Occurrence of 17α-ethynylestradiol in wastewater in Japan

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Abstract
This paper describes an analytical procedure for 17α-ethynylestradiol (EE2) in domestic wastewater. The procedure demonstrated in this study is innovative in terms of levels of detection of the EE2. The detection limit of this method was 0.05 ng/l in the original sample. The field survey using this method was conducted at four wastewater treatment plants in Japan. Te results showed actual levels of influent and effluent concentrations of EE2 in sewage. The levels of EE2 measured in the influent were in the range of 0.10 to 0.32 ng/l. In the final effluent, EE2 was only detected in one wastewater sample (0.13 ng/l). It was confirmed that the reduction ratio of EE2 in wastewater treatment plants was more than 50% except one wastewater treatment plant. The behavior of EE2 in treatment plants was also studied. The concentration of EE2 decreased at every plant investigated with the progress of treatment. It was confirmed that EE2 was reduced in the process from the biological reaction tank to final settling tank.

Keywords: behavior; 17α-ethynylestradiol; estrogen; removal; wastewater;

1. INTRODUCTION

In Japan the endocrine disruptor issue has arisen since the book "OUR STOLEN FUTURE" was introduced in 1997 [1]. A new problem has emerged in our water environment, namely, endocrine disruptors that may adversely affect the reproductive functions of human beings and wildlife. Contamination of water with endocrine disruptors poses new and potential environmental (and social) problems. The Japan Environment Agency (JEA) published strategic programs on environmental endocrine disruptors'98 (SPEED'98), in which basic policies and specific approaches to the problem were documented [2]. In this document, the JEA listed more 67 chemicals that were suspected of causing abnormalities in animals at extremely low levels. Furthermore, naturally occurring estrogens (e.g., estrone; E1 and 17β-estradiol; E2) and 17α-ethynylestradiol (EE2), which is the principal component of an oral contraceptive pill, tend to have higher estrogenic potentials than synthetic/industrial chemicals [3]. The Ministry of Land, Infrastructure and Transport (MLIT) was particularly concerned about female hormones originating from humans and animals. The study by the MLIT, thus far, found that estrogen represented by E1 and E2 existed in wastewater (including treated wastewater) at significant levels [4, 5, 6].

However, limited studies were conducted on the occurrence of EE2 in wastewater samples from wastewater treatment plants (WWTPs) in Japan. Tanaka et al. [6], Komori et al. [7], Nakada et al. [8] reported that EE2 existed in wastewater, generally below their detection limits or quantification limit of 0.5 ng/l, 1.2 ng/l, 2 ng/l, respectively. The predicted no effect concentration (PNEC) for estrogens was reported as follows:

3 ng/l for E1, 1 ng/l for E2 and 0.1 ng/l for EE2 [9]. In Japan, the concentration level of E1 and E2 in the WWTPs were already reported [4, 5, 6, 7, 8], but the level of EE2 is still unknown [6, 7, 8] in comparison with PNEC. In 1999, use of a pill was permitted for medical treatments. However, the existence concentration level that compare with PNEC is still unknown in Japan. The objectives of this study are determining occurrence of EE2 in wastewater and its fate in WWTPs after several years of the permission of pills by applying the GC/MS method (detection limit: 0.05 ng/l).

2. MATERIALS AND METHODS

2.1 Analytical method

A novel analysis method of EE2 was proposed to lower their detection limits in wastewater samples [10]. In this study, the analytical method by Ohiwa [10] was refined for the analysis of EE2 in wastewater sample. Sample preparation of this method consists of solid-phase extraction, clean-up and silyl-derivatization. The pretreated sample was analyzed using a GC/MS. A summary of the overall analytical scheme for WWTPs influent and effluent are illustrated in Figure 1.

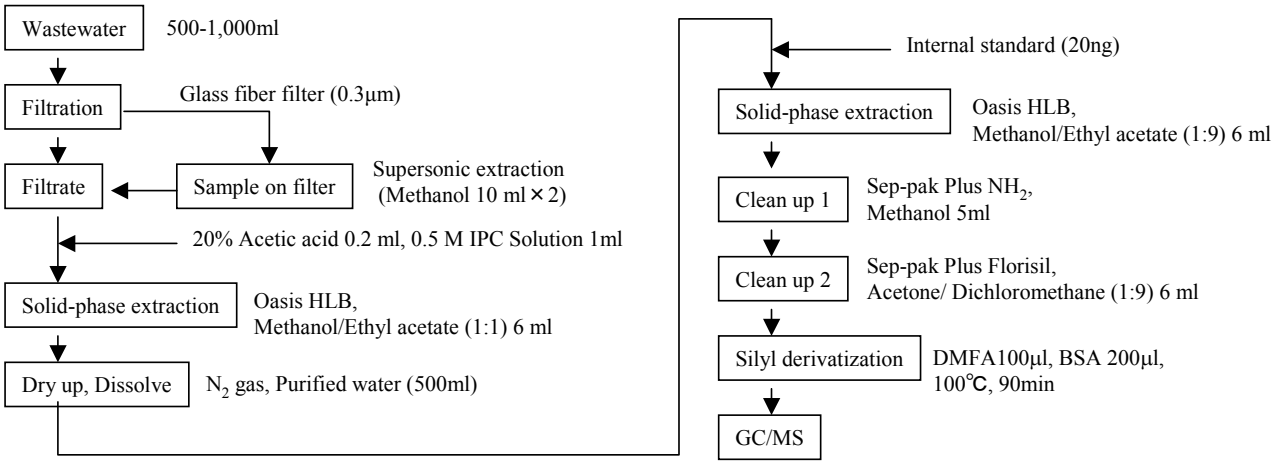

Figure 1 Summary of Analytical procedure

First, a 500-1,000 ml wastewater sample was filtered through a 0.3-μm pore size glass fiber filter. Residue on the filter was extracted by supersonic extraction with 20 ml of methanol. The methanol extract was then added to the filtrate. A volume of 0.2 ml of 20% acetic acid and 1 ml of 0.5 mol/l ion pair coupling (IPC) solution were added. After the mixing, the solution was passed through an Oasis HLB cartridge. Flow rate was maintained at 15 ml/min. The Oasis HLB cartridge was conditioned with methanol and purified water prior to extraction. The Oasis HLB cartridge was centrifuged with a gentle stream of nitrogen gas until it was dried completely. Then EE2 was eluted from the Oasis HLB cartridge with 6 ml of 0.05% NH_3 in methanol/ethyl acetate (1:1, v/v). The eluent was blown down to dryness with a gentle stream of nitrogen gas. The dry residue was dissolved in 500 ml of purified water, into which 20 ng of internal standard [i.e., 17α-ethynylestradiol-2,4,16,16-*d4* (EE2-*d4*)] was added. After the mixing, the solution was passed through another Oasis HLB cartridge, followed by the same procedure until dried completely. Then EE2 was eluted from the Oasis HLB cartridge with 6 ml of methanol/ethyl acetate (1:9, v/v). The eluent was blown down to dryness with a gentle stream of nitrogen gas. The dry residue was dissolved in 1 ml of methanol with supersonic extraction, and cleaned-up with Sep-Pak Plus NH_2 (Clean-up 1). EE2 was eluted from a Sep-Pak Plus NH2 cartridge with 5 ml of methanol. The eluent was collected and concentrated to just dryness under a gentle stream of nitrogen gas. The dry residue was dissolved in 1 ml of hexane/dichloromethane with supersonic extraction, and cleaned-up with a Sep-Pak Plus Florisil (Clean-up 2). The cartridge was washed with 10 ml of hexane/dichloromethane (1:1, v/v). EE2 was eluted with 6 ml of acetone/dichloromethane (1:9, v/v).

The eluent was collected and concentrated to just dryness under a gentle stream of nitrogen gas. The dry residue was dissolved in 100μl of N,N-dimethylformamide (DMFA) and 200 μl of silylation agents [i.e., N,O-bis(trimethylsilyl)acetamide (BSA)] with supersonic extraction. Trimethylsilyl derivatization (TMS) was conducted under 100 degree Celsius condition for 90 minute. The eluent was concentrated to just dryness under a gentle stream of nitrogen gas. The dry residue was dissolved in 2 ml of ethyl ether. The eluent was concentrated to just dryness under a gentle stream of nitrogen gas. The dry residue was dissolved in 100 μl of nonane which was then analyzed by GC/MS.

Operating conditions of the GC/MS are presented in Table 1. Detection limit of the method was 0.05 ng/l in the original sample, which was defined as three times the standard deviation (3σ) of the measurements divided by the concentration of the standard solution. The method using GC/MS was confirmed to be more sensitive than the conventional LC/MS/MS for wastewater samples, because the former method could quantify EE2 in the secondary effluent, but the latter method could not [6, 7].

Table 1 Analytical condition of GC/MS

GC	Type of HPLC	HP6890 Series
	Column	ENV-5MS 0.25mmϕ × 30m
	Column Temp.	150℃ (1min) - (10 ℃/min) - 250 ℃ - (5 ℃/min) - 300 ℃ (5min)
	Carrier gas	Helium, 99,9999%, 100kPa
	Sample size	1 μl
MS	Type of MS	Autospec Ultima
	Ionization	EI mode
	Electron energy	70 eV
	Measurement ion (SIM R=1,000)	EE2 , 425.24
		EE2-*d4* , 429.26

2.2 Pre-field survey of EE2 in wastewater

We conducted pre-field survey of EE2 in treated wastewater (i.e., secondary effluent) at ten WWTPs in winter, for a period from January to February 2005. Capacities of these wastewater treatment plants range from 3,000 to 910,000 m^3/day. Nine WWTPs apply conventional activated sludge process, and the other applys step feed aeration activated sludge process. The pre-field survey results showed actual levels of effluent concentration of EE2 in wastewater in Japan. In the effluent samples, the concentrations of EE2 ranged from ND to 0.28 ng/l (median, 0.07 ng/l).

2.3 Field survey of EE2 in wastewater

In the following survey, we focused on four-selected WWTPs, where a typical treatment plant system, the conventional activated sludge process, is used. Basic information on the studied WWTPs is given in Table 2. The capacities of these WWTPs ranged from 35,200 to 910,000 m^3/day. The surveys were conducted in summer (Aug. and Sep. 2006) and winter (Dec. 2006 and Jan. 2007). At each of these plants, spot samples were collected from each unit process (i.e., influent, primary effluent, secondary effluent and final effluent) at the sampling points shown in Figure 2. One gram of L-ascorbic acid was added to 1 litter of sample to prevent oxidation. These samples were collected in one-litter glass bottles, refrigerated, and transported to the laboratory within one day.

Table 2 Outlines of four wastewater treatment plants (WWTPs)

WWTP	Population served	Capacities (m^3/day)	Aeration period (hr)	Settling time (hr)	MLSS (mg/l)	BOD-SS loading (kg/kg·day)	Return sludge ratio (%)
A	81,000	35,200	7	4	1,300	0.28	69
B	239,000	123,200	5	5	1,700	0.39	55
C	678,000	450,000	9	3	1,400	0.25	52
D	883,000	910,000	8	5	990	0.47	52

From Statistics of Sewerage in Japan, Public Administration Version, 2003

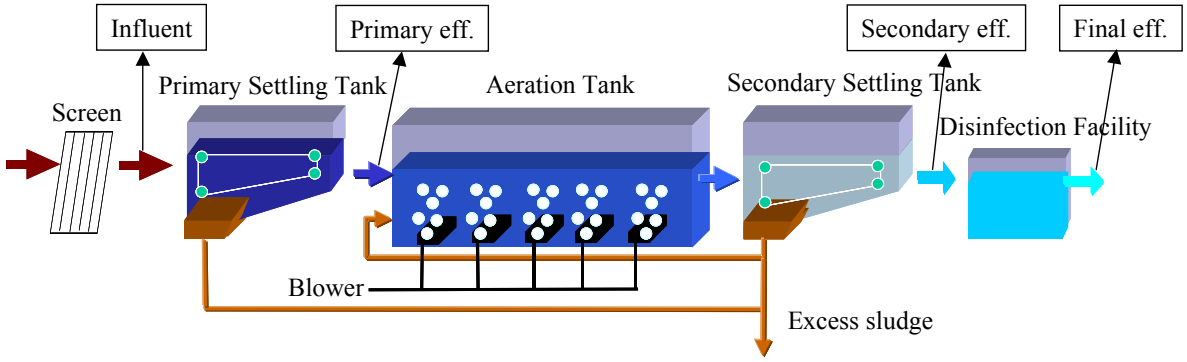

Figure 2 Sampling points in WWTPs

3. RESULTS AND DISCUSSION

3.1 Concentration of sewage influent and effluent (treated wastewater)

Field survey results of EE2 in wastewater are shown in Table 3. The levels of EE2 measured in the influent at the WWTPs investigated were in the range of 0.10 to 0.32 ng/l, and the average was 0.19 ng/l. To the best of our knowledge, no previous study has measured EE2 in the influent in Japan. Previous studies in other countries showed that the EE2 concentrations in the influent were <0.5 - 13 ng/l in Italy [11, 12], 2.5 - 7.1 ng/l in France [13, 14] and 6.2 - 10.1 ng/l in Germany [15]. The EE2 influent concentrations in our research were approximately fifty times lower than those of previous reports. In the secondary effluent and the final effluent, EE2 were only detected in three (0.09 - 0.18 ng/l) and one (0.13 ng/l) samples among eight samples, respectively. Previous studies showed that the EE2 concentrations in the effluent were ND - 1.7 ng/l in Italy [11, 12], 0.14 - 4.5 ng/l in France [13, 14], <1 - 1 ng/l (median) in Germany [15, 16], 9 ng/l (median) in Canada [16], <0.2 - 7.5 ng/l in Netherlands [17] and ND - 7 ng/l in UK [18]. The EE2 effluent concentrations in our research were fifty to hundred times lower than those previous reports. The EE2 levels in wastewater in Japan are not as high as those in the Western countries even though six years has passed since the use of EE2 was permitted in 1999.

Table 3 Survey results of EE2 in wastewater treatment plants (WWTPs)

		A		B		C		D	
		Aug. 2006	Dec. 2006	Aug. 2006	Dec. 2006	Sep. 2006	Jan. 2007	Sep. 2006	Jan. 2007
EE2 (ng/l)	Influent	0.16	0.21	0.11	0.19	0.10	0.32	0.17	0.23
	Primary eff.	ND	0.26	0.08	0.20	0.19	0.21	0.19	0.15
	Secondary eff.	ND	ND	ND	ND	0.09	0.09	ND	0.18
	Final eff.	ND	ND	ND	ND	ND	ND	ND	0.13

3.2 Behavior of EE2 in the wastewater treatment process

Figure 3 shows the behavior of EE2 in the wastewater treatment process in summer and winter surveys. Since the behavior shown here was based on spot samples, it was only approximate. In the summer and winter surveys, the concentration of EE2 decreased with the progress of the treatment. It was also confirmed that EE2 was reduced in the process from the biological reaction tank to final settling tank. The removal of EE2 was smaller in winter (67%) than in summer (83%), probably due to the effect of water temperature on biodegradation. It is essential that more data be accumulated on the behavior of EE2 to enhance the accuracy.

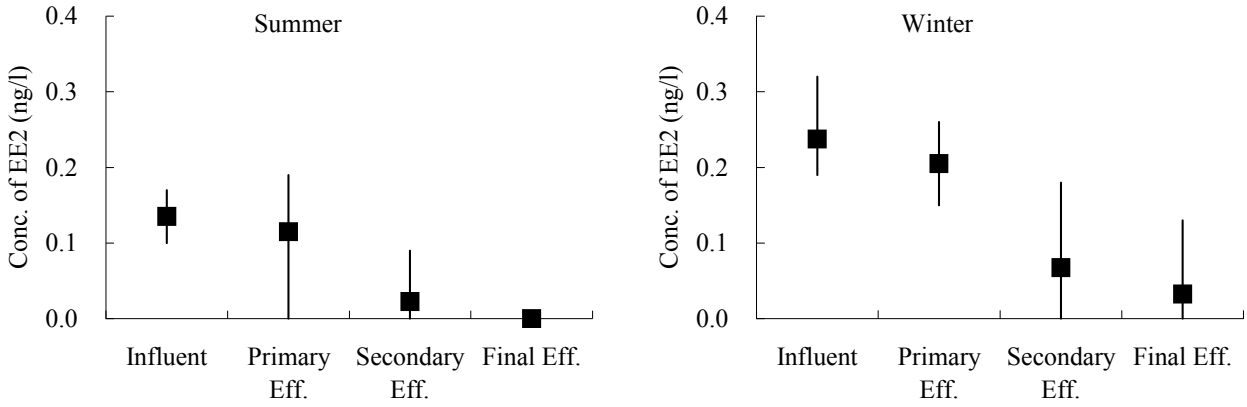

Figure 3 Survey results of EE2 in wastewater treatment plants (WWTPs)

4. CONCLUSION

The surveys were conducted at four wastewater treatment plants for the purpose of determining occurrence of EE2 in wastewater and its fate in WWTPs after several years of the permission of the use of the pills by applying the GC/MS method (detection limit: 0.05 ng/l). Findings thus obtained can be summarized as follows:

(1) The levels of EE2 measured in the influent were in the range of 0.10 to 0.32 ng/l, and the average was 0.19 ng/l. In the final effluent, EE2 were only detected in one WWTP sample (0.13 ng/l). The EE2 levels in wastewater in Japan are not as high as in the Western countries.

(2) Reduction ratio of EE2 in wastewater treatment plants was more than 50% except one wastewater treatment plant.

(3) The concentration of EE2 decreased at every plant investigated with the progress of the treatment. It was confirmed that EE2 was reduced in the process from the biological reaction tank to final settling tank.

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