The quantification and characterization of endocrine disruptor bisphenol-A leaching from epoxy resin

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Abstract Bisphenol-A (BPA), a known endocrine disruptor, is a main building block of epoxy resin which has been widely used as a surface coating agent on residential water storage tanks. Therefore, BPA leaching from the epoxy resin can adversely affect human health. In this study, BPA leaching from three epoxy resins were quantified at 20, 50, 75 and 100°C both in deionized water and the specified test water, respectively. BPA leached to the test water was identified using GC-MS and quantified with GC-FID after a sequential extraction and concentration. The results showed that BPA leaching has occurred in all three samples tested. The quantity of BPA from unit area of epoxy resin coating was in the range of 01.68~273. 12 μg/m^2 for sample A, 29.74~1734.05 μg/m^2 for sample B and 52.86~548.78 μg/m^2 for sample C depending on the test temperature, respectively. In general, the amount of BPA leashing increased as the water temperature increases. This result implies a higher risk of BPA leaching to drinking water during a summer season. In addition, microbial growth, measured by colony forming units, in epoxy coated water tanks was higher than that in a stainless steel tank. The results suggest that compounds leaching from epoxy resin may support the growth of microorganisms in a residential water holding tank.

Keywords Bisphenol-A; endocrine disruptor; epoxy resin; microbial growth

Water Science and Technology **Vol 46 No 11–12 pp 381–387** © IWA Publishing 2002

Introduction

An endocrine disrupter is an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and regulation of developmental processes (U.S.EPA, 1997). It has been reported that endocrine disrupters (EDS) seriously affect generative function of animals and human health (Colborn *et al.*, 1996). Bisphenol-A (BPA), a diphenyl compound containing two hydroxyl groups in *para* positions [2,2-bis(4-hydroxyphenyl) propane], is a well known EDS. It is a major component of epoxy resin which is used for protective coatings on food containers and in paints and adhesives, and in anti-oxidants and tanning agents (Tsutsui *et al.*, 1998). In addition, BPA is widely used as a surface-coating agent on residential drinking water storage tanks. For this reason, human exposure to BPA through contaminated drinking water is highly possible if BPA leaches from the epoxy resin.

To quantify and characterize BPA leaching from epoxy resin, three epoxy resins were tested by a modified KS D 8502 method, both in deionized water and in the specified test water, at several different temperatures. BPA leached to the water was identified using GC/MSD and quantified with GD/FID after a sequential extraction and concentration. The microbial growth, measured by colony forming units, in epoxy coated water tanks was also monitored and compared to that in a stainless steel tank to find biodegradability of the compounds leached form the three epoxy resins.

Methods

Leaching test and analytical methods

BPA leaching test was performed with three sample epoxy resins by the modified method

in KS D 8502 (KSS, 1994). Each epoxy resin was applied on three 120×70×3 mm glass plates, which were placed in a tightly closed 2 L stainless steel container with a teflon liner. The container includes either deionized water or the specified test water containing 30 mg/L of hardness (pH 7.0). Leaching test was conducted in an autoclave at 50, 75 and 100°C for 6 h and at 20°C for 24 h, respectively. All tests were performed in duplicate. In the first test, each 2L-test solution was extracted with dichloromethane (DCM) only. In the second test, however, the test solution was extracted with DCM after filtration through a 0.45 μm glass fiber filter (Metrigard, Gelman) to remove any epoxy resin fines. The extraction and concentration is noted below (U.S.EPA, 1996). A 1 L aliquot of sample was measured and placed into a 2 L separation funnel. The sample was then extracted with three portions of 100, 50 and 50 mL of pesticide grade DCM. (Fisher Co.) The extracts were collected and concentrated using a 500 mL Kuderna-Danish concentrator at 75°C to a volume of approximately 2 mL. The concentrate was derivatized, in order to meet target detection limits, by adding 200 μl of BSTFA (*N*, *O* bis(trimethylsilyl)-trifluoroacetamide) reagent (Pierce Co.) to 1 mL of the concentrate and the volume was brought to 2 mL in a graduated vial.

BPA was quantified using a GC/FID (Model 9001, Finnigan) equipped with a RTX-5 capillary column (30 nm×0.53 mm i.d., 0.5 μm film thickness). Separation was performed using the following temperature program: 4 min at 40°C, to 300°C with 20°C/min, and 5 min at 300°C. Nitrogen was used as the carrier gas, and the linear velocity was maintained at 45 cm/sec. BPA was qualified using a GC/MSD (Magnum, Finnigan Mat.), equipped with a 30 m×0.25 mm DB-5 capillary column (film thickness 0.25 μm). The nitrogen flow velocity was 25.7 ml/min and temperature program was the same as described above for GC/FID studies. In addition, the pH of leaching sample was monitored using a pH meter (Model 550, Orion), and the chemical oxygen demands was determined by *Standard Methods* (APHA *et al.*, 1995). Throughout the experiments, plastic containers were not used except for the teflon liner.

Precision, recovery and detection limit

Internal standard (10 mg/L BPA) was used to test the recovery of BPA. The recovery of BP ranged from 62.6 to 118%, which was within the acceptable range (U.S. EPA, 1996). The method detection limit (MDL) for the GC/FID method was evaluated by spiking seven replicates of reagent grade water with BPA at a concentration three times that of the estimated method detection limit. Results show that MDL of BPA was determined to be 297 ppb. The instrument detection limit (IDL) for the GC/FID was calculated from the mean and standard deviation of nine replicates of the lowest working standard and following the same calculation procedures used for the MDL. The IDL was evaluated to be 77 ppb.

Microbial growth

If compounds are leached from epoxy resin, it can be used for microbial growth. Therefore, a microbial growth study was conducted with a 1 L glass container coated with each of the epoxy resin samples and a 1 L stainless steel container, containing only tap water, at room temperature. The microbial populations in each container were quantified using colony forming unit (CFU) on Nutrient Broth (Difco Co.) after 7 days of incubation at 20±0.1°C. One mL of container sample was collected and used for suspended microbial counts using pour plate technique, whereas 1 cm^2 of surface sample was collected using a sterilized cotton swab for attached microbial counts using spread plate technique (*Standard Methods*, 1995).

Results and discussion

Effect of temperature on the leaching of COD

After the leaching test, the pH of the water ranged near neutral to weak alkaline (6.68–8.49). COD concentration was used as a gross parameter for leached organic compounds as epoxy resin contains many additive filler agents, such as amines, that are difficult to quantify. With leaching of these compounds, cross-linking in epoxy resin weakens and consequently BPA leaches to water. Therefore, COD concentration is used as an indicator of epoxy resin stability in water (KSS, 1994). As shown in Fig. 1, COD_{cr} and COD_{Mn} concentrations in between deionized water (D.I.W.) and specified test water (S.T.W.) did not show any significant difference. Sample A resin was the most stable, samples B and C were unstable at high temperature and low temperature, respectively. Koyama (Koyama *et al.*, 1995) reported that the weight loss of epoxy resin increased in proportion to the aging time at 160°C, reaching 0.61% at 10^4 hr in thermal aging test. In this study, COD concentrations in water sample increased with increasing temperature, though the extent was different among the three samples. In addition, the COD_{cr} concentrations in the test water were 1.5–2.0 times higher than that of COD_{Mn} concentrations. This result suggests that the compounds leached from the epoxy resin samples are difficult to oxidize, in general.

A linear relationship was observed between the total GC peak area of extracts and COD_{cr} concentration, as shown in Figure 2. The correlation constant was calculated to be 0.63, which can be increased more than 0.90 if we exclude one outlier. Therefore, the COD_{cr} concentration in test water was solely due to the compounds leached from the epoxy resin samples.

Effect of temperature on the leaching of BPA

BPA leached from three epoxy resins was quantified and summarized in Table 1. BPA concentrations in D.I.W. for samples A, B and C ranged 1.67~6.98 μg/L, 0.32~89.79 μg/L, and 1.56~24.81 μg/L, respectively. BPA concentrations in S.T.W. for samples A, B and C

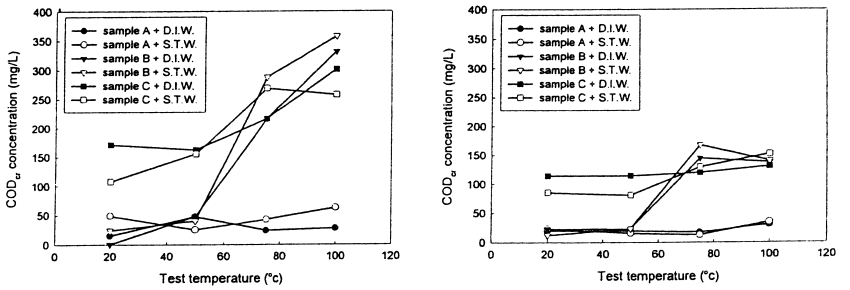


Figure 1 Effect of temperature on the leached COD concentrations in the test water

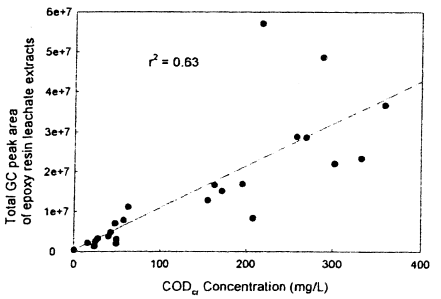


Figure 2 Relationship between total GC peak area and cod_{CR}

Table 1 Effect of temperature on the leaching of BPA from three epoxy samples (leaching time, 24 h at 20°C, 6 h at the other temperature)

	Sample A						Sample B						Sample C					
	D.I. water			S.T. water			D.I. water			S.T. water			D.I. water			S.T. water		
Temp (°C)	1st	2nd	TMS	1st	2nd	TMS	1st	snd	TMS	1st	2nd	TMS	1st	2nd	TMS	1st	2nd	TMS
20	ND	ND	0.64	ND	0.57	0.60	1.43	0.32	0.71	3.48	1.58	4.60	2.10	4.86	0.45	2.86	2.81	1.06
	(0)	(0)	(0)	(0.1)	(0.1)	(0.1)	(0)	(0)	(0.9)	(0.5)	(0.3)	(0.2)	(0.4)	(0)	(0.30)	(0.8)	(0.1)	
50	ND	ND	–	1.66	0.69	–	1.48	0.69	–	10.47	15.10	–	12.11	5.70	–	01.64	3.29	–
	(0)	(0)	–	(0.2)	(0.1)	–	(0.4)	(0)	–	(5.0)	(0.6)	–	(0)	(0.4)	–	(0)	(1.1)	–
75	2.64	1.67	–	1.85	3.16	–	89.79	25.20	–	10.64	66.60	–	1.56	7.23	–	14.16	8.01	–
	(0)	(0)	–	(0.3)	(0.2)	–	(0.126)	(1.4)	–	(7.8)	(7.0)	–	(0.3)	(0.6)	(0.7)	–		
100	5.06	6.98	0.88	14.15	14.53	20.01	54.14	52.22	237.4	92.39	92.35	179.8	11.03	24.81	24.96	27.83	29.20	25.40
	(24)	(0.3)	(0)	(0.3)	(2.4)	(0.6)	(14.6)	(3.2)	(0.36)	(1.9)	(13.1)	(0.39)	(26)	(25)	(0.6)	(1.5)	(0.06)	(8.6)

* ( ) Standard deviation

All unit: μg/L

ranged 0.57~14.53 μg/L, 1.58~92.39 μg/L, and 2.81~29.2 μg/L, respectively. Statistical test (t' test) did not show any significance between the concentrations in D.I.W. and S.T.W. BPA leached to the test water was increased with increasing the leaching COD concentration, which is in accordance with the COD concentration.

Both figures in Figure 3 shows the amount of leached BPA normalized to the unit epoxy coated surface area in D.I.W. and S.T.W., respectively. Depending on the samples, as much as 10–20 fold differences in the concentration of leached BPA were observed. The quantity of leached BPA leaching from unit area of epoxy resin coating was in the range of 10.677~273.120 μg/m^2 for sample A, 29.737~1734.045 μg/m^2 for sample B and 52.857~548.778 μg/m^2 for sample C depending on the test temperature, respectively. In general, the amount of BPA leaching increased as the water temperature increases. This result implies a higher risk of BPA leaching to drinking water during a summer season.

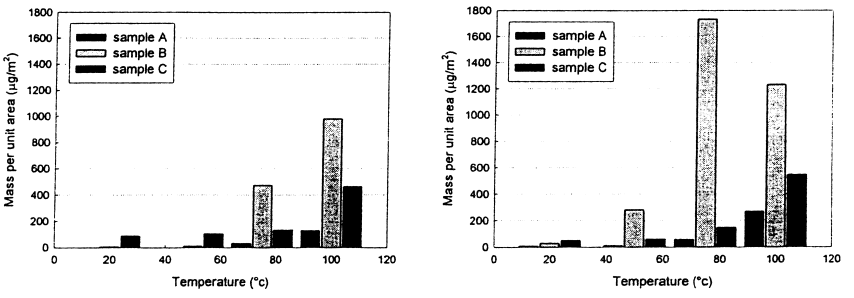
Identification of BPA

The extracted samples were analyzed using GC/MSD to provide the presence of noble BPA. Both figures in Figure 4 show that mass spectra of noble BPA and BPA in sample B, respectively. The results showed that the value of *m/z* in noble BPA and in the sample BPA matched at 228, 213, 119, 107, and 73, respectively.

Microbial growth

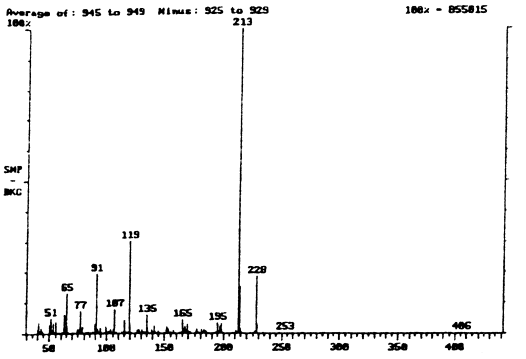
The results of the CFU test showed significant difference in microbial growth depending on the epoxy resin sample. Initially, microbial population was 369±167 (CFU/mL, and 84±40 CFU/mL in a container coated with epoxy resin and in a stainless steel container, respectively. Compared to the result of the blank test, these initial microbial concentrations were negligible. A shown in Figure 5a, however, suspended microorganisms in sample A container gradually increased over time, up to 3,396±518 CFU/mL after 6 days. On the contrary, microbial concentration in sample B, that showed the highest COD concentration reached the highest of 732±773 CFU/mL after 5 days and started to diminish. Microbial concentration in a stainless steel container was 100 CFU/mL after 5 days, and was the lowest in all containers. Combined with the leaching test, it seems that reins with higher leaching tendency did not result in higher microbial growth. BPA and other compounds used in epoxy resin are considered either recalcitrant or resistant to biodegradation. The COD_{cr} to COD_{Mn} ratio also showed that leached compounds are hard to oxidize. Therefore, it seems that most compounds leached from the epoxy resins are either toxic or nongrowth supporting to the microorganisms.

However, the microbial growth measured in epoxy coated containers was higher than that in a stainless steel tank suggesting that compounds leaching from epoxy resin may

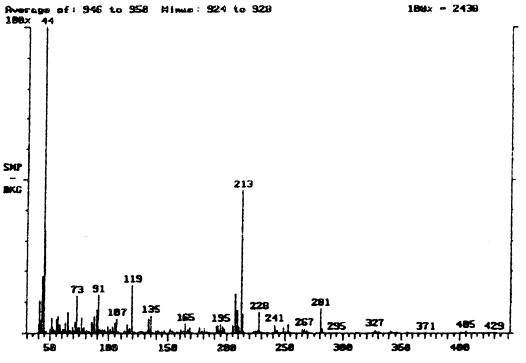


(a) deionized water (b) specified test water

Figure 3 The amount of BPA leaching normalized to the unit coating area

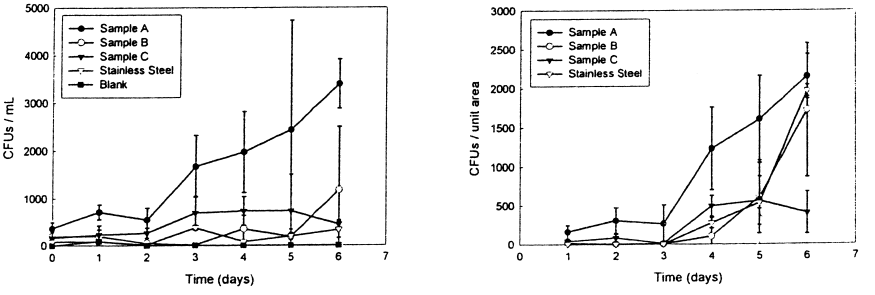


(a) Mass spectra of BPA standard 10mg/L



(b) Mass spectra of Sample B

Figure 4 Mass spectra of noble BPA and BPA in sample B



(a) suspended microorganisms (b) attached microorganisms

Figure 5 The changes of microorganisms measured by colony forming units in epoxy resin coated container vs. stainless steel container

support the growth of microoganisms in a residential water holding tank. Attached microbial populations were similar to suspended microbial populations over the course of study, as shown in Figure 5(b).

Summary

1. After leaching test with epoxy resin, pH value of the water immersed with three-epoxy resins ranged from near neural to weak alkaline (6.68 to 8.49).
2. A linear relationship was developed between the total GC peak area of extracts and COD_{c4} concentration in water. In addition, the amount of BPA leaching was also

proportional to the concentration of COD. Therefore, COD can be an indirect indicator of BPA leaching from the epoxy resins used in this study.

3. The amount of BPA leaching increased as the temperature of the test water increased. This result implies a higher risk of BPA leaching to drinking water during a summer season.
4. The microbial growth in epoxy coated water container was higher than that in a stainless steel container. The results suggest that those reaching compounds can support the growth of microbial populations in water. However, microbial concentration was the highest in the sample which showed least leaching characteristics. It seems that leached compounds are toxic and not growth supporting to microorganisms.
5. Therefore, much attention and stringent measures should be imposed on the use of epoxy resin as a surface-coating agent on drinking water holding reservoirs.

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