OCCURRENCE OF ESTROGEN-LIKE SUBSTANCES IN WASTEWATER IN JAPAN

Yuji Okayasu[1], Hiroaki Tanaka[1], Koya Komori[1], Makoto Yasojima[1]

[1]Water Environmental Research Group, Public Works Research Institute,
[1]1-6, Minamihara, Tsukuba-City, Ibaraki, 305-8516
[1] okayasu@pwri.go.jp, htanaka@pwri.go.jp, komori@pwri.go.jp, yasoji44@pwri.go.jp

ABSTRACT

The field study was conducted at twenty WWTPs in Japan about following estrogens: estrone(E1); 17β-estradiol(E2); 17α-ethynylestradiol(EE2); estriol(E3); estrone-3-sulfate(E1 -S); β-estradiol 3-sulfate (E2-S); estriol 3-sulfate (E3-S); estrone β-D-glucuronide(E1-G); β-estradiol 17-(β-D)-glucuronide(E2-G); estriol 3- (β-D)-glucuronide(E3-G); β-estradiol 3-sulfate 17-glucuronide(E2-S&G); and estradiol 3,17-disulfate(E2-diS). The median concentrations of the estrogens ranged from ND to as high as >100 ng/L. In the influent samples, the concentration of E1, E2 and E3 are the same levels as those were previously reported. The conjugated estrogens are higher than those of the free estrogens. The reduction of the free estrogens in WWTPs was good. The concentrations of a few conjugated estrogens (E1-S and E2-S) were declined in the WWTPs, while the levels of other conjugated estrogens (E3-S, E1-G, E2-G, E3-G, E2-S&G and E2-diS) were increased in the WWTPs. Morepver, the other field study was conducted at twelve WWTPs in Japan about following substances: nonylphenol(NP); nonylphenol ethoxylates(NPEOs); and nonylphenoxy acetic acids(NPECs). The concentrations of NP and Long-EO-chain NPEOs were declined in WWTPs while the levels of short –EO-chain NPECs were increased in the WWTPs.

KEYWORDS

Endocrine disruptors, 17β-estradiol, estrone, estrogen, estrogen conjugates, nonylphenol, nonylpehol ethoxylate, nonylphenoxy acetic acid.

INTRODUCTION

In recent years a new problem has emerged in our water environment, namely, endocrine disruptors (EDs) 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 programs 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 more than 70 chemicals that are suspected to cause abnormalities in animals at extremely low levels. The Ministry of Land, Infrastructure and Transport (MLIT) of Japan has decided to grasp EDs conditions in the water environment conducting extensive studies with major rivers and WWTPs (MLIT, 2001a). Among over 70 suspected substances, the MLIT selected 27 compounds for the river studies and 25 substances for the WWTPs studies, based on the annual production of the chemicals and the levels detected in the environment. The MLIT particularly concerned female hormones originating from humans

and animals. The study by the MLIT, thus far, found that estrogen represented by 17β-estradiol (E2) exists in river water and wastewater (including treated wastewater) at significant levels (MLIT, 2001a; Tanaka et al., 2001b, 2003).

Analytical methods currently available for EDs are limited their applications to certain chemicals. The method for the analysis of E2 in the early stage of the MLIT survey had been based on enzyme linked immunosorbent assay (ELISA), which can detect E2 as low as 0.2 ng/L. However, due to the potential "cross-reaction" problem, ELISA is limited in its applications to certain conditions when it is applied to domestic wastewaters. Recently, estrone (E1) has emerged as concerned EDs in water environment (MLIT, 2001b; Goda *et al.*, 2001), and many other estrogen-like chemicals appear to have estrogenic effects on fish. Furthermore, naturally occurring estrogens (e.g., E1 and E2) and nonylphenol (NP) tend to have higher estrogenic potentials than other synthetic, industrial chemicals (Yakou *et al.*, 1999; Tanaka *et al.*, 2001b). Although E2 and 17α-ethynylestradiol (EE2) can be analyzed simultaneously using the GC/MS method (Huang *et al.*, 2001), this method is rather cumbersome requiring a derivatization process. PWRI refined the analytical method developed by Komori *et al.* (2001) for the analysis of specific estrogens (i.e., E2, E1, and EE2) present in wastewater. This method uses a LC/MS/MS, but the derivatization process is not required. Estrogens are excreted by male as well as female animals. Prior to excretion, most estrogens are hydroxylated and conjugated to glucuronides, sulfates, and acetates. Because very few analytical methods (Ternes *et al.*, 1999a; Belfroid *et al.*, 1999) are capable of analyzing estrogenic compounds, relatively little work has been directed toward investigating impacts and occurrence of estrogens in water environment. PWRI refined an analytical procedure (Komori *et al.* 2003) that allows routine analysis of estrogens and their conjugates (i.e., glucuronides and sulfates conjugates) in wastewater based on the method by Komori *et al.* (2002),

On the other hand, nonylphenol (NP) is known to be byproduct of nonylphenol ethoxylates (NPEOs) which are used as detergent for industrial use (Ahel *et al.*, 1994). It is important that not only NP but also NPEOs and their related substances are analyzed when behavior of NP in wastewater treatment process is surveyed. NPEOs are biodegraded to shorter-EO-chain NPEOs or NPECs under aerobic condition, and then biodegraded to NP under anaerobic condition. NP is a suspected endocrine disrupting chemical. Moreover, shorter-EO-chain NPEO has higher toxicity than longer EO chain NPEO (Comber *et al.*, 1993). PWRI developed analytical methods (Yasojima *et al.*, 2002a, 2002b) which can analyze NP, NPEO (EO chain length 1-15) and NPEC (EO chain length 1-10) in wastewater.

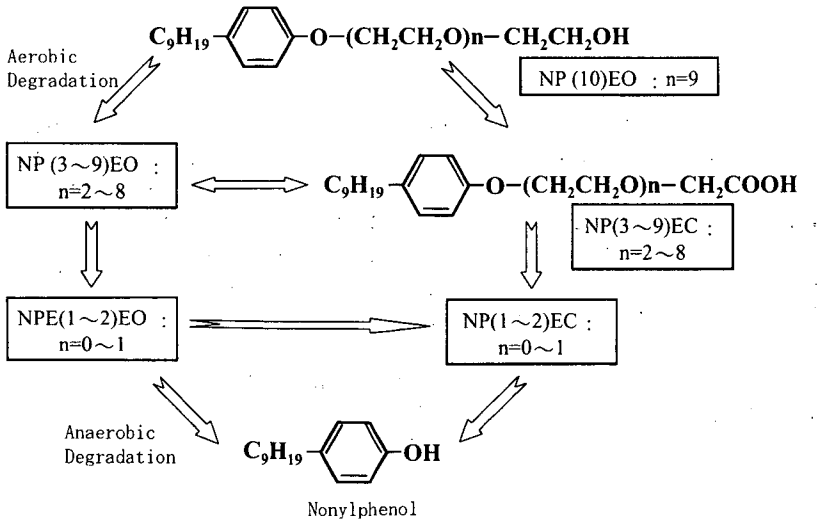

Figure 1 Degradation pathway of NP and its related substances

As mentioned above, result of YES (Yeast Estrogen Screening Test) shows that estrogens (E1, E2), EE2 and NP has high estrogenicity. This paper describes results of field study on estrogen, conjugated estrogens, NP and its related substances in wastewater treatment process.

METHOD

Field survey of estrogens and their conjugates

The field surveys were conducted at twenty WWTPs where influent and secondary effluent were collected for the analysis of estrogens and their conjugates (i.e., E1, E2, EE2, estriol (E3), estrone-3-sulfate (E1-S); β-estradiol 3-sulfate (E2-S); estriol 3-sulfate (E3-S); estrone β-D-glucuronide (E1-G); β-estradiol 17-(β-D)-glucuronide (E2-G); estriol 3-(β-D)-glucuronide (E3-G); β-estradiol 3-sulfate 17-glucuronide (E2-S&G); and estradiol 3,17-disulfate (E2-diS) . The capacities of these WWTPs range from 12,000 to 680,000 m^3/day. Thirteen of them apply a conventional activated sludge process. Three WWTPs employ an anaerobic-oxic activated sludge proceee (A/O process). Other WWTPs adapted various combined process: 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 process. Grab samples were collected at WWTP sites. One gram of L-ascorbic acid was added to 1 litter of sample to prevent oxidation. All samples were collected in one-litter glass bottles, refrigerated, and transported to the laboratory within one day. Concentrations of estrogens and estrogen conjugates were measured by the method of Komori *et al.*,(2003).

Field survey of nonylphenol (NP) and its related substances

12 WWTPs were selected for the survey of nonylphenol and its related substances (i.e., NP, NPnEO (n=1-15), NPnEC (n=1-10)). All the WWTPs applied a conventional activated sludge process. The capacities of these WWTPs range from 17,300 to 168,000 m^3/day. Grab samples were taken from the influent and the secondary effluent. One gram of L-ascorbic acid was added to 1 litter of sample to prevent oxidation. All samples were collected in one-litter glass bottles, refrigerated, and transported to the laboratory within one day. Concentrations of NP and NPEOs were measured by HPLC. HPLC was performed by the method of Komori *et al.*, (2002). Concentrations of NPnECs were measured by LC/MS/MS. LC/MS/MS was performed by the method of Yasojima *et al* (2002b).

RESULT AND DISCUSSION

Field survey of estrogens and their conjugates

The measured concentrations of the target compounds in wastewater are presented in Table 1. In the WWTP influent, we found: 10 - 57 ng/l (median, 24 ng/l) of E1; ND - 21 ng/l (median, 5.7 ng/l) of E2; 27 – 220 ng/l (median, 110 ng/l) of E3; 12 - 170 ng/l (median, 42 ng/l) of E1-S; 26 - 410 ng/l (median, 110 ng/l) of E2-S; 6.5 - 79 ng/l (median, 22 ng/l) of E3-S; ND - 88 ng/l (median, 11 ng/l) of E1-G; 5.3 - 100 ng/l (median, 18 ng/l) of E2-G; 4.1 - 73 ng/l (median, 22 ng/l) of E3-G; 0.8 - 38 ng/l (median, 5.5 ng/l) of E2-S&G; and 21 - 670 ng/l (median, 77 ng/l) of E2-diS. In the secondary effluent, we observed: ND - 180 ng/l (median, 12 ng/l) of E1; ND - 11 ng/l (median, ND) of E2; ND - 5.8 ng/l (median, 1.5 ng/l) of E3; 7.5 - 34 ng/l (median, 13 ng/l) of E1-S; 27 - 94 ng/l (median, 52 ng/l) of E2-S; 37 - 160 ng/l (median, 69 ng/l) of E3-S; 34 - 140 ng/l (median, 74 ng/l) of E1-G; 47 - 210 ng/l (median, 91 ng/l) of E2-G; 37 - 150 ng/l (median, 72 ng/l) of E3-G; 3.7 - 17 ng/l (median, 8.9 ng/l) of E2-S&G; and 160 - 1500 ng/l (median, 360 ng/l) of E2-diS. EE2 was not detected in any of the samples analyzed (including WWTP influent and secondary effluent). The concentrations

of E1, E2, and E3 were the same levels as those reported in the literature (Tanaka *et al.*, 2003; MLIT, 2001b; Huang *et al.*, 2001; Komori et al., 2001; Ternes *et al.*, 1999a and Belfroid et al., 1999). Reductions of E2 and E3 (free, unconjugated estrogens) in the WWTPs were very good having nearly 100% and 98% reductions (calculated using median value), respectively. Reduction of E1 is 47%, which was considerably smaller than those of E2 and E3. This observation is consistent with Ternes *et al.* (1999b) who reported that the degradation rate of E1 is smaller than that of E2. Belfroid *et al.* (1999) reported that hormone-glucuronides exist generally below their detection limits in the effluent of WWTPs. However, the concentrations of the conjugated estrogens that we measured were higher than those of the unconjugated (free) estrogens in spite of the lower recovery ratios. The average concentrations of E1-S and E2-S (conjugated estrogens) were reduced in the WWTPs, but other conjugated estrogens (i.e., E3-S, E1-G, E2-G, E3-G, E2-S&G and E2-diS) were increased. The removal efficiencies of E1-S and E2-S (conjugated estrogens) were approximately 68% and 51%, respectively.

Table 1. Concentrations of Selected Estrogens Detected in Wastewater Samples from Twenty WWTPs (ng/l)

		min	25%	median	75%	max
influent	E1	10	17	24	29	57
	E2	ND (<0.5)	1.9	5.7	8.6	21
	EE2	ND (<1.2)	ND	ND	ND	ND
	E3	27	52	110	130	220
	E1-S	12	21	42	78	170
	E2-S	26	52	110	220	410
	E3-S	6.5	12	22	41	79
	E1-G	ND (<1.3)	5.2	11	24	88
	E2-G	5.3	12	18	31	100
	E3-G	4.1	11	22	38	73
	E2-S&G	0.8	2.2	5.5	12	38
	E2-diS	21	41	77	120	670
effluent	E1	ND (<0.8)	3.1	12	46	180
	E2	ND (<0.5)	ND	ND	ND	11
	EE2	ND (<1.2)	ND	ND	ND	ND
	E3	ND (<1.4)	0.9	1.5	2	5.8
	E1-S	7.5	8.8	13	17	34
	E2-S	27	44	52	56	94
	E3-S	37	56	69	77	160
	E1-G	34	58	74	82	140
	E2-G	47	76	91	110	210
	E3-G	37	55	72	90	150
	E2-S&G	3.7	6.1	8.9	9.6	17
	E2-diS	160	240	360	510	1500

ND : Not detected (less than detection limit)

Field survey of nonylphenol (NP) and its related substances

The measured concentrations of the target compounds in wastewater are presented in. In the secondary effluent, we observed: 0.10-1.0μg/L (median, 0.20μg/L) of NP. Table 2 and Figure 2. In the WWTP influent, we found: 0.50-20μg/L (median, 1.7μg/L) of NP.
Regarding NP related substances, in the WWTP influent, NPEOs from NP1EO to NP15EO (mainly NP6EO-NO8EO) were detected but there were hardly any NPECs. In the secondary effluent, there were hardly any NPEO whose EO chain length is more than 5 and NPECs from NP1EC to NP4EC were detected. These results indicate that reduction of long-chain-NPEOs (EO chain length is more than 5) in STPs were very good but reduction of short-chain- NPEOs were small. Moreover, it was indicated that NPECs were produced in aerobic wastewater treatment process and degradation rate of long-chain-NPEC (EO chain length is more than 5) is large but degradation rate of short-chain NPEC is smaller.

Table 2 Results of NP and its relates substances in WWTPs

		Influent				Secondary Effluent			
		Detection limit	min	median	max	Detection limit	min	median	max
Nonylphenol	NP	0.10	0.50	1.7	20	0.10	0.10	0.20	1.0
Nonylphenol Ethoxylate	NP1EO	0.04	0.82	2.1	17	0.04	0.04	0.26	0.49
	NP2EO	0.04	0.54	2.9	11	0.04	0.16	0.30	1.5
	NP3EO	0.06	1.2	4.5	14	0.06	0.07	0.14	0.81
	NP4EO	0.04	3.5	9.8	21	0.04	0.60	0.98	1.7
	NP5EO	0.05	2.0	7.3	23	0.05	N.D.	N.D.	0.08
	NP6EO	0.07	2.4	7.8	24	0.07	N.D.	N.D.	0.14
	NP7EO	0.05	2.4	7.9	23	0.05	N.D.	N.D.	0.14
	NP8EO	0.08	2.8	7.5	24	0.08	N.D.	N.D.	N.D.
	NP9EO	0.07	2.6	6.2	20	0.07	N.D.	N.D.	N.D.
	NP10EO	0.15	2.0	5.2	18	0.15	N.D.	N.D.	N.D.
	NP11EO	0.07	1.1	3.9	15	0.07	N.D.	N.D.	N.D.
	NP12EO	0.14	0.73	2.7	12	0.14	N.D.	N.D.	N.D.
	NP13EO	0.18	0.23	1.7	11	0.18	N.D.	N.D.	N.D.
	NP14EO	0.16	0.35	1.2	6.7	0.16	N.D.	N.D.	N.D.
	NP15EO	0.23	0.34	1.5	3.4	0.23	N.D.	N.D.	N.D.
Nonylphenoxy Acetic Acid	NP1EC	0.002	0.085	0.15	0.78	0.002	0.35	1.2	3.4
	NP2EC	0.002	0.11	0.36	4.7	0.002	1.1	3.5	10
	NP3EC	0.002	0.098	0.20	2.5	0.002	0.51	1.2	4.4
	NP4EC	0.002	0.086	0.14	0.99	0.002	0.13	0.52	2.5
	NP5EC	0.002	0.088	0.14	0.88	0.002	0.065	0.24	1.2
	NP6EC	0.002	0.11	0.14	0.48	0.002	0.009	0.049	0.62
	NP7EC	0.002	0.054	0.13	0.50	0.002	0.006	0.019	0.31
	NP8EC	0.002	0.052	0.12	0.53	0.002	0.005	0.028	0.27
	NP9EC	0.002	0.055	0.11	0.46	0.002	N.D.	0.028	0.13
	NP10EC	0.002	0.057	0.11	0.45	0.002	N.D.	0.023	0.069

Unit: μg/l N.D.: non-detection

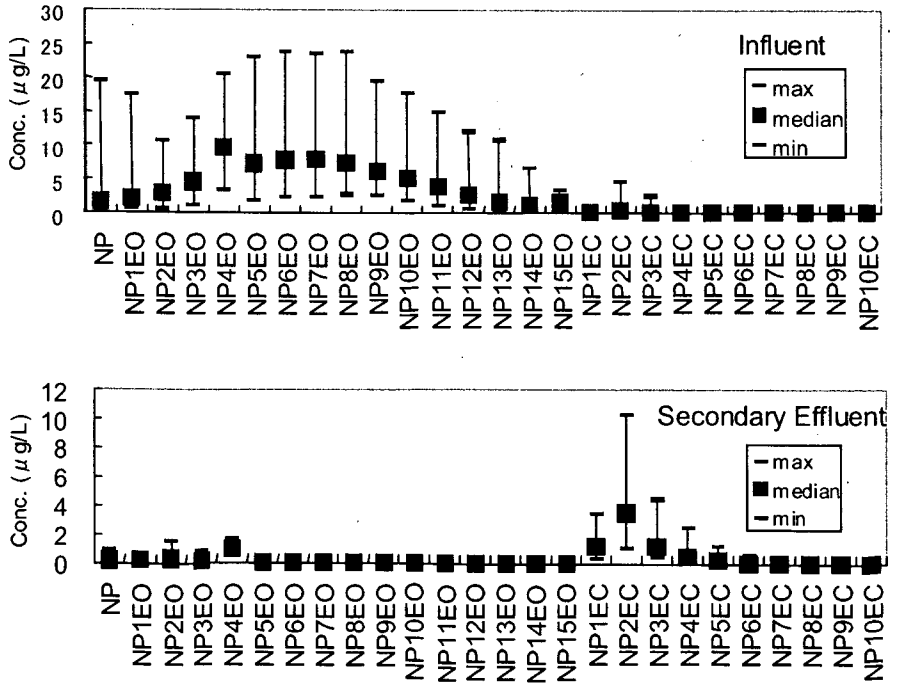

Figure 2. Change of NP and its related substances in WWTPs

In order to understand behavior of target compounds in wastewater treatment process, we focus on A STP of the 12 STPs as an example. The observed concentrations of target compounds in A STP are presented in Figure 3, Figure 4.

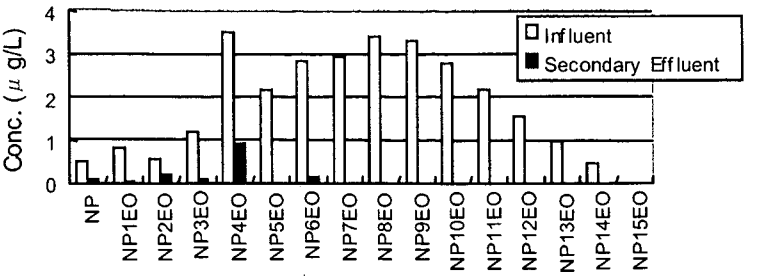

Figure 3 Change of NPEOs in A WWTP

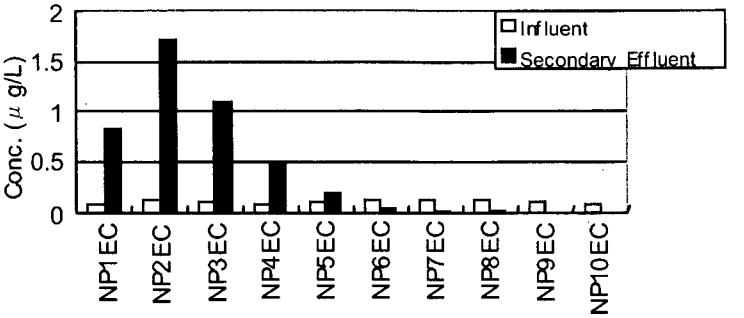

Figure 4 Change of NPECs in A WWTP

Regarding NPEOs, in the WWTP influent, NPEOs from NP1EO to NP14EO were detected and it is indicated that NPEOs were degraded easily in wastewater treatment because concentrations of NPEOs decreased drastically in the secondary effluent. It is unknown why values of NP4EO in both the influent and the secondary effluent were relatively large.
Regarding NPECs, in the WWTP influent, concentrations were below 0.1μg/L, but concentrations of NPECs increased during wastewater treatment. Concentration of NP2EC was largest and NPEC from NP1EC to NP5EC were observed in the secondary effluent. One possible explanation about accumulation of NPECs in wastewater treatment process is difference between degradation rate of NPEO and NPEC. On the other hand, it is indicated that long-EO-chain NPEO changed to short-EO-chain NPEO and further to NPEC because long-EO-chain NPEC were not produced. Concentrations of NP in the secondary effluent were smaller than those in the influent. It was not unclear whether NP was biodegraded or removed by adsorption to sludge because concentrations on/in sludge were not measured.

CONCLUSIONS

1) LC/MS/MS method by Komori *et al.*,(2003) was applied to the wastewater samples collected from twenty WWTPs. The concentrations (median) of estrogens and their conjugates in the WWTP influent range from non-detection (ND) to as high as >100 ng/L. In the influent samples, the concentrations of E1, E2 and E3 were the same levels as those were previous reported. Belfroid *et al.* (1999) reported that hormone-glucuronides exist generally below their detection limits in effluent of WWTPs. However, the concentrations of conjugated estrogens that we measured were higher than those of free estrogens.
2) The reduction of the free estrogens in the WWTPs was very good with approximately 100% and 98% for E2 and E3, respectively, while removal efficiency for E1 (47%) was less significant than E2 and E3, suggesting that the degradation rate of E1 was smaller than that

of E2 in the wastewater treatment processes. The concentrations of the conjugated estrogens (E1-S and E2-S) were declined in the WWTPs, while the levels of other conjugated estrogens (E3-S, E1-G, E2-G, E3-G, E2-S&G and E2-diS) were increased in the WWTPs.
3) HPLC method and LC/MS/MS method by Yasojima et al., (2002a, 2002b) were applied to the wastewater samoles from twelve WWTPs. The concentrations (median) of nonylpheol and its related substances in the WWTP influent range from non-detection (ND) to as high as >20μg/L. In the influent samples, the concentrations of NP were the same levels as those were previous reported.
4) The reduction of the long-EO-chain NPEOs in the WWTPs was very good with approximately 100%, respectively, while removal efficiency for short-EO-chain NPEOs was less significant than long-EO-chain NPEO, suggesting that the degradation rate of short-EO-chain NPEOs were smaller than those of long-EO-chain NPEO in the wastewater treatment processes. The concentrations of the NP were declined in the WWTPs, while the levels of short-EO-chain NPECs were increased in the WWTPs.

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