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Bisphenol A in the Surface Water and Freshwater Snail Collected from Rivers Around a Secure Landfill

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Bisphenol A (BPA; 4,4'-isopropylidenediphenol; CAS Registry No. 80-05-7) is widely used as a material for the production of epoxy resins and polycarbonates (Steinmetz et al., 1997; Staples et al. 1998; Kang et al., 2003). It is known as one of endocrine disruptors (Steinmetz et al., 1997) and has an acute toxicity to aquatic organisms in the range of 1 to 10 mg/L for freshwater and marine species (Alexander et al., 1988). The solubility of BPA in water ranged from 120 to 300 μg/ml (Staples et al., 1998). The BPA levels in river water were 8 ng/ml or less except one river water (21 ng/ml) (Belfroid et al., 2002) according to several studies of America (Staples et al., 2000), Germen (Bolz et al., 2001; Heemken et al., 2001; Fromme et al., 2002), Japan (Matsumoto et al., 1977; Matsumoto, 1982), and the Netherlands (Hendriks et al., 1994; Belfroid et al., 2002).

BPA is degraded by bacteria distributed in river water under aerobic conditions (Dorn et al., 1987; Klečka et al., 2001; Kang and Kondo, 2002a, b), but not under anaerobic conditions (Kang and Kondo, 2002a). Half-lives of BPA for surface water were 2.5 to 4 days in a study by Dorn et al. (1987), 3 to 6 days by Klečka et al. (2001), and 2 to 3 days by Kang and Kondo (2002a). These different results of half-lives for BPA biodegradation may be caused by the difference of temperature and bacterial counts (Kang and Kondo, 2002b). Although the significant amount (>90%) of BPA is removed during wastewater treatment, BPA from effluent samples of wastewater treatment plants was detected (Staples et al., 1998; Fürhacker et al., 2000; Körner et al., 2000). These wastewaters containing BPA have been known as the main source of BPA contamination in aquatic environment. The secure landfill is used to dispose hazardous waste, and the leachate from waste is controlled and treated before being discharged. Recently, high levels of BPA were identified from leachates of waste landfill (Yamada et al., 1999; Yamamoto et al., 2001; Behnisch et al., 2001; Filho et al., 2003) of secure landfill. Yamamoto et al. (2001) reported that the levels of BPA in the leachates of hazardous waste landfill ranged from 1.3 to 17,200 ng/ml (average 269 ng/ml).

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However, since these leachates are discharged following treatment, the concentration of BPA in effluent is considerably decreased. For example, Yamada et al. (1999) found that the levels of BPA in four landfill leachates ranged from 15 to 5,400 ng/ml, but were from 0.5 to 5.1 ng/ml in effluents after treatment. The present study investigated the levels of BPA in surface water and freshwater snail *Semisulcospira libertina libertine* taken from rivers around a secure landfill.

MATERIALS AND METHODS

The samples (surface river water and freshwater snail) were collected from two rivers (the Kogawauchi and the Kiyotake River) at 0.5, 1. 2 and 5 km from the effluent site of secure landfills. The Kogawauchi River is a tributary of Kiyotake River. About a 1 L sample was gathered in the sterile glass bottle and kept at <4˚C (no freezing). BPA analysis from river water sample was performed within 12 hours after sampling. The freshwater snails in the sterile glass were stored at <4˚C (no freezing). After transporting to the lab, the freshwater snails were immediately homogenized and the homogenats kept at -20˚C. The freshwater snails and surface water for blank samples were collected from the upstream of the Kaeda River in Miyazaki city. Since upstream of the Kaeda River is an area designated for the protection of nature, there are no landfills, industrial, plants, or massed areas.

Bisphenol A (BPA) (>99%) was purchased from Nacalai Tesque (Kyoto, Japan). Sep-Pak Florisil (900 mg) and OASIS HLB cartridge (60 mg) were obtained from Waters (Millipore, Milford, MA, USA). Water used as HPLC solvent was distilled and purified with a Milli-Q water purification system (Nihon Millipore, Yonezawa, Japan). Standard stock solution (1000 μg/ml) of BPA was prepared in acetonitrile-water (40:60, v/v) and working solution (10 to 1000 ng/ml) was prepared by further diluting the stock solution in acetonitrile-water (40:60, v/v). Acetone, hexane, acetonitrile, and methanol (all HPLC grade) were purchased from Kanto Chemical (Tokyo, Japan) and *n*-heptane from Nacalai Tesque.

A Waters 600E multisolvent delivery system (Millipore) with a column Symmetry Shield RP_{18} 3.5 μm (4.6 × 150 mm) (Millipore) was used for the analysis of BPA in standards and sample extracts. Fluorescence detector (Model F-1050, Hitachi, Tokyo, Japan) was set with excitation at 275 nm and emission at 300 nm. The mobile phase was acetonitrile-water (40:60, v/v) with a flow rate of 1 ml/min under isocratic conditions. The column temperature was 40˚C at a Column heater U-620 Type 30 (Sugaichemi, Osaka, Japan).

A 100 ml volume from each of water samples was applied to the OASIS HLB cartridge, which had previously been conditioned with 10 ml methanol and 10 ml water. The cartridge was washed with 10 ml of water and BPA was eluted by 5 ml methanol. The methanol extract was evaporated to dryness under nitrogen in a 50°C water bath. The residue was dissolved with 1 ml mobile phase and a 50-µl volume of the solution was analyzed by HPLC.

Five grams of the homogenized freshwater snail samples were mixed twice with 30 ml of acetonitrile and 3 g of anhydrous sodium sulfate, and were homogenized again. The vessel containing the mixture was placed in an ultrasonic bath (Bransonic, Yamato, Tokyo, Japan) for 20 min. The mixture was filtered through a paper filter with 3 g of anhydrous sodium sulfate and was evaporated just to dryness using a flow of nitrogen in a 50°C water bath. The residue was dissolved in 10 ml of hexane, was shaken twice with 20 ml of acetonitrile saturated with hexane by a mechanical shaker (Model Taiyo SR-2W, Taiyo, Osaka, Japan) for 10 min, and the phases were then allowed to separate for 15 min. The acetonitrile layer (the lower layer) was evaporated to dryness under nitrogen in a 50°C water bath. The residue was dissolved in 10 ml of acetone-*n*-heptane (3:97, v/v) and was applied to the Florisil cartridge, which had previously been conditioned with 10 ml of acetone-*n*-heptane (20:80, v/v) and 10 ml of acetone-*n*-heptane (3:97, v/v). After washing with 10 ml of acetone-*n*-heptane (5:95, v/v), BPA was eluted from the cartridges with 10 ml of acetone-*n*-heptane (20:80, v/v). The eluate was evaporated to dryness, was dissolved with 1 ml mobile phase, and then was analyzed by HPLC.

RESULTS AND DISCUSSION

BPA was not detected in the surface water (<0.5 ng/ml) and freshwater snails (2 ng/g) taken from the upstream of the Kaeda River. For recovery test, a 5 g portion of freshwater snail and surface river water sample spiked with 10 or 100 ng/g BPA were analyzed as described above. The recoveries of BPA for the freshwater snail were > 80%. Good recovery results were obtained by using the ultrasonic extraction and solid-phase extraction. Moreover, the recoveries of BPA for the surface water were > 99%. The limits of detection set as the 3-fold height of noise were 2 ng/g for the freshwater snail and 0.5 ng/ml for the water sample, respectively (Table 1).

BPA in the surface water was detected from samples collected in September 2002 at the concentration of 0.9 ng/ml, but no BPA (<0.5 ng/ml) was found from the others (Table 2). Previous studies reported that rivers around secure landfills

could be contaminated by BPA in spite of the treatment of leachates (Yamada et al., 1999; Behnisch et al., 2001; Yamamoto et al., 2001). Even if once only, our study also shows that there is the possibility of BPA contamination in rivers around secure landfills.

Table 1. Recovery results of BPA in surface water and freshwater snail.

Sample (n=5)	BPA concentration added (ng/ml)	Recoveries (%)[a]	Detection limits
Surface water	10	104 ± 5	0.5 ng/ml
	100	99 ± 3	
Freshwater snail	10	81 ± 7	2 ng/g
	100	84 ± 5	

a, Mean ± SD

The concentration of BPA in the freshwater snail ranged from <2 to 11 ng/g at sampling sites of <0.5 Km (Table 2). In April and June 2003, BPA was identified from samples at the sampling sites that no BPA (<0.5 ng/ml) was detected in the surface water. These are very interesting results because no, or very little, BPA level in the surface river water dose not mean no, or very little, BPA contamination in the aquatic organism.

Table 2. BPA levels in surface water and freshwater snail.

Samples (n=5)	BPA concentration (ng/ml)				Sampling date
	Sampling site (Km)				
	<0.5	1	2	5	
Surface water	0.9 ± 0.3 [a]	<0.5	<0.5	<0.5	September 2002
	<0.5	<0.5	<0.5	<0.5	December 2002
	<0.5	<0.5	<0.5	<0.5	April 2003
	<0.5	<0.5	<0.5	<0.5	June 2003
Freshwater snail	11.0 ± 3.4	<2	<2	<2	September 2002
	- [b]	-	-	-	December 2002
	3.1 ± 0.8	<2	<2	<2	April 2003
	4.7 ± 2.1	<2	<2	<2	June 2003

a, Mean ± SD; b, No collection of sample

A recent study (Belfroid et al., 2002) reported that the levels of BPA in fish varied from 2 to 75 ng/g dry weight (DW) in the liver and 1 to 11 ng/g in the muscle, but ranged from <0.01 to 0.33 ng/ml in the surface water. BPA were also detected in fish (1 to 6 ng/g, DW) that lived at locations where no BPA (<0.18 ng/ml) was observed in the surface water. Larsson et al. (1999) found that the

bile of fish near the sewage treatment works contained estrogenic substances at levels 10^4-10^6 times higher than water levels.

The soil adsorption coefficient (K_{oc}) values ranged from 314 to 1524 by the calculation using a water solubility of 120 ng/L and an octanol-water partition coefficient (K_{ow}) of 3.32 (Howard 1989). These absorption values mean that BPA released to the ground or to the surface water can be absorbed to soil or to sediments. It is the fact that the levels of BPA in sediments are higher than that in surface waters (Bolz et al., 2001; Heemken et al., 2001; Fromme et al., 2002). The freshwater snails feed on algae, water plants, and detritus from rock surfaces and sediments using a long and rough tongue. These habits may be related to higher levels of BPA in freshwater snail than in surface water. On the other hand, the levels of BPA in freshwater snails were below detection limit at sampling sites of from 1 to 5 Km (<2 ng/g) (Table 2). This may be caused by the dilution of BPA by the larger volume of river water and/or by BPA biodegradation by bacteria.

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