PERGAMON

Chemosphere 44 (2001) 973–979

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Identification and quantification of chlorinated bisphenol A in wastewater from wastepaper recycling plants

Hitoshi Fukazawa [a,b], Kentaro Hoshino [a], Tatsushi Shiozawa [a],
Hidetsuru Matsushita [b], Yoshiyasu Terao [a,*]

[a] *Institute for Environmental Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan*
[b] *Shizuoka Institute of Environment and Hygiene, 4-27-2 Kitaando, Shizuoka 420-8637, Japan*

Received 23 June 2000; accepted 5 September 2000

Abstract

Chlorinated derivatives of bisphenol A were detected in the final effluents of eight paper manufacturing plants in Shizuoka, Japan, where thermal paper and/or other printed paper is used as the raw material. Their amounts were determined by gas chromatography/mass spectrometry (GC/MS) after treatment with N, O-bis(trimethylsilyl)trifluoroacetamide, and ranged from traces to 2.0 μg/l. They are likely produced by chlorination of bisphenol A, which was released into the effluents from the pulping process of wastepaper, during or after bleaching with chlorine. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Bisphenol A; Sodium hypochlorite; Wastepaper recycling; GC/MS

1. Introduction

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl)propane has been reported to interact with estrogen receptors, suggesting that it may serve as endocrine disrupter at environmental concentrations below acute toxic levels (Alexander et al., 1988; Olea et al., 1996; Routledge and Sumpter, 1996; Coldham et al., 1997; Steinmetz et al., 1997). Bisphenol A is a common raw material used in the polymer and plastics industries, and is also used to produce paper, such as thermal paper and carbonless copy paper. Therefore, many factories that recycle wastepaper are thought to release bisphenol A into wastewater.

Bisphenol A is easily chlorinated by sodium hypochlorite, which is used as a bleaching agent in paper factories as well as a disinfection agent in sewage treatment plants. Thus it is important to investigate the release of chlorinated bisphenol A into the environment and its toxicity.

There are more than 100 wastepaper recycling plants in Shizuoka prefecture, Japan. If these plants incorporate a chlorine-bleaching process, chlorinated bisphenol A may be produced and released into the wastewater. In the present study, we have quantitatively analyzed the chlorinated bisphenol A released from the wastepaper recycling process.

2. Experimental

2.1. Materials and spectral measurement

Bisphenol A and bisphenol A-d_{16} were purchased from Kanto Chemical, Japan. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (GC grade) was obtained from Wako Pure Chemicals Industry, Japan. All

---

[*] Corresponding author. Tel.: +81-54-264-5788; fax: +81-54-264-5788.
E-mail address: terao@u-shizuoka-ken.ac.jp (Y. Terao).

PII: S0045-6535(00)00507-5

solvents used were of pesticide analysis grade. Water was treated by a Milli-Q Water Purification System after distillation and then purified by an activated carbon column. ^{1}H-NMR and ^{13}C-NMR spectra were taken of solutions in chloroform-d with a JEOL JNM-GSX270 ^{1}H: 270 MHz, ^{13}C: 67.5 MHz) or a JNM-GSX500 (^{1}H: 500 MHz , ^{13}C: 125 MHz) Fourier-transform spectrometer. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet and br = broad. Chemical shifts are shown in ppm using tetramethylsilane as an internal standard. High-resolution mass spectra were measured using a JEOL JMS-DX300 or a JEOL JMS-AX505w mass spectrometer.

2.2. Synthesis of chlorinated bisphenol A

Sodium hypochlorite solution (100 ml, available chlorine >5%) was added dropwise to a stirred solution of bisphenol A (13.7 g, 60 mmol) in methanol (100 ml) at room temperature, and the stirring was continued for 11 h. After adding 1 M-sodium sulfite solution (50 ml), the mixture was condensed under reduced pressure to one-half of the initial volume, acidified with 10% hydrochloric acid and extracted with dichloromethane. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and evaporated to dryness. An oily residue was subjected to chromatography on a silica gel column (eluent: chloroform and 1% methanol in chloroform) to give the following crystalline products:

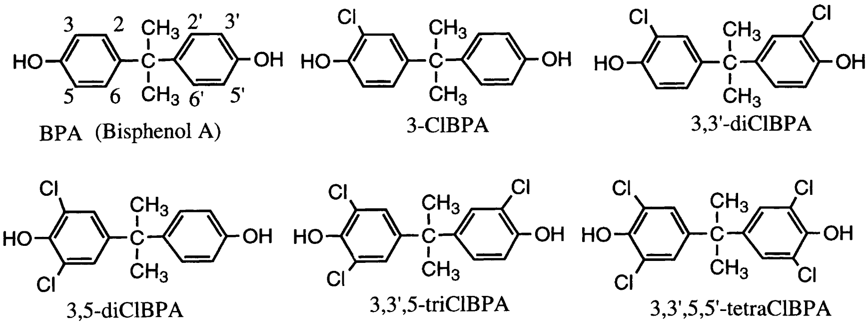
2-(3-Chloro-4-hydroxyphenyl)-2-(4-hydroxyphenyl) propane (3-ClBPA); 4.9 g (31%), mp 111–112°C. HRMS *m/z*: 262.0801 (calcd for $C_{15}H_{15}ClO_2$, 262.0761). ^{1}H-NMR ($CDCl_3$) δ: 1.60 (6H, s, $2 \times CH_3$), 5.24 (1H, br, OH), 5.54 (1H, br, OH), 6.73 (2H, dt, $J = 2.6$, 8.9 Hz, $2 \times$ ArH), 6.89 (1H, d, $J = 8.4$ Hz, ArH), 7.00 (1H, dd, $J = 2.4$, 8.4 Hz, ArH), 7.07 (2H, dt, $J = 2.6$, 8.9 Hz, $2 \times$ ArH), 7.17 (1H, d, $J = 2.4$ Hz, ArH).

2,2-Bis(3-chloro-4-hydroxyphenyl)propane (3,3′-diClBPA); 4.1 g (23%), mp 90–92°C. HRMS *m/z*: 296.0345 (calcd for $C_{15}H_{14}Cl_2O_2$, 296.0372) ^{1}H-NMR ($CDCl_3$) δ: 1.60 (6H, s, $2 \times CH_3$), 5.47 (2H, br, $2 \times$ OH), 6.91 (2H, d, $J = 8.8$ Hz, $2 \times$ ArH), 7.00 (2H, dd, $J = 2.3$, 8.8 Hz, $2 \times$ ArH), 7.16 (2H, d, $J = 2.3$ Hz, $2 \times$ ArH).

2-(3-Chloro-4-hydroxyphenyl)-2-(3,5-dichloro-4-hydroxyphenyl)propane (3,3′,5-triClBPA); 2.3 g (12%), mp 74–75°C. HRMS *m/z*: 329.9974 (calcd for $C_{15}H_{13}Cl_3O_2$, 329.9981). ^{1}H-NMR ($CDCl_3$) δ: 1.59 (6H, s, $2 \times CH_3$), 5.51 (1H, br, OH), 5.79 (1H, br, OH), 6.93 (1H, d, $J = 8.5$ Hz, ArH), 6.98 (1H, dd, $J = 2.3$, 8.5 Hz, ArH), 7.08 (2H, s, $2 \times$ ArH), 7.16 (1H, d, $J = 2.3$ Hz, ArH). 2,2-Bis(3,5-dichloro-4-hydroxyphenyl)propane (3,3′,5,5′-tetraClBPA); 0.67 g (3%), mp 135–136°C. HRMS *m/z*: 363.9615 (calcd for $C_{15}H_{12}Cl_4O_2$, 363.9594). ^{1}H-NMR ($CDCl_3$) δ: 1.59 (6H, s, $2 \times CH_3$), 5.78 (2H, br, $2 \times$ OH), 7.07 (4H, s, $4 \times$ ArH).

2.3. Synthesis of 2-(3,5-dichloro-4-hydroxyphenyl)-2-(4-hydroxyphenyl) propane (3,5-diClBPA)

A solution of pivaloyl chloride (11.1 ml, 90 mmol) in dichloromethane (20 ml) was added to a stirred solution of bisphenol A (13.7 g, 60 mmol) and triethylamine (18.2 ml, 130 mmol) in dichloromethane (100 ml) at room temperature. After stirring for 15 h, cold water was added and the organic layer was separated, washed with brine and dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure gave a solid residue, which was subjected to chromatography on a silica gel column (eluent: 10% ethyl acetate in hexane) to give crystals of 2-(4-hydroxyphenyl)-2-(4-pivaloyloxyphenyl)propane (BPA-monopivalate); 16.8 g (90%), mp 138–139°C. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (9H, s, $3 \times CH_3$), 1.50 (6H, s, $2 \times CH_3$), 4.61 (1H, br OH), 6.58 (2H, dt, $J = 2.1$, 8.7 Hz, $2 \times$ ArH), 6.81 (2H, dt, $J = 2.1$, 8.7 Hz, $2 \times$ ArH), 6.94 (2H, dt, $J = 2.2$, 8.8 Hz, $2 \times$ ArH), 7.07 (2H, dt, $J = 2.2$, 8.8 Hz, $2 \times$ ArH). Sodium hypochlorite solution (35 ml, available chlorine >0.05%) was added dropwise to a stirred solution of BPA-monopivalate (15.2 g, 50 mmol) in methanol (100 ml). After stirring for an additional hour, the reaction was quenched by adding 1 M-sodium sulfite solution (20 ml). The reaction mixture was condensed under reduced



BPA (Bisphenol A)

3-ClBPA

3,3'-diClBPA

3,5-diClBPA

3,3',5-triClBPA

3,3',5,5'-tetraClBPA

Fig. 1. Chemical structures of bisphenol A and the chlorinated derivatives.

pressure to give an oily residue, which was extracted with dichloromethane. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After removal of the solvent, the residue was subjected to chromatography on a silica gel column (eluent: 10% of ethyl acetate in hexane) to give crystals of 2-(3,5-dichloro-4-hydroxyphenyl)-2-(4-pivaloyloxyphenyl)propane (3,5-diClBPA-monopivalate); 6.5 g (34%), mp 148–149°C. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (9H, s, 3 × CH_3), 1.50 (6H, s, 2 × CH_3), 5.82 (1H, br, OH), 6.86 (2H, dt, $J = 2.2$, 8.8 Hz, 2 × ArH), 6.98 (2H, s, 2 × ArH), 7.07 (2H, dt, $J = 2.2$, 8.8 Hz, 2 × ArH). To a solution of 3,5-diClBPA-monopivalate described above (1.9 g, 10 mmol) in methanol (20 ml) a 10% aqueous solution of potassium carbonate (20 ml) was added. The mixture was stirred at room temperature for 2 h, and the methanol was then evaporated under reduced pressure to give an aqueous residue, which was acidified with 10% hydrochloric acid and extracted with dichloromethane. The organic layer was washed with brine and dried over

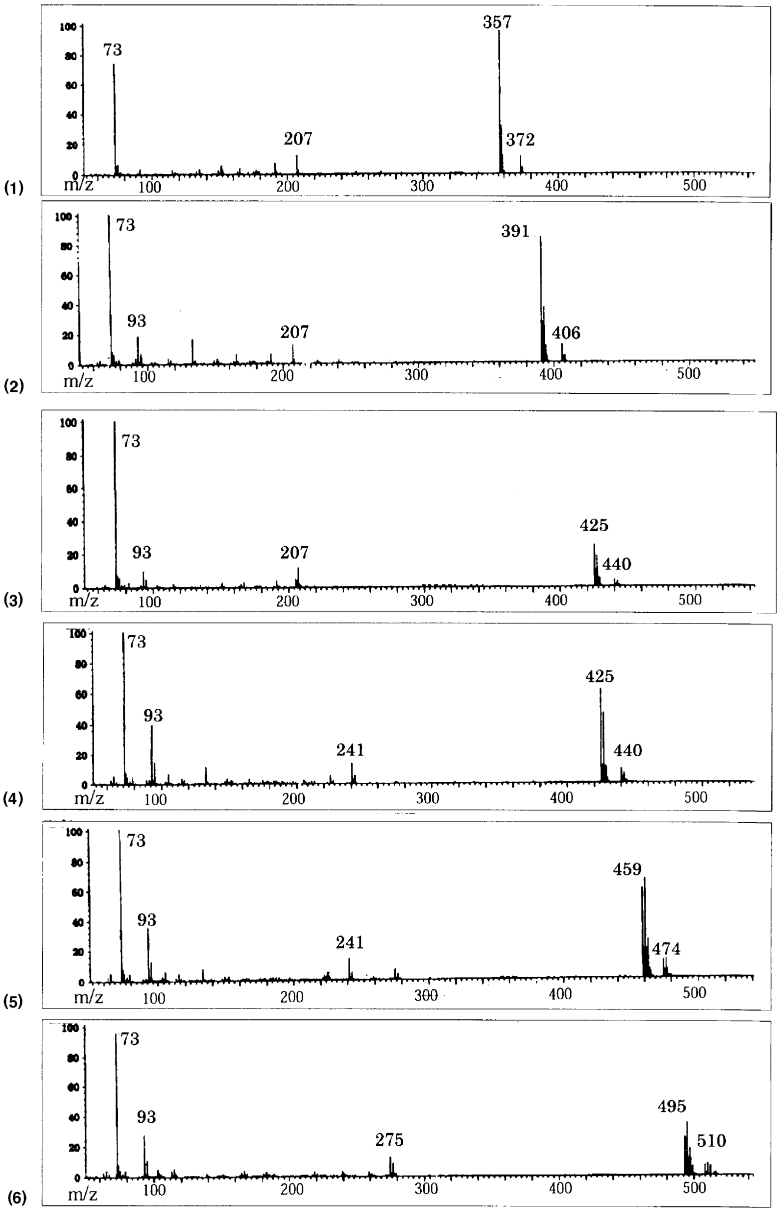


Fig. 2. Mass spectra of bis(trimethylsilyl) ethers of BPA (1), 3-ClBPA (2), 3,5-diClBPA (3), 3,3′-diClBPA (4), 3,3′,5-triClBPA (5), 3,3′,5,5′-tetraClBPA (6).

anhydrous magnesium sulfate. Removal of the solvent under reduced pressure gave a solid which was recrystallized from hexane to give pure 3,5-diClBPA, 1.6 g (55%); mp 110–112°C. HRMS *m/z*: 296.0403 (calcd for $C_{15}H_{14}Cl_2O_2$, 296.0372). ^{1}H-NMR ($CDCl_3$) δ: 1.60 (6H, s, 2 × CH_3), 4.95 (1H, br, OH), 5.75 (1H, br, OH), 6.75 (2H, dt, $J = 2.2$, 8.7 Hz, 2 × ArH), 7.06 (2H, dt, $J = 2.2$, 8.7 Hz, 2 × ArH), 7.09 (2H, s, 2 × ArH).

2.4. GC/MS apparatus and operating conditions

Gas chromatography/mass spectrometry (GC/MS): Hewlett Packard HP-5890 II gas chromatograph and Hewlett Packard HP-5971A mass spectrometer. The GC/MS conditions were as follows: column, HP-5 trace analysis (5% diphenyl and 95% dimethylarylenesiloxane, coating film thickness 0.1 μm, 0.25 mm i.d. × 30 m, Hewlett Packard); injection mode, splitless; injection temperature, 250°; column head pressure, 7 psi; column temperature, 100–270°; program rate, 15°/min; ionizing voltage, 70 eV. MS measurement was performed in scan mode for a qualitative analysis and in the selected ion monitoring (SIM) mode for a quantitative analysis. The chlorinated derivatives of bisphenol A were detected after trimethylsilylation with BSTFA, and the ions monitored were BPA, *m/z* 357, 372, 207; 3-ClBPA, *m/z* 392, 393, 406; 3,3′- and 3,5-diClBPA, *m/z* 425, 427, 440; 3,3′,5-triClBPA, *m/z* 459, 461, 474; 3,3′,5,5′-tetraClBPA, *m/z* 493, 495, 510.

2.5. Detection of chlorinated bisphenol A formed by the reaction with sodium hypochlorite in aqueous medium

An acetone solution of 20 μg of bisphenol A was added to 20 ml of sodium hypochlorite solution (10 mg/l of chlorine) adjusted to pH 11 with sodium hydroxide solution. The solution was allowed to stand with occasional shaking for an appropriate time at room temperature. After adding 0.01 M-sodium thiosulfate solution, the reaction mixture was acidified with hydrochloric acid (pH 4), and then extracted twice with 2 ml of dichloromethane. The dichloromethane solution was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to about 1 ml, and finally to 0.3 ml under a nitrogen stream. After adding 200 μl of BSTFA solution, the solution was allowed to stand for 30 min to complete the trimethylsilylation. The reaction solution was concentrated again to 0.3 ml under a nitrogen stream, diluted up to 1 ml with dichloromethane and subjected to GC/MS (SIM) analysis.

2.6. Sample preparation

A water sample (100 ml) was acidified with hydrochloric acid (pH 4) and extracted twice with 20 ml of dichloromethane. The dichloromethane solution was dried over anhydrous sodium sulfate and treated in the same way as described above.

2.7. Biodegradation of bisphenol A and chlorinated bisphenol A

In a 5-l glass flask covered with aluminum foil, 2 l of supernatant of activated sludge were diluted with the same volume of pure water. The aqueous solution was stirred slowly with a magnetic stirrer for 24 h. To the solution an acetone solution containing 16 μg of bisphenol A, 3-ClBPA, 3,3′-diClBPA, 3,3′,5-triClBPA and 3,3′,5,5′-tetraClBPA was added. After stirring for 30 min, two 100 ml samples were taken and immediately extracted twice with 20 ml of dichloromethane. Each extract was subjected to GC/MS analysis as described above. Samples taken after 1, 2, 3, 5 and 7 days of stirring were treated as described above. The temperature was kept at 24–26°C during the test.

3. Results and discussion

3.1. Synthesis and structural determination of chlorinated bisphenol A

Treatment of bisphenol A with an excess of sodium hypochlorite gave mono-, di-, tri- and tetra-chlorinated

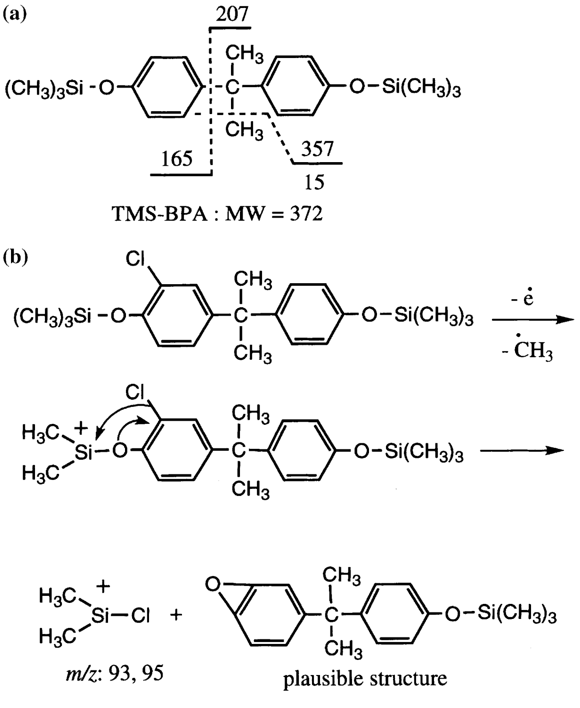


Fig. 3. Fragmentation of bis(trimethylsilyl) ethers of bisphenol A and the chlorinated derivatives.

bisphenol A, which were separated by chromatographic methods. Since 3,5-diClBPA was scarcely produced by this reaction, it was synthesized via 2-(4-hydroxyphenyl)-2-(4-pivaloyloxyphenyl)propane. Their structures were determined on the basis of their high-resolution mass and ^{1}H-NMR spectra. Fig. 1 shows the chemical structures of the chlorinated derivatives of bisphenol A obtained.

3.2. Mass spectrometric fragmentation patterns of chlorinated bisphenol A

Mass spectra of bis(trimethylsilyl) ethers of bisphenol A and chlorinated bisphenol A are shown in Fig. 2. The molecular ion peaks (M^+) in the mass spectra of these compounds are very small. However, the intense (M^+–CH_3) peaks can be used without difficulty to

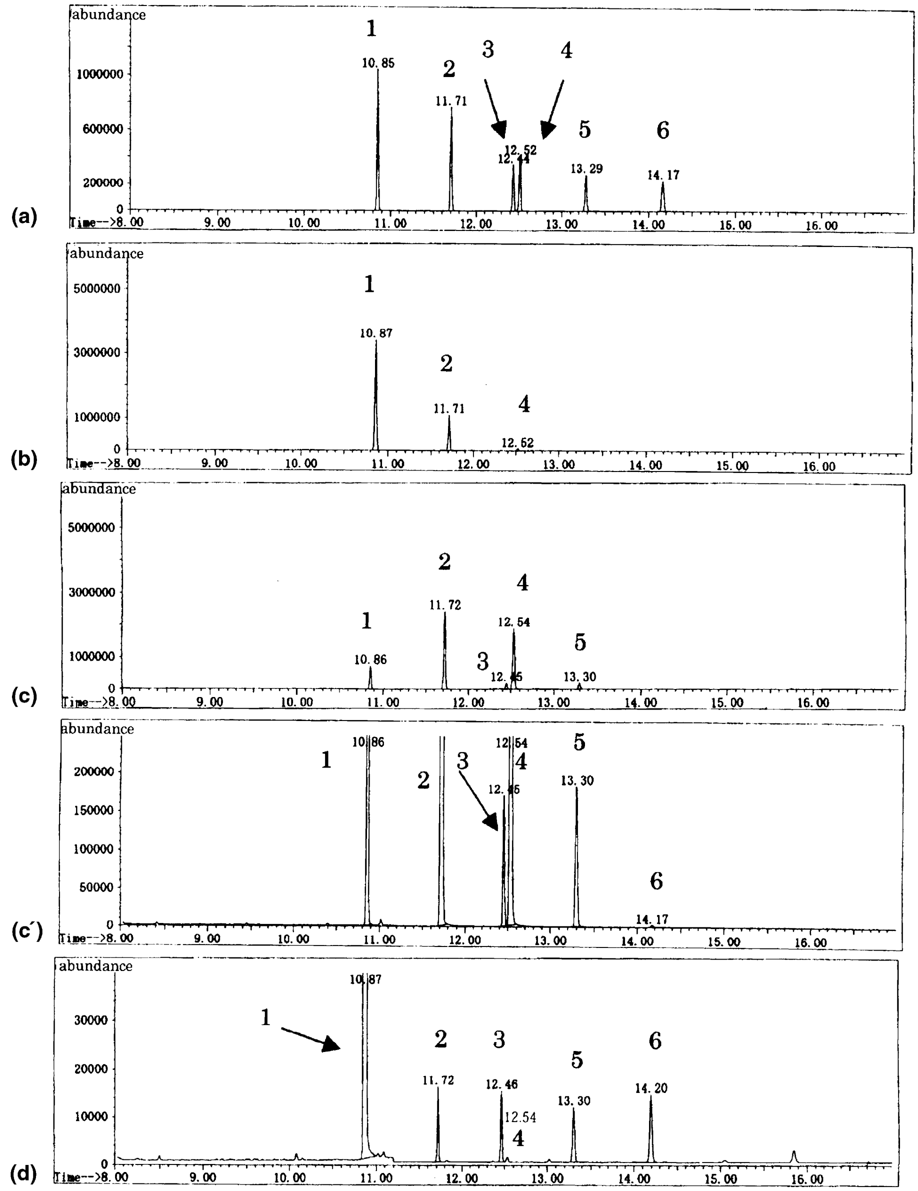


Fig. 4. Total ion chromatograms (TIC) of the solution of bis(trimethylsilyl) ethers of bisphenol A and chlorinated derivatives: (a) the standard solution, (b) the sample taken after the reaction of bisphenol A with NaClO for 5 min, and (c) for 60 min, (c′) expansion of (c), (d) the sample in plant 3. Peak number: 1 = BPA, 2 = 3-ClBPA, 3 = 3,5-diClBPA, 4 = 3,3′-diClBPA, 5 = 3,3′,5-triClBPA, 6 = 3,3′,5,5′-tetraClBPA.

Table 1
Concentration[a] of BPA and chlorinated derivatives in final effluents from paper recycling plants

Plant no.	BPA	3-Cl BPA	3,5-diCl BPA	3,3′-diCl BPA	3,3′,5-triCl BPA	3,3′,5,5′-tetraCl BPA
1	8	0.3	0.4	Trace		
2	41	Trace				
3	43	0.8	1.0	Trace	1.2	1.4
4	63	0.5	0.4	0.5	0.9	1.3
5	119	2.0	Trace			
6	195	0.2				
7	202	Trace				
8	370	0.3				

[a] μg/l, trace: <0.2 μg/l.

determine the molecular weights. The loss of a methyl group was demonstrated to originate from the propyl moiety on the basis of the spectrum of bis(trimethylsilyl) ether of BPA-d_{14}, in which a (M^+–CD_3) peak was observed at *m/z* 368. In the spectrum of bisphenol A, another characteristic fragment peak at *m/z* 207 results from the elimination of a trimethylsilyloxyphenyl radical from the molecular ion. The mass spectra of chlorinated bisphenol A show similar patterns, in which chlorine-substituted trimethylsilyloxyphenyl groups are preferentially lost; *m/z* 207 (M^+–$(ClC_6H_3OSi(CH_3)_3)$ in 3-ClBPA and (M^+–$Cl_2C_6H_2OSi(CH_3)_3$) in 3,5-diClBPA, *m/z* 241 (M^+–$ClC_6H_3OSi(CH_3)_3$) in 3,3′-diClBPA and (M^+–$Cl_2C_6H_2OSi(CH_3)_3$) in 3,3′,5-triClBPA, and *m/z* 275 (M^+–$Cl_2C_6H_2OSi(CH_3)_3$) in 3,3′,5,5′-tetraClBPA. These fragment peaks are useful for identifying chlorinated bisphenol A in the GC/MS analysis. In addition, two peaks at *m/z* 93 and 95 were observed in the spectrum of each chlorinated bisphenol A. These peaks correspond to a dimethylsilyl chloride ion, which might be formed from the molecular ion by cleavage of a methyl group of the trimethylsilyl moiety and simultaneous migration of an *ortho* chlorine (Fig. 3). Similar migration was reported to occur in the case of 2-chloro-1-(trimethylsilyl)ethane (Hill and Mokotoff, 1984).

3.3. Chlorination of bisphenol A with sodium hypochlorite in aqueous solution and GC/MS analysis of the chlorinated products

As a model experiment for the chlorination of bisphenol A with sodium hypochlorite in an aqueous medium, we carried out the reaction under alkaline conditions, since wastepaper is treated with an alkaline solution in the pulping process at paper recycling factories. Chlorinated bisphenol A was analyzed after trimethylsilylation with BSTFA to avoid contamination and improve the limits of detection. The total ion chromatograms (TIC) of the products obtained after 5 and 60 min are shown in Fig. 4.

After only 5 min, 3-ClBPA and a small amount of 3,3-diClBPA were produced (b, in Fig. 4). After 60 min, peaks of 3-ClBPA, 3,3′- and 3,5-diClBPA, 3,3′,5-triClBPA and 3,3′,5,5′-tetraClBPA were observed in the TIC of the extract (c, in Fig. 4). These findings suggest that bisphenol A reacts easily with sodium hypochlorite in an aqueous medium and chlorinated products are detected in wastewater samples by these methods.

3.4. Analysis of chlorinated bisphenol A in final effluents from wastepaper recycling plants

We investigated the presence of chlorinated bisphenol A in the final effluents of eight paper manufacturing plants, where wastepaper is used as the raw material and the bleaching process involves the use of sodium hypochlorite. Bisphenol A was detected at high concentrations, as shown in Table 1, and is believed to have originated from waste containing thermal paper and/or other printed paper.

Chlorinated derivatives of bisphenol A were detected in concentrations ranging from traces to 2.0 μg/l in the final effluents of these eight plants, as shown in Table 1. Patterns of the chlorinated bisphenol A released into the final effluents were classified into three groups: (1) Only mono-chlorinated bisphenol A was detected (plant nos.

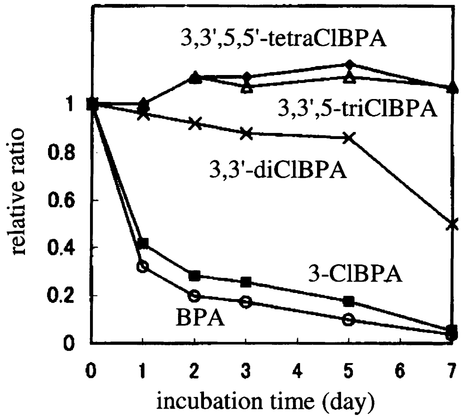


Fig. 5. Biological degradation of bisphenol A and the chlorinated bisphenol A in the supernatant of the activated sludge in plant 4.

2, 6, 7 and 8). (2) Mono-chlorinated bisphenol A and dichlorinated bisphenol A were detected (plant nos. 1 and 5). (3) mono-, di-, tri- and tetra-chlorinated bisphenol A were all detected (plant nos. 3 and 4).

It seems likely that treatment with sodium hypochlorite in the bleaching process causes the chlorination of bisphenol A. The differences in chlorinated bisphenol A detected in the effluents of these plants may be explained in terms of differences in the pulping process and treatments applied to wastewater.

On the other hand, the relatively large amount of tri- and tetra-chlorinated bisphenol A detected in plants 3 and 4 is unexpected, since an activated sludge process was used before the final effluents in both plants. Bisphenol A is reportedly biodegradable in river water (Dorn et al., 1987; Staples et al., 1998). Therefore, to clarify the biodegradability of chlorinated bisphenol A, a biodegradation test was performed using the supernatant of activated sludge in one of these plants, and the results are summarized in Fig. 5.

Bisphenol A and 3-ClBPA were biodegraded rapidly; more than 50% disappeared after one day. However, the poly-chlorinated derivatives, 3,3′,5-triClBPA and 3,3′,5,5′-tetraClBPA, were scarcely biodegraded under the same conditions. The di-chlorinated bisphenol A, 3,3′-diClBPA, underwent biodegradation, but very slowly. These findings suggest that poly-chlorinated bisphenol A, if produced, preferably contaminates the final effluent without biodegradation. Furthermore, a yeast estrogen screen test (Arnold et al., 1996) has recently shown that chlorinated bisphenol A has an approximately 10–100-fold higher affinity for the estrogen receptor than bisphenol A (Terao et al., 2001).

From an environmental point of view, the most significant conclusion that can be drawn from the present results is that sodium hypochlorite should not be used as a bleaching agent in wastepaper recycling plants.

References

Alexander, H.C., Dill, D.C., Smith, L.W., Guiney, P.D., Dorn, P.B., 1988. Bisphenol A: acute aquatic toxicity. Environ. Toxicol. Chem. 7, 19–26.

Arnold, S.F., Robinson, M.K., Notides, A.C., Guillette Jr., L.J., McLachlan, J.A., 1996. A yeast estrogen screen for examining the relative exposure of cells to natural and xenoestrogens. Environ. Health Perspect. 104, 544–548.

Coldham, N.G., Dave, M., Sivapathasundaram, S., McDonnell, D.P., Connor, C., Sauer, M.J., 1997. Evaluation of a recombinant yeast cell estrogen screening assay. Environ. Health Perspect. 105, 734–741.

Dorn, P.B., Chou, C.-S., Gentempo, J.J., 1987. Degradation of bisphenol A in natural waters. Chemosphere 16, 1501–1507.

Hill, S.T., Mokotoff, M., 1984. Chlorine migration in the mass spectra of *tert*-butyldimethylsilyl derivatives of chloro alcohols. J. Org. Chem. 49, 1441–1442.

Olea, N., Pulgar, R., Plerez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M., Sonnenschein, C., 1996. Estrogenicity of resin-based composites and sealants used in dentistry. Environ. Health Perspect. 104, 298–305.

Routledge, E.J., Sumpter, J.P., 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environ. Toxicol. Chem. 15, 241–248.

Staples, C.A., Dorn, P.B., Klecka, G.M., O'Block, S.T., Harris, L.R., 1998. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36, 2149–2173.

Steinmetz, R., Brown, N.G., Allen, D.L., Bigsby, R.M., Ben-Jonathan, N., 1997. The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo. Endocrinology 138, 1780–1786.

Terao, Y., et al., 2001. Chemosphere, in preparation.