rapid filtration and carbon adsorption; or an anaerobic–anoxic–oxic process (A_2/O process) with rapid filtration and step aeration. Grab samples were collected twice at these WWTP sites from April to May 2002 (Survey A) and from February to March 2003 (Survey B). One gram of L-ascorbic acid was added to 1 L of sample to prevent oxidation. All samples were collected in 1-L glass bottles, refrigerated, and transported to the laboratory within 24 h. Concentrations of NP and NPnEO were measured by HPLC. Concentrations of NPECs were measured by LC/MS/MS.

Results and discussion

Field survey results of NP and its related substances in the WWTPs are shown in Tables 3 and 4, and Figure 3. The result of Survey A and Survey B showed a similar trend. The NP concentrations range from 0.5 to 66 μg/L (median, 1.9 μg/L) in the plant influents and from ND to 2.6 μg/L (median, 0.3 μg/L) in the secondary effluents. Regarding the NP-related substances, in the influent, the NPnEO species (from NP1EO to NP15EO) were detected but the NPnEC species were rarely detected. In the secondary effluent, there were hardly any NPnEOs whose EO chain length was more than 5, but the NPnEC species (from NP1EC to NP4EC) were detected. The survey results indicate that the NPnEO species were degraded easily in the WWTPs as the concentrations of NPnEO decreased drastically in the secondary effluent. It is unknown why the values of NP4EO in both the plant influent and the secondary effluent were relatively large. One possible explanation for the accumulation of the short EO chain NPnEO and NPnEC with the largest concentration of NP2EC in the wastewater treatment processes is because of the difference between the degradation rate of NPnEO and that of the short EO chain NPnEO and NPnEC. It can be conceived that the short EO chain NPnEO and NPnEC can be biodegraded under aerobic conditions owing to the fact that their concentrations in the secondary effluent were relatively low in this study. On the other hand, the results indicate that the long EO chain NPnEO was transformed to the short EO chain NPnEO and further to NPnEC because the long EO chain NPnEC was not produced. The concentrations of NP in the secondary effluents were smaller than those in the influents. It was not clear whether NP was biodegraded or removed by adsorption to sludge because NP concentrations on/in sludge were not measured.

Table 3 Concentrations of NP, NPnEO and NPnEC in wastewater samples from 20 WWTPs: Survey A (μg/L)

	Detection limit	Influent					Secondary effluent				
		Min	25%	Median	75%	Max	Min	25%	Median	75%	Max
NP	0.1	0.5	1.0	1.9	3.3	66	ND	0.1	0.3	0.5	2.6
NP1EO		0.8	1.7	2.4	5.2	17	ND	0.2	0.3	0.5	2.0
NP2EO		0.5	1.1	2.5	5.4	11	0.1	0.2	0.3	0.8	2.5
NP3EO		1.1	1.7	3.9	5.3	14	ND	0.1	0.1	0.3	0.8
NP4EO		3.5	6.4	8.2	11.0	21	0.4	0.6	0.9	1.0	1.7
NP5EO		2.0	2.6	5.8	9.7	23	ND	ND	ND	0.1	0.3
NP6EO		2.4	3.4	6.5	11.4	24	ND	ND	ND	ND	0.3
NP7EO		2.4	3.7	7.2	12.1	23	ND	ND	ND	ND	0.1
NP8EO	0.1	2.8	3.8	8.3	13.4	24	ND	ND	ND	ND	ND
NP9EO		2.6	3.8	7.4	12.9	20	ND	ND	ND	ND	ND
NP10EO		2.0	3.6	7.0	10.8	18	ND	ND	ND	ND	ND
NP11EO		1.1	2.7	5.5	8.5	15	ND	ND	ND	ND	ND
NP12EO		0.7	1.7	3.7	6.3	12	ND	ND	ND	ND	ND
NP13EO		0.2	1.3	2.6	5.3	11	ND	ND	ND	ND	ND
NP14EO		ND	0.7	1.8	3.6	6.7	ND	ND	ND	ND	ND
NP15EO		ND	0.3	0.7	1.7	3.4	ND	ND	ND	ND	ND
NP1EC	0.01	0.07	0.10	0.15	0.23	0.78	0.35	0.89	1.4	3.1	6.1
NP2EC		0.11	0.19	0.27	0.39	4.7	0.88	1.7	3.5	5.5	23
NP3EC		0.10	0.14	0.18	0.22	2.5	0.51	0.91	1.4	2.9	8.6
NP4EC		0.09	0.12	0.14	0.18	0.99	0.13	0.23	0.53	0.87	2.5
NP5EC		0.08	0.09	0.14	0.16	0.88	0.07	0.10	0.24	0.45	1.2
NP6EC		0.09	0.12	0.14	0.20	0.48	0.01	0.03	0.05	0.11	0.62
NP7EC		0.05	0.10	0.12	0.17	0.50	0.01	0.01	0.02	0.04	0.31
NP8EC		0.05	0.09	0.11	0.15	0.53	0.01	0.02	0.04	0.06	0.36
NP9EC		0.05	0.08	0.10	0.13	0.46	ND	0.01	0.03	0.08	0.34
NP10EC		0.05	0.08	0.11	0.14	0.45	ND	0.01	0.02	0.04	0.13

ND, below detection limit of 0.1 μg/L (NPnEO) and 0.01 μg/L (NPnEC)

Table 4 Concentrations of NP, NPnEO and NPnEC in wastewater samples from 20 WWTPs: Survey B (μg/L)

	Detection limit	Influent					Secondary effluent				
		Min	25%	Median	75%	Max	Min	25%	Median	75%	Max
NP	0.1	0.3	0.5	0.9	1.9	14	ND	0.1	0.2	0.3	0.9
NP1EO		0.4	0.7	1.2	1.5	7.1	0.2	0.2	0.3	0.5	2.8
NP2EO		0.3	0.7	1.3	2.1	7.7	0.1	0.2	0.4	1.0	4.0
NP3EO		ND	0.7	1.3	2.7	18	ND	ND	0.2	0.4	1.2
NP4EO		2.3	4.7	6.3	11	31	0.7	1.1	1.6	1.7	3.4
NP5EO		1.3	2.7	3.5	6.6	38	ND	ND	ND	0.1	0.2
NP6EO		0.5	2.4	3.1	6.0	46	ND	ND	ND	ND	ND
NP7EO		0.8	3.2	4.1	7.5	52	ND	ND	ND	0.1	0.1
NP8EO	0.1	0.8	3.7	5.1	9.1	61	ND	ND	ND	ND	ND
NP9EO		1.1	3.7	5.3	8.7	57	ND	ND	ND	ND	ND
NP10EO		1.1	3.5	5.6	8.1	51	ND	ND	ND	ND	0.2
NP11EO		0.9	2.9	4.9	6.5	42	ND	ND	ND	ND	0.1
NP12EO		ND	1.0	4.3	6.0	33	ND	ND	ND	ND	0.1
NP13EO		ND	1.1	2.6	4.5	23	ND	ND	ND	ND	0.2
NP14EO		ND	1.0	1.4	2.3	14	ND	ND	ND	ND	0.1
NP15EO		ND	0.5	0.9	1.6	5.6	ND	ND	ND	ND	ND
NP1EC	0.01	0.05	0.13	0.18	0.30	0.87	0.15	0.46	0.97	1.7	3.9
NP2EC		0.07	0.13	0.24	0.60	3.9	0.11	0.58	0.92	2.5	10
NP3EC		0.05	0.08	0.21	0.31	1.8	0.08	0.45	0.75	1.1	4.3
NP4EC		0.07	0.09	0.18	0.29	0.87	0.10	0.28	0.34	0.50	2.0
NP5EC		0.07	0.09	0.18	0.27	0.60	0.04	0.11	0.15	0.21	1.0
NP6EC		0.08	0.11	0.21	0.32	0.61	0.02	0.03	0.06	0.11	0.32
NP7EC		0.07	0.10	0.19	0.30	0.58	ND	0.01	0.01	0.02	0.13
NP8EC		0.06	0.11	0.19	0.28	0.47	ND	0.01	0.01	0.02	0.14
NP9EC		0.06	0.09	0.16	0.24	0.36	ND	ND	0.01	0.02	0.12
NP10EC		0.06	0.09	0.14	0.23	0.33	ND	ND	0.01	0.01	0.11

ND, below detection limit of 0.1 μg/L (NPnEO) and 0.01 μg/L (NPnEC)

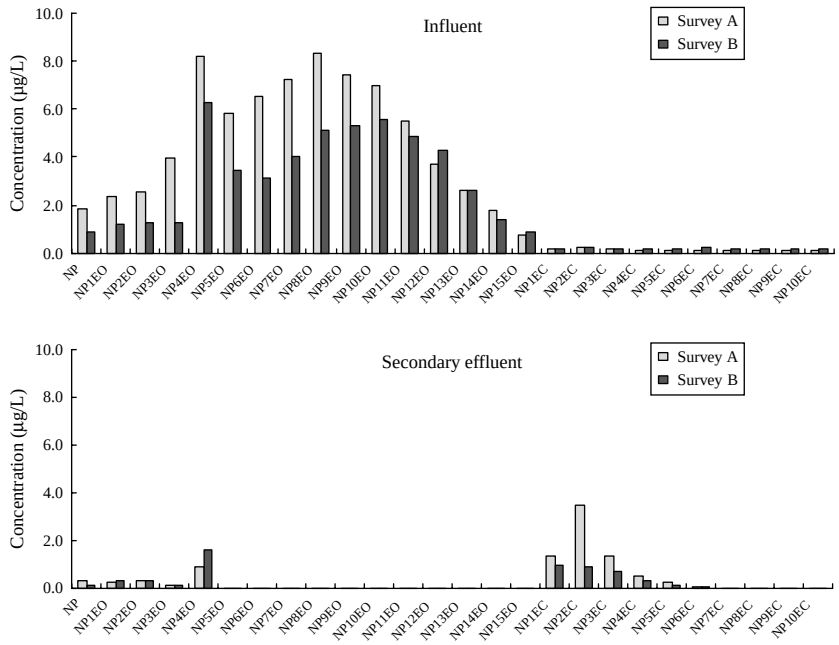

Figure 3 Concentrations of NP, NPnEO and NPnEC in WWTPs

Conclusions

We analysed the concentration of NP, and the selected NPnEO and NPnEC species at 20 WWTPs. The concentrations (median) of NP and its related substances in the WWTPs' influents ranged from 0.1 to 8.3 μg/L, which were the same levels as those previously reported (Brunner *et al.*, 1988; Tanaka *et al.*, 2003). The reduction of the long EO chain NPnEO in the WWTPs was almost complete, while the removal efficiency for the short EO chain NPnEO was less significant than the long EO chain NPnEO, suggesting that the degradation rate of the short EO chain NPnEO was lower than that of the long EO chain NPnEO in the wastewater treatment processes. Also, the short EO chain NPnEC was accumulated in the secondary effluents, indicating that the degradation rates of NPnEO and NPnEC were different in the WWTPs.

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