

## Concentrations of Benzotriazole UV Stabilizers and Polycyclic Musks in Wastewater Treatment Plant Samples in Japan

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**Abstract**—Benzotriazole UV stabilizers (BUVSs) and polycyclic musks were analyzed in influent, effluent, and sewage sludge samples collected from five wastewater treatment plants (WWTPs) in Japan. BUVSs were detected in most samples analyzed, and UV-326 was the dominant compound in influents (mean: 46 ng/L), followed by UV-328 (34 ng/L). UV-327 was detected in two influents, and UV-320 did not identify in any samples. These results imply a large amount of production and usage of UV-326 compared with other BUVSs in Japan. The high concentrations of polycyclic musks were determined in influents, at mean levels of 1,100 ng/L for HHCB and 450 ng/L for AHTN. The concentrations of BUVSs in the effluents were generally low (<5 ng/L), suggesting an elimination of these compounds during wastewater treatment processes. The removal rates of UV-326 and UV-328 were more than 90% in the effluents, but high concentrations of BUVSs were detected in sewage sludge samples of WWTPs, at the levels of 1,100, 170, and 510 ng/g (dry wt.) for UV-326, UV-327, and UV-328, respectively. This indicates an adsorption of BUVSs with organic carbon in sewage sludge. The mean concentrations of HHCB and AHTN in sludge samples were 21,000 and 7,700 ng/g (dry wt.), which are one to two orders of magnitude higher than those of BUVSs. This study implies that WWTP may be a potential source of BUVSs and polycyclic musks into the aquatic ecosystems. Investigations on mass balance and inventory analysis are needed in order to understand the source and pathway of these compounds in the environment.

**Keywords:** benzotriazole UV stabilizers (BUVSs), polycyclic musks, wastewater treatment plant (WWTP)

### INTRODUCTION

The benzotriazole UV stabilizers (BUVSs) are used in a variety of plastics, such as building, automobile and household materials to prevent yellowing and degradation of the products. In Japan, 2-(3-*t*-butyl-2-hydroxy-5-methylphenyl)-5-chlorobenzotriazole (UV-326, CAS#: 3896-11-5), 2,4-di-*t*-butyl-6-(5-chloro2*H*-benzotriazol-2-yl) phenol (UV-327; 3864-99-1), and 2-(2*H*-benzotriazol-2yl)-4,6-di-*t*-pentylphenol (UV-328; 25973-55-1) have been registered in the market (Fig. 1). The annual production and import of UV-327 were 2,310 tons during

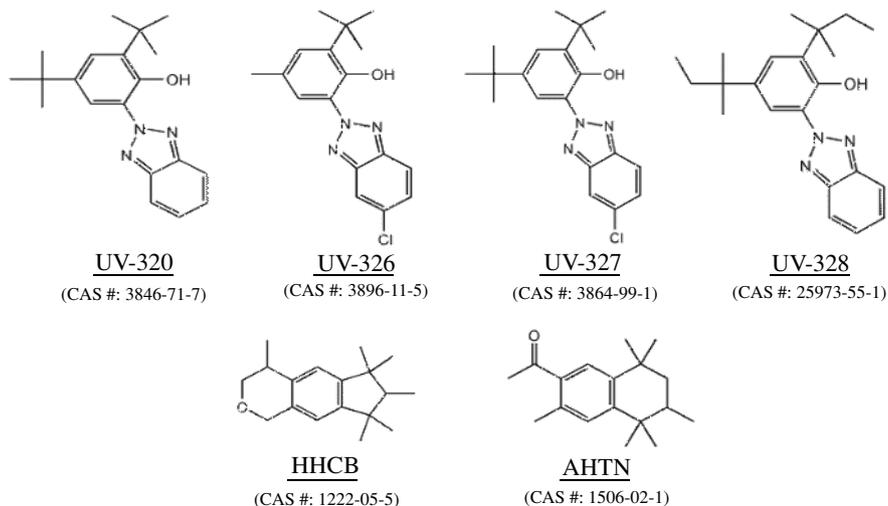


Fig. 1. Benzotriazole UV stabilizers and polycyclic musks analyzed in this study.

April 2004 to March 2008 (Ministry of Economy, Trade and Industry, Japan, 2005, 2006a, 2007, 2008).

It has reported that BUVSs are identified in various environmental matrices in the aquatic ecosystems. The severe contamination of BUVSs was found in river water and sediment proximal to chemical manufacturing plant in Rhode Island, US (Jungclaus *et al.*, 1978; Lopez-Avila and Hites, 1980). The highest concentrations of UV-327 and UV-328 in river sediments were 100 and 300  $\mu\text{g/g}$ , respectively. Recently, the occurrence of BUVSs has been reported in marine organisms collected from Japanese coastal water. The concentrations of BUVSs in benthic species, such as clams, oysters, and gastropods are in the order of several hundreds of  $\text{ng/g}$  on a lipid weight basis (Nakata *et al.*, 2009a). The higher trophic species, such as sharks, rays, and mallards, accumulate BUVSs, which indicates their persistent and bioaccumulative profiles in a marine food-chain. BUVSs were analyzed in blue and green mussels from 10 Asian countries and region. UV-326, UV-327, and UV-328 were detected in most samples analyzed, indicating a widespread contamination of BUVSs in Asian coastal waters (Fig. 2; Nakata *et al.*, 2009b). Temporal trends of BUVSs were examined in marine mammal tissues collected from Japanese coastal waters during past three decades. These archived samples have stored in the Environmental Specimen Bank (*es-BANK*) at Ehime University, Japan. The concentrations of BUVSs in marine mammals have been increasing since the 1990s, which strongly suggest a continuous input of BUVSs into the marine environment (Shinohara *et al.*, 2009). However, there is little information about the source identification of BUVSs into the marine ecosystems.

Regarding to polycyclic musks, HHCB (CAS#: 1222-05-5) and AHTN (CAS#:

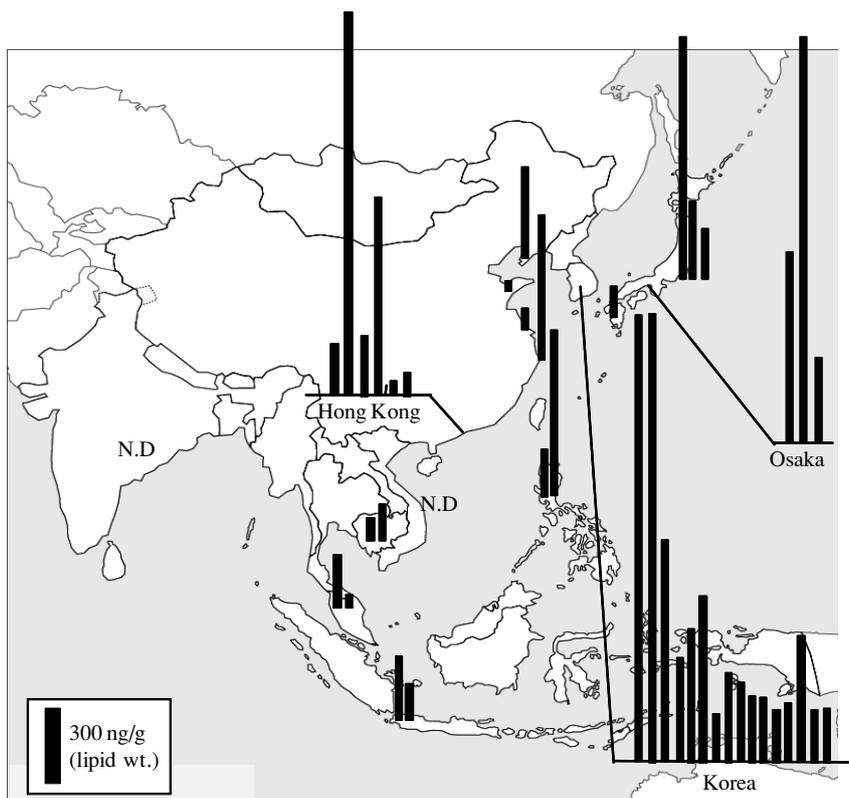


Fig. 2. Geographical distributions of UVSs concentrations in mussels from Asian coastal waters (Nakata *et al.*, 2009b). Mussel samples were kindly donated by the Environment Specimen Bank (*es-BANK*) in Ehime University, Japan.

1506-02-1) have been used in cosmetics and personal care products, such as perfume, body lotion, shampoo, and deodorant (Fig. 1). However, it has been reported that HHCb accumulates in higher trophic organisms of the marine ecosystems (Nakata, 2005). Temporal trends of HHCb concentrations in marine mammals suggested a continuous contamination of this compound during past 20 years (Nakata *et al.*, 2007).

Wastewater treatment plant (WWTP) has been considered as a major source of anthropogenic chemicals in the aquatic environment. The high concentrations of polycyclic musks were detected in influent, effluent and sewage sludge samples of WWTP, which indicate the presence of significant source of these compounds (Fookien, 2004). However, little data is available on the occurrence and concentrations of UVSs in the WWTP matrices. Based on these backgrounds, we analyzed UVSs, such as UV-320 (2-(3,5-di-*t*-butyl-2-hydroxyphenyl)-benzotriazole; CAS#: 3846-71-7), UV-326, UV-327, UV-328 and polycyclic

musks, HHCB and AHTN in influent effluent, and sewage sludge samples collected from five WWTPs in Japan, in order to identify the possible source of these compounds in the aquatic ecosystems.

## MATERIALS AND METHODS

### *Samples*

Influent, effluent and sludge samples were collected from five WWTPs, which named East, South, West, North, and Central, located in a town (population: 680,000) in Japan during May and October, 2009. The wastewater flows are 140000, 29300, 9300, 53300, and 63200 m<sup>3</sup>/day in East, South, West, North, Central WWTPs, respectively. The treatment process of wastewater is controlled by the activated sludge method in all WWTPs. Influent samples were collected in East WWTP at 9:00, 12:00, 15:00, 18:00 and 21:00 ( $n = 5$ ), to understand a time-dependent variation of BUVSs concentrations. Influent and effluent samples were also obtained from South, West, North, and Central WWTPs ( $n = 1$ /sample), to calculate removal rates of BUVSs. Two sewage sludge samples were also collected from five WWTPs ( $n = 10$ ). All samples were stored at  $-20^{\circ}\text{C}$  until chemical analysis.

### *Analytical method*

Influent samples (500 to 1,000 ml) of WWTPs were glass fiber filtered and spiked with a surrogate standard, deuterated phenanthrene. Effluents (2,000 to 2,500 ml) were not filtered, due to small amounts of suspended particles in samples. Liquid-liquid extraction was performed by using a one liter glass separatory funnel which included 500 ml of wastewater samples and 100 ml of hexane. The funnel was shaken for 15 min and hexane layer was collected in a clean glass flask. The influent was extracted with hexane again, and the extracts were combined and concentrated to approximately 5 ml by using rotary evaporator. The sample extract was passed through a glass column packed with 5 g of 5% deactivated silica gel (Wakogel C-200, Wako Pure Chemical Co. Ltd., Japan) for cleanup. The eluate was concentrated to 200  $\mu\text{l}$  and injected into a gas chromatograph interfaced with mass spectrometer (GC-MSD, Agilent 6890 and 5973 series). The GC column used was a HP-5MS fused silica capillary column (30 m  $\times$  0.25 mm id, 0.25 mm film thickness; Agilent Technologies). The ions were monitored at  $m/z$  308, 323, 252 for UV-320,  $m/z$  300, 315, 272 for UV-326,  $m/z$  342, 344, 357 for UV-327,  $m/z$  322, 351, 336 for UV-328,  $m/z$  243, 258, 214 for HHCB,  $m/z$  243, 258, 159 for AHTN, and  $m/z$  188 and 94 for  $d_{10}$ -phenanthrene. The details of the analytical conditions of GC-MS have been described previously (Nakata *et al.*, 2009a).

Sludge samples were freeze-dried, and approximately 2 g of dried samples were extracted by using a Soxhlet apparatus with a mixture of dichloromethane and hexane (8:1) for 6 hours. The extract was concentrated and it was passed through a gel permeation chromatography (GPC) column packed with a Biobeads

S-X3 (Bio-Rad Laboratories, heracules, CA). The eluate was cleaned by silica-gel column and it was injected into the GC-MSD.

### Quality control

A standard mixture containing all BUVSs and polycyclic musks analyzed was used to determine the recovery rates of the compounds through the whole analytical procedure. Three replicate analyses were performed, and the average recoveries of UV-320, UV-326, UV-327, UV-328, HHCB and AHTN were  $115 \pm 9.3$ ,  $115 \pm 13$ ,  $107 \pm 15$ ,  $98 \pm 11$ ,  $100 \pm 0.4$ ,  $92 \pm 1.6\%$ , respectively. The recoveries of surrogate standard, *d*-phenanthrene, were  $86 \pm 11\%$  in this study. A procedural blank was analyzed with every set of six samples to confirm interfering peaks in chromatograms and to correct sample values. The concentrations were reported as below the limit of detection, if the peak height was not greater than a signal to noise ratio of 3 times the blank values. The detection limits of BUVSs and musks in liquid samples ranged 2.1 to 8.7 ng/L in this study.

## RESULTS AND DISCUSSION

### *Levels and time-dependent variation of BUVSs and musks concentrations in influents*

BUVSs and polycyclic musks were detected in all influents collected from East WWTP at every three hours during 9:00 and 21:00 (Table 1). Among the BUVSs, UV-326 showed the highest concentration in influents (mean  $\pm$  sd:  $24 \pm 3.7$  ng/L), followed by UV-328 ( $18 \pm 3.9$  ng/L) and UV-327 ( $12 \pm 5.6$  ng/L). This implies a large production and use of UV-326 compared with UV-327 in Japan, although no statistical data is available on the domestic production of UV-326. UV-320 was not detected in any samples because the domestic production and use has been banned since 2007 (Ministry of Economy, Trade and Industry, Japan, 2006b). In case of polycyclic musks, HHCB showed the highest concentrations, ranging from 850 to 1,500 ng/L in influents of East WWTP, followed by AHTN ( $600 \pm 190$  ng/L). While the musks concentrations were approximately 1 to 2 orders of magnitude higher than those of BUVSs in influents, these levels were generally lower than those found in China (1,250 to 3,000 ng/L; Zhou *et al.*, 2009), Korea (2,560 to 4,520 ng/L; Lee *et al.*, 2010), and US (1,780 to 12,700 ng/L).

Table 1. Concentrations (ng/L) of BUVSs and polycyclic musks in influents of East WWTP.

Time of sampling	9:00	12:00	15:00	18:00	21:00	average $\pm$ sd
UV-326	26	24	23	19	28	$24 \pm 3.7$
UV-327	17	11	10	20	5.6	$12 \pm 5.6$
UV-328	23	20	17	14	15	$18 \pm 3.9$
HHCB	1100	970	910	850	1500	$1100 \pm 260$
AHTN	600	520	490	460	930	$600 \pm 190$

Table 2. Concentrations of BUVs and polycyclic musks in influent, effluent (ng/L) and sewage sludge (ng/g dry wt.) samples collected from five WWTPs in western Japan.

Sampling site	East						South						West					
	Influent		Effluent		Sludge		Influent		Effluent		Sludge		Influent		Effluent		Sludge	
	5	1	1	1	2	2	1	1	1	1	1	2	1	1	1	1	2	
UV-326	24	4.1	59	3.0	900	1400	78	3.1	1800									
UV-327	12	<8.7	<8.7	<8.7	120	160	<8.7	<8.7	200									
UV-328	18	2.1	46	2.5	570	540	52	2.9	570									
HHCB	1100	243	1000	72	20000	23000	1100	130	27000									
AHTN	600	120	400	32	8500	7900	450	49	8600									

Sampling site	North						Central						Average ± sd					
	Influent		Effluent		Sludge		Influent		Effluent		Sludge		Influent		Effluent		Sludge*	
	1	1	1	1	2	2	1	1	1	1	2	9	5	5	10			
UV-326	34	4.5	35	3.4	770	760	46 ± 22	3.6 ± 0.65	1,100 ± 460									
UV-327	9.2	<8.7	<8.7	<8.7	200	180	NA	NA	170 ± 33									
UV-328	31	2.8	23	2.8	430	450	34 ± 15	2.6 ± 0.32	510 ± 67									
HHCB	1400	660	780	67	20000	14000	1100 ± 220	230 ± 250	21,000 ± 4,800									
AHTN	520	250	280	27	7800	5700	450 ± 120	96 ± 94	7,700 ± 1,200									

\*Mean and sd of carbon % in sewage sludges were 31 ± 2.2%.

NA: Not available.

L; Reiner *et al.*, 2007).

BUVSs concentrations in influents of East WWTP were less variable; UV-326 concentrations were 26, 24, 23, 19, 28 ng/L at the sampling time of 9:00, 12:00, 15:00, 18:00, and 21:00, respectively (Table 1). Similarly, little time-dependent differences were found in UV-327 and UV-328 concentrations in influents of East WWTP. The high concentrations of HHCB and AHTN were detected in samples at 21:00 (1,500 ng/L for HHCB, 930 ng/L for AHTN), but no significant difference in the concentrations was found in influents collected between 9:00 and 18:00. The less variation in BUVSs and synthetic musks concentrations in influents imply that a comparison of these concentrations are possible in wastewater samples collected during daytime, between 9:00 and 18:00.

#### *BUVSs and musks in influents, effluents and sewage sludge samples*

The concentrations of BUVSs and musks in influents, effluents and sludge samples collected from five WWTPs were shown in Table 2. The mean concentrations of UV-326, 327 and 328 in influents were 46, 11, and 34 ng/L, respectively, which were 1 to 2 orders of magnitude lower than those of HHCB (mean: 1,100 ng/L) and AHTN (450 ng/L). On the other hand, UV-326 and UV-328 concentrations in effluents were significantly lower than those in influents for all WWTPs (Table 2). HHCB and AHTN concentrations in effluents were also lower than those in influents. These results suggest an elimination of these compounds during wastewater treatment processes. Because total volumes of influents and effluents were similar in all WWTPs, the removal rates (RR) of BUVSs and musks in the WWTPs were calculated as a following equation:

$$\text{RR (Removal rate; \%)} = (C_{\text{influent}} - C_{\text{effluent}}) / C_{\text{influent}} \times 100$$

where  $C_{\text{influent}}$  and  $C_{\text{effluent}}$  are the concentrations of compounds in influent and effluent of WWTPs, respectively.

The mean RR of BUVSs showed high and constant, at the values of  $90 \pm 5.5\%$  for UV-326 and  $91 \pm 3.2\%$  for UV-328. Similar results were found in HHCB and AHTN; more than 80% for both compounds in all WWTPs, except for the North facility. The significant reductions of BUVSs concentrations in effluents may imply their adsorption with organic carbon in sewage sludge. The mean carbon contents of sewage sludges were  $31 \pm 2.2\%$  (Table 2), and significant correlations between BUVSs concentrations and organic carbon contents were found in sediments collected from Japanese coastal water (Nakata *et al.*, 2009a).

BUVSs and polycyclic musks were detected in all sewage sludge samples. The mean concentrations of UV-326, UV-327, and UV-328 were 1,100, 170, and 510 ng/g (dry wt.; Table 2), respectively. HHCB and AHTN concentrations in sludges were extremely high, 21,000 ng/g for HHCB and 7,700 ng/g for AHTN. The partitioning coefficients ( $K_p$ ) were calculated as a moisture content of 80% in sludges, and the values are  $7,200 \pm 3,900$  L/kg for UV-326 and  $4,200 \pm 970$  L/

kg for UV-328. These values were higher than those of HHCb ( $3,200 \pm 2,600$  L/kg) and AHTN ( $2,600 \pm 1,900$  L/kg), which may imply strong binding affinity of BUVSs with organic materials rather than polycyclic musks. While little information is available on adsorption efficiencies of BUVSs,  $K_p$  of BUVSs obtained in this study may be important to understand the concentrations and distribution of these compounds in both WWTPs and in the aquatic environment.

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## REFERENCES

- Fookien, C. (2004): Synthetic musks in suspended particulate matter (SPM), sediment and sewage sludge. p. 29–47. In *Synthetic Musk Fragrances in the Environment*, ed. by G. G. Rimkus, Springer, Berlin.
- Jungclaus, G. A., V. Lopez-Avilla and R. Hites (1978): Organic compounds in an industrial wastewater: A case study of their environmental impact. *Environ. Sci. Technol.*, **12**, 88–96.
- Lee, I.-S., S.-H. Lee and J.-E. Oh (2010): Occurrence and fate of synthetic musk compounds in water environment. *Wat. Res.*, **44**, 214–222.
- Lopez-Avila, V. and R. Hites (1980): Organic compounds in an industrial wastewater: Their transport into sediments. *Environ. Sci. Technol.*, **14**, 1382–1390.
- Ministry of Economy, Trade and Industry, Japan (2005): Available online at [http://www.meti.go.jp/policy/chemical\\_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20051108.pdf](http://www.meti.go.jp/policy/chemical_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20051108.pdf) (access on March 18, 2009).
- Ministry of Economy, Trade and Industry, Japan (2006a): Available online at [http://www.meti.go.jp/policy/chemical\\_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20061010.pdf](http://www.meti.go.jp/policy/chemical_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20061010.pdf) (access on March 18, 2009).
- Ministry of Economy, Trade and Industry, Japan (2006b): 2-(3,5-di-*t*-butyl-2-hydroxyphenyl)-benzotriazole. Available online at [www.meti.go.jp/committee/materials/downloadfiles/g60705a03j.pdf](http://www.meti.go.jp/committee/materials/downloadfiles/g60705a03j.pdf) (access on March 18, 2009).
- Ministry of Economy, Trade and Industry, Japan (2007): Available online at [http://www.meti.go.jp/policy/chemical\\_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20071004.pdf](http://www.meti.go.jp/policy/chemical_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20071004.pdf) (access on March 18, 2009).
- Ministry of Economy, Trade and Industry, Japan (2008): Available online at [http://www.meti.go.jp/policy/chemical\\_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20081031.pdf](http://www.meti.go.jp/policy/chemical_management/kasinhou/kanshikagakubusshitsu/kanpo-1-20081031.pdf) (access on March 18, 2009).
- Nakata, H. (2005): Occurrence of synthetic musk fragrances in marine mammals and sharks from Japanese coastal waters. *Environ. Sci. Technol.*, **39**, 3430–3434.
- Nakata, H., H. Sasaki, A. Takemura, M. Yoshioka, S. Tanabe and K. Kannan (2007): Bioaccumulation, temporal trend, and geographical distribution of synthetic musks in the marine environment. *Environ. Sci. Technol.*, **41**, 2216–2222.
- Nakata, H., S. Murata and J. Filatreau (2009a): Occurrence and concentrations of benzotriazole UV stabilizers in marine organisms and sediments from the Ariake Sea, Japan. *Environ. Sci. Technol.*, **43**, 6920–6926.
- Nakata, H., S. Murata, R. Shinohara, J. Filatreau, T. Isobe, S. Takahashi and S. Tanabe (2009b): Occurrence and concentrations of persistent personal care products, organic UV filters, in the marine environment. p. 239–246. In *Interdisciplinary Studies on Environmental Chemistry—Environmental Research in Asia for Establishing a Scientist's Network*, ed. by Y. Obayashi, T.

- Isobe, A. Subramanian, S. Suzuki and S. Tanabe, TERRAPUB, Tokyo.
- Reiner, J., J. D. Berset and K. Kannan (2007): Mass flow of polycyclic musks in two wastewater treatment plants. *Arch. Environ. Contam. Toxicol.*, **52**, 451–457.
- Shinohara, R., H. Nakata, S. Murata, T. Isobe, S. Takahashi and S. Tanabe (2009): Temporal trends and seasonal variation of organic UV filters in coastal waters of Japan. *Abstract of 18th Symposium on Environmental Chemistry*, p. 354–355.
- Zhou, H., X. Huang, M. Gao, X. Wang and X. Wen (2009): Distribution and elimination of polycyclic musks in three sewage treatment plants of Beijing, China. *J. Environ. Sci.*, **21**, 561–567.

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