Determination of Bisphenol-A in Reusable Polycarbonate Food-Contact Plastics and Migration to Food-Simulating Liquids

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Bisphenol-A (BPA) is a principal reactant in the preparation of polycarbonate (PC) plastics and has been shown in in vitro cell proliferation studies to exhibit estrogen-like characteristics. Reusable baby bottles, water carboys, and other housewares are often made of PC. A high-pressure liquid chromatographic (HPLC) protocol was used to determine residual BPA in PC and BPA migrated to food simulants in contact with PC under controlled time/temperature conditions. Confirmation of BPA was performed by gas chromatography/mass spectrometry (GC−MS). Residual amounts of BPA found in PC food contact articles ranged from 7 to 58 μg/g. In migration tests the plastic was exposed to water, ethanol/water mixtures, and Miglyol (a food oil simulant) in sealed vials at a constant temperature of 65 °C, for up to 10 days. BPA in food simulants ranged from 13 to 368% of BPA available to migrate from the polymer. GC−MS methods were applied to the analysis of water stored in reusable PC 5-gal water carboys. The amount of BPA found in the water ranged from ND to 5 ppb.

Keywords: *Bisphenol-A; polycarbonate; migration; baby bottles; hydrolysis*

INTRODUCTION

Polycarbonate (PC) plastic is used in food storage containers including water carboys, baby bottles, and kitchen appliances (Seymour and Carraher, 1988). In reusable food containers, additives and residual chemicals in the plastic may migrate to food. Bisphenol-A (4,4′-isopropylidene diphenol, CAS Registry No. 80-05-7, more commonly known as BPA) is a principal reactant in the preparation of PC. BPA has also been implicated as a potential endocrine disrupter by positive in vitro test results for estrogen-like behavior (Krishnan et al., 1993). The present work reports residual BPA in finished housewares at levels up to 58 μg/g. Actual migration of BPA from reusable items may exceed available residual levels when additional BPA is formed through polymer hydrolysis in repeated heating operations with aqueous foods (Gachter and Muller, 1990; Gardner and Martin, 1979; Lee, 1964; Bikales, 1969). Analytical methods for determining BPA residues in plastic articles, as well as in foods and food simulants, are required to evaluate dietary exposure to BPA from reusable PC articles. High-pressure liquid chromatography (HPLC) methods are reported in the literature (Gandara et al., 1990; Losada et al., 1991) for the determination of BPA in epoxy resins and the diglycidyl ether of BPA (DGEBA) migrating from films to aqueous food simulants. However, neither of these methods was suitable for accomplishing our goals. Neither method dealt with residual BPA in PC nor its migration to water and food simulating solvents as in our investigation. This paper describes the analytical procedures developed and the results of several migration scenarios.

Residual BPA in PC housewares such as baby bottles was determined by dissolving the polymer in methylene chloride and precipitating the PC by adding methanol. The residual BPA in the methylene chloride/methanol solvent was determined by HPLC after filtration. BPA was identified by comparing its liquid chromatographic retention volume with that of an authentic BPA standard.

Migration of BPA was assessed by placing the polymer in contact with water and food simulants under time and temperature test conditions. Either the PC bottle was filled with water or simulant or a portion of the article was exposed to the water or simulant in a sealed vial and held at a constant temperature for the duration of the migration experiment. Then the food-simulating liquids or foods were analyzed directly by HPLC and the identity of the analyte was confirmed by GC−MS. Residual BPA in water stored in 5-gal carboys was first concentrated on a solid phase extraction cartridge and eluted in a small volume of chloroform.

EXPERIMENTAL PROCEDURES

Reagents. All solvents were HPLC grade and purchased from Burdick and Jackson, Inc. (Muskegon, MI). Bisphenol-A purchased from Aldrich Chemical Co. (St. Louis, MO) was of 99+% purity. Water was distilled and then purified by a Milli-Q water purification system (Millipore Corp., Milford, MA). A fatty food simulating liquid, Miglyol 812 (a distillation fraction of coconut oil), was purchased from Huls America, Inc. (Piscataway, NJ). Ninety-five percent ethanol, a food simulating liquid, was purchased from Pharmaco (Bayonne, NJ). A powdered infant formula (Gerber Products Co., Freemont, MI) was purchased from a local supermarket and prepared daily as needed according to instructions on the label.

HPLC Apparatus and Operating Conditions. The HPLC system consisted of a Spectra Physics Model 8800 pump (Spectra-Physics, San Jose, CA), a Rheodyne Model 7125 injector valve (Rheodyne, Inc., Cotati, CA) equipped with a 20-μL injection loop, a second Rheodyne 7125 injector valve equipped with a Shandon Hypercarb S 7-μm guard column (Keystone Scientific, Inc., Bellefonte, PA) as the injector loop, a Spectra-Physics SP8792 column heater set at 40 °C, a 7-μm particle size, 150 × 4.6 mm Shandon Hypercarb S graphitized carbon column (Keystone Scientific), a Shimadzu RF-551 fluorescence detector (Shimadzu Corporation, Tokyo, Japan) operated at 235 nm excitation and 317 nm emission, and a Spectra-Physics Chromstation/2 data system interfaced to an IBM PS/2 Model 80 personal computer. The HPLC mobile phase consisted of 25.0% methanol, 26.2% water, and 48.8% acetonitrile, pumped at a flow rate of 0.5 mL/min under isocratic conditions.

GC−MS Apparatus and Operating Conditions. The GC−MS system consisted of a Hewlett-Packard (HP) 5890B

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gas chromatograph (GC) with an HP 7673 Automated Liquid Sampler, a capillary split-splitless injector, a 30 m × 0.25 mm i.d. Rt_x-5 FSOT capillary column with 1.0 μm d_f (Restek Corp., Bellefonte, PA) and an HP 5970B mass selective detector (MSD) with capillary direct interface to GC. The GC operating parameters were: UHP helium carrier gas at ca. 10 psi column head pressure (1 mL/min); injection volume, 2 μL; split vent open after 1 min; temperatures injector 280 °C, interface 290 °C; oven program, initial temperature 100 °C, program at 10 °C/min to 280 °C and hold 3 min. (BPA t_R, ca. 19.10 min). The MSD is operated in the selected ion monitoring mode; ions *m*/*z* 119, 213, and 228 are monitored at 2.6 scans/s from 18.8 to 19.3 min, dwell 100 ms. The GC−MS system was controlled with a Pascal Chemstation Data System.

HPLC Separation and Quantitation. External calibration was performed by using chromatographic responses of at least five concentrations of bisphenol-A ranging from 5 to 500 ng/mL. The standard curve of concentrations versus chromatographic peak areas was calculated from a linear regression program.

GC−MS Separation and Quantitation. Confirmation of the identity of BPA in chloroform extracts of bottled water and selected food simulants was based on the ratio of integrated peak areas of ions *m*/*z* 119, 213, and 228, 0.18:1:0.30, respectively ±25%. Quantitation of BPA in chloroform extracts from bottled water was by external calibration, based on the integrated response of the BPA base peak (ion *m*/*z* 213).

Polycarbonate Food Contact Articles. Baby bottles and a training cup were purchased from several grocery and drug stores in the Washington, DC, metropolitan area. The bottles were the products of at least six different manufacturers (brands A through F). The 5-gal water carboys were also obtained locally.

Polymer Analysis. A 1.00-g portion of polymer was weighed into a 250-mL Erlenmeyer flask, 20.0 mL of methylene chloride added, and the flask placed in an ultrasonic water bath at ambient temperature. The flask and contents were kept in the ultrasonic water bath until the polymer was completely dissolved (usually 15 min). To the dissolved polymer, 50.0 mL of methanol was added, dropwise initially while the flask was agitated until precipitation of the polymer was complete, then the remaining methanol was added more rapidly, ca. 10 mL/min. The polymer precipitate was allowed to settle for 10 min. Then an aliquot of the supernate was removed and filtered through a 0.45-μm nylon-66 membrane filter (0.45-μm glass microfiber filters were substituted without problems). A 1-mL portion of the filtrate was diluted to 10.0 mL in a volumetric flask with methanol and analyzed by HPLC. A blank was prepared and analyzed by omitting the polymer.

To compare bottle wall thickness with residual BPA levels, ca. 1-g coupons were cut from adjacent areas of a bottle and their thickness measured with a micrometer in at least five different places. The average of the five readings was reported along with the residual BPA level found by HPLC.

Recovery Studies. A 1 mg/mL BPA stock solution was prepared in 10% (v/v) methanol/methylene chloride. To two identical sets of five 100.0-mL volumetric flasks, 0.075 mL and 0.150 mL of the BPA stock solution were added, respectively. A flask in each of the two sets was diluted to volume with water, 10% (v/v) ethanol/water, 50% (v/v) ethanol/water, 95% (v/v) ethanol/water, and Miglyol, respectively. The final concentration for each solvent pair was 0.75 and 1.50 μg/mL. A third set of five flasks were prepared without addition of stock solution to serve as blanks. Aliquots of 25.0 mL each were pipetted from each flask into clean 40-mL glass vials and sealed with Teflon septa and screw caps. These vials were placed into a preheated 65 °C forced air oven for 240 h. At the end of the 240-h period a 0.100-mL aliquot was removed from each vial, diluted to 5.0 mL in methanol, and analyzed by HPLC.

Migration from Baby Bottles. Four migration experiments, numbered 1 through 4 below, were performed with the PC baby bottles. These conditions were representative of exaggerated, repeat use, typical use, and more extreme typical use with extreme time and temperature conditions.

Exaggerated Conditions. The PC bottles were immersed in boiling HPLC grade water for 10 min. Then, in triplicate, ca. 6.0 × 4.0 cm portions were cut from each bottle. A portion of the bottle was reserved for determination of residual BPA. The polymer was folded to permit it to fit through the mouth of a tared 40-mL screw cap glass vial. The weight of the folded polymer and vial were determined and the weight of the polymer was then calculated. A 25.0-mL aliquot of food simulant was pipetted into a vial containing the polymer, then all of the vials, including blanks and fortified blanks, were placed in an oven heated to 65 °C. The vials were agitated on a vertical rotisserie apparatus controlled by a Variac (Limm and Hollifield, 1995; A. D. Little, Inc., 1990) set to operate at the slowest possible rotation speed. Each vial was sampled periodically (24, 48, 72 h, etc.) by removing a 0.01- to 0.05-mL aliquot with a microliter syringe. The aliquot was diluted with methanol in a volumetric flask so that the response by HPLC for BPA fell within the response observed for the 5 and 500 ng/mL BPA standards.

Repeat-Use Migration Experiment. A PC bottle was immersed in boiling HPLC grade water for 30 min to "sterilize" it, then removed from the water and cooled to room temperature. In triplicate, ca. 6.0 × 10.0 cm (6 g) of the bottle was cut into strips that fit through the mouth of a tared 40-mL vial. The weight of the polymer and vial were determined and the weight of the polymer was calculated. Ten milliliters of 10% (v/v) ethanol/water was pipetted into the vials. The vials containing polymer and simulant were then placed into a preheated 100 °C forced air oven equipped with the rotisserie apparatus as in the previous experiment. Careful capping of the vials was essential to prevent leakage and/or septum blowout. The vials were removed from the oven after 30 min. All of the original simulant was decanted from the vials, leaving behind only the polymer. The vials and polymer were rinsed with 10 mL of 10% ethanol and the wash discarded. Each vial was refilled with 10.0 mL of fresh 10% ethanol and the entire procedure repeated three more times for a total of four 30-min heating intervals. An aliquot of the simulant from each heating cycle was analyzed by HPLC.

Typical Use Conditions with a Whole Bottle. One gram of polymer from the threaded portion of a PC baby bottle was removed, leaving enough thread to secure the cap. The surface area of exposure and the BPA residual in the 1-g portion were determined. Each bottle was washed with soap and water, rinsed, boiled in HPLC grade water for 5 min, filled with formula or apple juice, and then refrigerated at 4 °C for up to 72 h. At regular time intervals small aliquots (up to 0.25 mL) of the beverage were removed with a microliter syringe. The aliquots were diluted in a 5.0-mL flask with methanol and analyzed by HPLC.

More Sensitive Experiment for Typical Use Conditions. In triplicate, 6 × 4 cm pieces were cut from a bottle that had been analyzed for residual BPA, washed, and then boiled in HPLC grade water for 5 min. Each bottle piece (folded to fit through the mouth of the vial) was tared into a 40-mL vial, the weight of the polymer (ca. 2 g) and vial was determined and the weight of polymer was calculated. To each vial, 20.0 mL of water or 10% (v/v) ethanol/water (care was taken to ensure that all of the polymer was immersed) was added and the vial sealed. The vials were placed into a preheated 100 °C forced air oven for 30 min, then cooled to room temperature, an aliquot was then removed with a microliter syringe, diluted with methanol, and analyzed by HPLC. The vials were placed in a refrigerator (4 °C) and aliquots were removed after 48 and 72 h. The vials were brought to room temperature briefly before aliquots were removed.

Migration from Water Carboys. The 20-mL solid phase extraction (SPE) cartridge containing 5 g C-8 reversed phase on silica (Varian C8 1225-6024, or equivalent) was conditioned with 3 × 20 mL volumes of chloroform, then with 3 × 20 mL volumes of methanol. The column was primed with 6 × 20 mL volumes of distilled deionized water (DDH_2O). A 1-L aliquot of bottled water was transferred from the PC carboy to a separatory funnel. The funnel was placed above the cartridge and a flow of ca. 3 mL/min was introduced through the cartridge using a vacuum system. The total volume of test

Table 1. Residual BPA in Selected Polycarbonate Baby Bottles

brand[a]	μg/g	origin	brand[a]	μg/g	origin
A	7.4	USA	D_7	32.8	USA
B	17.3	USA	D_8	34.0	USA
C	10.4	USA	D_9	43.1	USA
D_1	9.5	USA	D_{10}	36.3	USA
D_2	31.3	USA	D_{11}	29.1	USA
D_3	31.8	USA	D_{12}	33.7	USA
D_4	14.0	USA	E_1	22.5	Hong Kong
D_5	44.3	USA	E_2	17.1	Thailand
D_6	46.6	USA	F (juice cup)	57.7	Thailand

[a] D_1-D_{12} represent different lots of the same brand.

Table 2. Homogeneity of a Brand D Baby Bottle

thickness mm (mils)	residual BPA (μg/g)	thickness mm (mils)	residual BPA (μg/g)
0.63 (25)	33.1	0.998 (39)	38.7
0.79 (31)	31.3	0.68 (27)	32.0
1.0 (40)	34.0		

av = 33.8
% CV = 8.6

solution was passed through the cartridge. The funnel was washed with 2 × 20 mL volumes DDH_2O and the wash allowed to pass through the cartridge. The total aqueous eluate was discarded and any residual water was removed from the cartridge by vacuum. BPA was eluted from the cartridge into a calibrated 25-mL conical tube with 20 mL chloroform, the chloroform volume reduced to 1 mL over steam, and BPA determined by GC−MSD.

RESULTS

Bisphenol-A was determined in the PC baby bottles and cup at levels ranging from 7 to 58 μg/g (Table 1). The wide range of residue levels from article to article may be due in part to processing and/or storage conditions (Gachter and Muller, 1990; Gardner and Martin, 1979) as well as raw material sources.

The thickness of the PC bottle walls ranged from 0.38 (15 mils) to 1.5 mm (60 mils) within a single bottle. The observed variability in thickness could result from processing conditions that could also affect the levels of residual BPA in the polymer. The residue levels and bottle thicknesses were compared for several brand D bottles (Table 2). Our data indicate that there was no relation between the thickness and residual BPA levels, i.e., BPA was uniformly distributed in the plastic of each brand D bottle. For this reason all further experiments were conducted with brand D bottles.

We also measured the levels of BPA that migrate to foods and food simulants from PC bottles under both typical and exaggerated time and temperature conditions. Recovery of BPA was first measured with food simulants under the most severe conditions, 240 h at 65 °C. Good recoveries for three replicates of all simulants (CVs averaged 5%) were seen.

A migration experiment with parameters identical to those described in the recovery experiment above was performed with folded 6 × 4 cm pieces of polymer cut from a pre-boiled bottle and placed into glass screw cap vials containing 25.0 mL of the selected food simulants (Table 3 and Figure 1). Migration as high as 368% of the original residue level (31.5 μg/g) of BPA in the PC was observed for the 50% ethanol food simulant. The polymer exposed to 95% ethanol clouded the simulant after 162 h. Further analysis on the 95% ethanol simulant showed that the BPA level dropped after 162 h; after 240 h, the food simulant had turned into a gel. This may be the result of condensation of the free BPA into low molecular weight oligomers (Gachter and Muller, 1990). The levels of BPA found in 50 and 95% ethanol in water solutions were significantly higher than the expected levels for 100% migration of the original BPA. This indicated that hydrolysis of the polymer affected BPA migration when these simulants were heated to 65 °C. The food simulant tests also indicate the importance of mixing. Migration of BPA to Miglyol and water was greater when test portions were mixed (agitated) as opposed to static tests. It is likely that partitioning may have suppressed the total BPA migration in the static trial (Limm and Hollifield, 1995).

Table 3. 240-h Migration Data for BPA from PC[a] at 65 °C

simulant	agitation	μg/cm² migrated[b]	% migrated	% rec	% CV
water	Y	1.0	43.7	103	7
water	N	0.23	13.2	103	8
8% EtOH	Y	0.87	50.2	108	11
10% EtOH	N	0.91	45.7	108	10
50% EtOH	N	5.9	368	117	23
95% EtOH	N	2.2[c]	132[c]	116	19
Miglyol	Y	1.5	76.0	103	14
Miglyol	N	0.03	1.2	103	9

[a] BPA residue in polymer used in this experiment was 31.5 μg/g. [b] Average of three trials, corrected for recoveries. [c] 162-h data. After 162 h, the solutions were cloudy, which was not characteristic of the other solutions at any time during the experiment. The 240-h measurement indicated a loss of BPA of 19.6 μg/g of polymer.

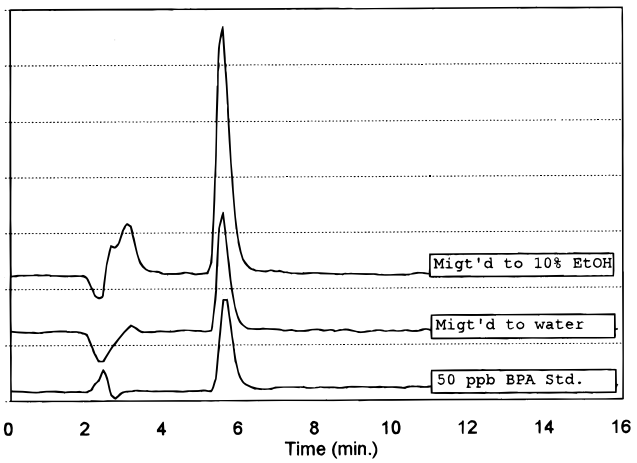

Figure 1. Chromatogram of Bisphenol-A migrated to water and 10% ethanol.

Since most PC articles are intended to be used again (in some rare instances, formula may be sterilized in bottles in a boiling water bath), an experiment was conducted to provide information on migration over several use cycles. A bottle was analyzed for residual BPA and the remainder of the bottle was cut into pieces that would fit through the mouth of a 40-mL vial. The sensitivity of the analytical method was enhanced by placing 6 g of a bottle into each vial with 10 mL of 10% ethanol. The vials containing polymer and simulant were agitated while heated in the oven at 100 °C for 30 min. The vials were removed from the oven and cooled to room temperature; the simulant was removed and analyzed for BPA. Fresh simulant was added to the vial for the next interval. The data suggest that after an initial "bloom" of BPA on the polymer surface was washed away, migration of BPA decreased rapidly and then leveled out with each subsequent interval in the experiment (Table 4).

In two other experiments, BPA migration from PC bottles to foods and food simulants was measured under normal use conditions. (These conditions were less severe than the exaggerated time/temperature condi-

Table 4. Repeated Use Migration Experiment[a]

	residual BPA observed to migrate		
interval no.	% of residual	μg/cm^2	% CV
1	4.9	0.21	11
2	1.7	0.07	8
3	0.9	0.04	12
4	0.9	0.04	9

[a] Polycarbonate (ca. 6 g) with 44.3 μg residual BPA per gram of polymer exposed to 10 mL of 10% ethanol at 100 °C for 30-min intervals. Polymer was rinsed with 10% ethanol between intervals and fresh 10% ethanol was used for each interval.

tions described earlier.) In the first of these experiments, whole bottles were cleaned, rinsed, held in boiling water for 5 min, and then filled with apple juice or formula and refrigerated at 4 °C for 72 h. This is a less sensitive experiment due to dilution with water required by matrix effects (detection limit of 100 ng/mL, which is equivalent to ca. 2% of residual BPA migrating from the bottle). Measurable BPA was not present in either the juice or formula.

In the second normal use experiment, bottle pieces were placed in 40-mL vials (as in exaggerated migration experiments) with food simulants, heated (sterilized) at 100 °C in an oven for 30 min, and refrigerated for 72 h. Greater contact between the polymer and given volume of simulant resulted in increased sensitivity. Sensitivity for BPA increased by measuring double-sided as opposed to a single-sided migration experiment with whole bottles. The amount of BPA that migrated into 10% ethanol and water by using the double-sided exposure was 2 ng/cm^2. This migration amount was equivalent to 2 ng/g in the formula, when converted by using the internal surface area in contact with the food (249 cm^2) when a bottle is completely filled (250 mL). Essentially all of the BPA migration occurred in the 30-min 100 °C sterilization step, with little or no measurable migration during the 72-h refrigeration.

These results, on a relative basis, were consistent with the amount of BPA measured in water which was autoclaved in polycarbonate for 30 min at 120−125 °C (Krishnan et al., 1993). Here, under autoclave conditions, the higher migration reported, 3 ng/g, was related to the higher temperature.

The limit of detection (defined as 3 times the standard deviation of a signal produced by the detector when measuring a blank) for BPA was determined to be 2 ng/mL in ethanolic simulants and water. Limits of detection for BPA in fruit juices, infant formula, and Miglyol were higher, ca. 100 ng/mL as a result of matrix effects.

No residual BPA determinations were performed on 5-gal (19 L) PC water carboys. However, BPA residues from 1-L volumes of water bottled in PC carboys with different fill dates were concentrated on SPE cartridges, eluted with chloroform, concentrated, and analyzed by GC−MSD. All analyses were performed in early July, 1995. BPA residues were found in all the waters tested, and based on fill dates the concentration of BPA in the waters increased with contact time. BPA concentrations in the bottled waters ranged from 0.1 to 4.7 ng/L, with a detection limit <0.05 ng/L. BPA recovery from fortified bottled water at the 1 and 3 ng/L level was 98−105% (Table 5).

Table 5. Bisphenol-A Residues Found in Water Bottled in 5-gal Polycarbonate Carboys

time[a] (weeks)	BPA found[b] (ng/L)	fortification (ng/L)	% recovery
39	4.7, 4.6	3	104, 105
12	0.5, 0.4		
3	0.1, 0.2		
distilled water	<0.1	1	98, 98

[a] Elapsed time water was in carboy prior to analysis. [b] All analyses performed in July 1995.

DISCUSSION

This investigation shows that residual BPA is present and can be quantitated in PC polymers. Residual BPA in PC baby bottles migrates to food simulants and can be determined at low ppb levels in liquid foods and food simulants by using HPLC and GC−MSD methods. Under exaggerated test conditions PC undergoes hydrolysis yielding additional BPA, which also migrates to food simulants. When whole PC baby bottles were tested by using typical fill conditions and less severe, normal use conditions, neither BPA migration nor hydrolysis were observed (limit of detection was 2 ng/mL). Future work should investigate the degree to which hydrolysis affects total BPA migration, which foods may augment hydrolysis of the polymer, and to what extent, if any, BPA bloom contributes to migration.

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