

Migration of Bisphenol A from Polycarbonate Baby and Water Bottles into Water under Severe Conditions

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The isotope dilution headspace solid-phase microextraction and gas chromatography–mass spectrometry method for bisphenol A (BPA) developed previously was used successfully in a BPA migration study at 70 °C of polycarbonate baby and reusable water bottles recently sold in Canada by using the whole bottles instead of pieces cut from the bottles. Migration of BPA from the PC bottles heated at 70 °C was found to increase over the time in the quadratic equations. Migration levels of BPA in water varied from 228 to 521 $\mu\text{g L}^{-1}$ or from 0.26 to 0.90 $\mu\text{g cm}^{-2}$ after being heated at 70 °C for 6 days. The average migration rates of BPA from the PC bottles into water at 70 °C ranged from 1.84 to 4.83 $\text{ng cm}^{-2} \text{h}^{-1}$.

KEYWORDS: Bisphenol A; migration; polycarbonate; baby bottle; water bottle

INTRODUCTION

Polycarbonate (PC) is a clear, strong, and rigid thermoplastic and is used in the production of food containers such as baby bottles, water bottles, and water carboys. PC is produced by the reaction of the monomer bisphenol A (BPA) with phosgene gas (carbonyl chloride) in the presence of sodium hydroxide to form the carbonate linkage in the polymer. Because of the incomplete reactions, the unreacted BPA will be present in the PC products. As summarized in **Table 1**, the residual levels of BPA in PC baby bottles ranged from <1 ppm to as high as 599 ppm, with average levels from 9.9 to 177 ppm (1–4). The residual BPA in the PC products may migrate into foods, especially at elevated temperatures, for extended periods of time. PC could also degrade slowly by hydrolysis of the carbonate linkages at elevated temperatures and in an alkaline pH environment (5), and the BPA released from this degradation will also migrate into foods.

Because BPA is a potential endocrine disruptor that mimics the action of the hormone estrogen (6), the EC Directive reduced the previous specific migration limit for BPA at 3 mg kg^{-1} in food or food simulant (7) to 0.6 mg kg^{-1} in an amending document relating to plastic materials and articles intended to come into contact with foodstuffs (8). The tolerable daily intake for BPA was established at 50 $\mu\text{g kg}^{-1}$ body weight day^{-1} by the United States and the European Food Safety Authority (9), while Health Canada established the provisional tolerable daily intake for BPA at 25 $\mu\text{g kg}^{-1}$ body weight day^{-1} .

Migrations of BPA from PC baby bottles into food simulants have been investigated under less severe or real use conditions with whole PC bottles. In the work of Mountfort et al. (10), the possible release of BPA from the PC baby bottles following a series of cycles of sterilization by alkaline hypochlorite, steam,

or washing in a dishwasher at 65 °C was investigated, but BPA was not detected in food simulants in all cases due to the less sensitive method used (limit of detection, 30 $\mu\text{g kg}^{-1}$). However, Brede et al. (11) found that the average BPA levels in food stimulant in PC baby bottles after repeated simulated use were 8.4 ± 4 (dishwashed 51 times) and 6.7 ± 4 $\mu\text{g L}^{-1}$ (dishwashed 169 times). Maragou et al. (12) observed that BPA migration levels ranged from 2.4 to 14.3 $\mu\text{g L}^{-1}$ for 31 PC baby bottles when filled with boiled water and left at room temperature for 45 min.

Migrations of BPA from PC baby bottles into food simulants were also investigated under severe conditions (for example, at high temperatures for extended periods of time) to simulate the worst-case migration scenario by using pieces cut from the whole PC bottles according to the recommendations of the U.S. FDA for repeated-use food-contact articles (13, 14). The migration of BPA from a 6 cm \times 4 cm piece of a PC bottle was detected at 1.0 $\mu\text{g/cm}^2$ for water as the food stimulant and as high as 5.9 $\mu\text{g/cm}^2$ for 50% ethanol as the food stimulant after heating at 65 °C for 10 days (1). BPA was not detected (<50 ng/in^2) in any of the food simulants after heating the pieces of a PC bottle at 100 °C for 6 h or at 49 °C for 10 days (3). Migration levels of BPA from pieces of 28 PC baby bottle products ranged from not detected (<0.05 $\mu\text{g/in}^2$) to 1.92 $\mu\text{g/in}^2$ in 10% ethanol after heating at 70 °C for 10 days and ranged from not detected (0.05 $\mu\text{g/in}^2$) to 6.54 $\mu\text{g/in}^2$ in corn oil after heating at 100 °C for 10 days (4). Although this is a simple approach to investigate BPA migration under severe conditions, the results obtained may not reflect the real situations. First, the PC bottle product is not homogeneous in thickness and residue BPA concentration; thus, the results from a single piece of the product will not represent the whole bottle (1). Second,

Table 1. Average, Median, Minimum, and Maximum Concentrations (ppm) of Residual BPA in PC Baby Bottles

average	median	minimum	maximum	refs
177	47	<1	599	(2)
28.8	32	7.4	57.7	(1)
9.9	not available	8.8	11.2	(3)
20.0	9	<3	141	(4)

BPA will migrate from both surfaces plus the four sides, depending on the thickness of the piece cut from the product by using this approach, which is in contrast to BPA migration from only one side of the bottle in the real situation. Migration of BPA will also decrease after initial "bloom" as observed by Biles et al. (1) due to the finite amount of residual BPA in the piece of product used, and this again does not reflect the real situation.

In this work, migration of BPA from PC bottles recently sold in Canada under severe conditions was investigated using the whole PC bottles. The PC reusable water bottles used by the general population were also included in this study in addition to the baby bottles, since investigations have rarely been conducted to investigate the migration of BPA from the PC reusable water bottles (15). Like the baby bottles, reusable water bottles are also intended to be used repeatedly and are subjected to routine cleaning procedures (such as washing with detergent at relatively high temperatures). Filling reusable water bottles with hot water and reheating the water in water bottles in a microwave are also routine practices among some populations.

MATERIALS AND METHODS

Reagents and Materials. Methanol (99.8%), toluene (99%), trimethylchlorosilane (99%), BPA (99%), and BPA- d_{16} (98 atom % D) were purchased from Sigma-Aldrich (Mississauga, Ontario). Sodium chloride (>99.0%) was obtained from VWR (Mississauga, Ontario).

To minimize losses of BPA onto glass surfaces, all glassware was deactivated by a procedure developed previously (16). In brief, glassware was heated in an oven at 200 °C to remove any water adsorbed on the glass surfaces, and the heated glassware was soaked overnight in a 5% trimethylchlorosilane solution in toluene. The glassware was rinsed with toluene and methanol and then dried in an oven at 100 °C for 1 h.

Individual standard solutions of BPA and BPA- d_{16} were prepared in methanol. For solid-phase microextraction (SPME) analysis, BPA standard solutions in water with concentrations of 5–600 $\mu\text{g L}^{-1}$ were prepared by injecting methanol solutions of BPA and BPA- d_{16} into 10 mL of water in a 20 mL amber vial. Tap water was used in preparation of BPA water standard solutions and for blanks since deionized water contains BPA at higher levels. All solutions were stored at 4 °C.

The 60 μm Carbowax-polyethylene glycol (CW-PEG) metal alloy SPME fiber was purchased from Supelco (Bellefonte, PA). The 20 mL SPME screw top amber vials with metal caps were purchased from Gerstel (Baltimore, MD).

Migration of BPA from PC Bottles. Five PC bottles with different brand names were purchased in a local store in Ottawa; three of the brands were baby bottles, and the other two brands were reusable water bottles. All bottles were rinsed with boiling water. For the migration experiment, boiling water was added to each bottle to its capacity, and the bottles were placed in an oven at 70 °C. Water samples (10 mL) were taken from each bottle after being incubated for 1, 2, 3, and 6 days and analyzed by headspace SPME and GC-MS.

Sample Preparation. An amount of 3 g of NaCl (preheated at 200 °C for at least 24 h) was weighed into a 20 mL amber vial. A 10 mL aliquot of water sample was added via pipettor into the 20 mL vial and was spiked (directly into the sample) with internal standard solution, and the vial was capped. The sample was vortexed for 10–15 s to speed up the dissolution of NaCl in the sample. Samples were loaded onto the autosampler tray and equilibrated at room temperature for 2 h.

SPME and Instrument Conditions. An Agilent 6890 gas chromatograph (GC) coupled to a 5973N mass selective detector (MSD) was used for the analysis. The GC-MSD was equipped with a MultiPurpose Autosampler (MPS 2) from Gerstel (Baltimore, MD), which was set up in SPME operation mode. At the beginning of the sequence of analysis, the sample vial was transported from the tray to the agitator held at 95 °C. After it was incubated for 1 min, the SPME fiber was inserted through the septum into the headspace. The vial penetration depth was set at 25 mm, and the tip of the SPME fiber was 2.0 cm above the surface of the sample solution. The agitation speed was set at 250 rpm. After the extraction for 60 min, the SPME fiber was inserted into the injector fitted with a 0.75 mm i.d. liner. The injector temperature was set at 250 °C. The injection penetration depth was set at 65 mm, and the SPME fiber was desorbed for 5 min in splitless mode. After desorption, the SPME fiber stayed in the injector for another 10 min for cleaning with the purge flow of helium to the split vent at 50 mL min^{-1} . Analytes were separated on a Zebron ZB-5 (5% phenyl and 95% dimethylpolysiloxane) capillary column (30 m \times 0.25 mm \times 1.0 μm) from Phenomenex (Torrance, CA). The flow rate of the helium carrier gas was 1.5 mL min^{-1} . The GC oven temperature program was set at an initial temperature of 50 °C for 5 min, raised to 280 °C at 15 °C min^{-1} , and held for 10 min.

The MSD was operated with electron impact ionization in selected ion monitoring (SIM) mode. The ions selected were 213 and 228 for BPA and 224 and 242 for BPA- d_{16} . The dwell time was 50 ms for each ion. The GC-MSD interface and MSD source temperatures were 280 and 230 °C, respectively.

Quantitation and Quality Control. Confirmation of BPA identity was based on the retention time being within ± 0.01 min of that of the standard and that the ion ratio must be within $\pm 25\%$ of that obtained using the standard. The calculation of BPA concentrations in samples was based on the calibration curves (5–600 $\mu\text{g L}^{-1}$) of ratios of native BPA peak areas over the deuterated BPA peak area plotted with the ratios of native BPA concentration over the deuterated BPA concentration. For each sequence of analysis, three water blanks were analyzed under the same conditions as the standards and samples, and the average blank level of BPA was subtracted from the results of standards and samples.

RESULTS AND DISCUSSION

The isotope dilution headspace SPME and GC-MS methods for determination of BPA in water were developed and validated previously (16). The instrument detection limit was 0.05 $\mu\text{g L}^{-1}$, and the method detection limit was 0.5 $\mu\text{g L}^{-1}$. Linearity of the instrument and the method was demonstrated with a six-point calibration curve over the concentration range of 5–600 $\mu\text{g L}^{-1}$ ($R^2 > 0.998$).

Water has often been used as the food simulant to mimic aqueous foodstuffs during migration studies and was also used as the food simulant in this study. The pH of the water used in the migration studies was about 7. **Table 2** shows the concentrations of BPA in water in the five PC bottles after being heated at 70 °C for different durations. Because a 10 mL water sample was taken from each bottle for analysis starting at 24 h, higher concentrations of BPA in water samples during the subsequent migration periods were partly due to the smaller volume of water in the bottle than the volume at the beginning. To eliminate this factor so that variations of BPA concentrations in water during different migration periods were purely due to migration, BPA concentrations in water at 48, 72, and 144 h were all corrected to the original volume of water.

Migrations of BPA increased with time for all PC bottles in a similar trend, and they all fitted well in the quadratic equations (second-degree polynomial) with R^2 values better than 0.9978. Migration levels of BPA were the highest for brand C baby bottles (516 $\mu\text{g L}^{-1}$ at day 6) and brand D water bottles (521 $\mu\text{g L}^{-1}$ at day 6) during the whole migration period tested,

Table 2. Concentrations ($\mu\text{g L}^{-1}$) of BPA in Water in Different PC Bottles Heated at 70 °C for 1, 2, 3, and 6 Days

bottle brand	type	surface area (cm^2)	volume (mL)	migration time (h)				equation	R^2
				24	48	72	144		
A	baby bottle	130	150	32.0	65.3	99	228	$y = 0.0026x^2 + 1.2098x$	0.9998
B	baby bottle	130	150	46.3	87.7	159	374	$y = 0.0065x^2 + 1.6686x$	0.9990
C	baby bottle	198	280	54.7	105	204	516	$y = 0.0121x^2 + 1.8535x$	0.9987
D	water bottle	349	600	51.3	105	213	521	$y = 0.0119x^2 + 1.9104x$	0.9978
E	water bottle	427	800	34.1	60.6	114	286	$y = 0.0062x^2 + 1.0971x$	0.9986

Table 3. Average, Minimum, and Maximum Migration Levels ($\mu\text{g cm}^{-2}$) of BPA in Water in PC Bottles Heated at 70 °C for 1, 2, 3, and 6 Days

BPA migration level ($\mu\text{g cm}^{-2}$)	migration time (h)				(1)	(4)
	24	48	72	144	migration at 65 °C for 240 h in water	migration at 70 °C for 240 h in 10% ethanol
average	0.064	0.12	0.23	0.57		
minimum	0.037	0.076	0.11	0.26	0.23 ^a	0.068
maximum	0.088	0.18	0.37	0.90	1.0 ^b	0.30

^a Without agitation. ^b With agitation.

followed by brand B baby bottles ($374 \mu\text{g L}^{-1}$ at day 6), brand E water bottles ($286 \mu\text{g L}^{-1}$ at day 6), and brand A baby bottles ($228 \mu\text{g L}^{-1}$ at day 6). Because all bottles were under the same migration conditions, the differences between the migration levels are likely due to the different levels of residue BPA in the products. Although residual BPA levels in these five PC bottles were not determined in this work, the relationships between residual levels of BPA in the products and the migration levels are well-established (2); higher residual levels of BPA in the products will lead to higher migration levels of BPA under the same conditions. Degradation of PC at elevated temperatures, which may be different from one product to another, could also be a factor.

To compare the BPA migration levels obtained with the whole-bottle method in this work with the results reported in the literature (using the pieces cut from bottle), units of BPA migration levels were converted from $\mu\text{g L}^{-1}$ to $\mu\text{g cm}^{-2}$ by using the surface areas and volumes of the bottles. The results are shown in **Table 3**, together with the results from Biles et al. (1) and Wong et al. (4) for comparison. The results from Biles et al. (1) were obtained from only one PC baby bottle by heating the bottle piece in a glass vial filled with water at 65 °C for 10 days (240 h). The migration level was $0.23 \mu\text{g cm}^{-2}$ without agitation, which is close to the minimum migration level but about four times lower than the maximum migration level found in this work after 6 days of migration at 70 °C. The migration level was about four times higher with agitation ($1.0 \mu\text{g cm}^{-2}$). The results of Wong et al. (4) were obtained from 28 PC baby bottles by heating the bottle pieces in glass vials filled with water at 70 °C for 10 days. The maximum migration level was $0.30 \mu\text{g cm}^{-2}$, three times less than the maximum migration level ($0.90 \mu\text{g cm}^{-2}$) obtained at 70 °C for 6 days in this work. It should be mentioned that it is not clear whether the surface areas of both sides of the bottle piece were used in the calculations of migration levels reported by Biles et al. (1) and Wong et al. (4), and their reported results will be even lower (reduced by half) if the surface area from only one side of the bottle piece was used in the calculations. However, because the migration study was conducted with only the whole bottle in this work, the discrepancies observed between this work and others are not conclusive, and they will be addressed with further investigations using both approaches (whole bottle vs pieces cut from bottle) with bottles from the same lot.

Table 4. Migration Rate ($\text{ng cm}^{-2} \text{h}^{-1}$) of BPA from PC Bottles Heated at 70 °C

bottle brand	migration rate ($\text{ng cm}^{-2} \text{h}^{-1}$)					
	24 h	48 h	72 h	144 h	average	RSD (%)
A	1.54	1.69	1.83	2.29	1.84	17.7
B	2.23	2.27	2.95	3.76	2.80	25.7
C	3.23	3.22	4.33	5.68	4.11	28.4
D	3.68	3.84	5.26	6.56	4.83	27.9
E	2.66	2.39	3.05	3.87	3.00	21.4

Migration rates, defined as the amount of BPA migrated from the unit surface area of the bottle per unit of time, were also calculated and are shown in **Table 4**. The average BPA migration rates ranged from $1.84 \text{ ng cm}^{-2} \text{ h}^{-1}$ for brand A bottles to $4.83 \text{ ng cm}^{-2} \text{ h}^{-1}$ for brand D bottles.

It should be mentioned that migration levels of BPA from PC bottles at lower temperatures will be much lower; it was observed in a previous study that levels of BPA in water contained in PC bottles ranged from 1.7 to $4.1 \mu\text{g L}^{-1}$ after being filled with boiling water and stored at room temperature for 24 h (16).

In summary, BPA migration from various PC baby and water bottles recently sold in Canada was investigated with the whole bottles under severe conditions. To minimize the exposure to BPA, the contact time of infant formula with the PC bottles should be kept to a minimum if they have to be warmed up in a microwave, and the leftovers should be discarded to avoid accumulation of BPA in the formula. For PC reusable water bottles, filling with hot water or heating in the microwave to high temperatures will increase the level of BPA migration and, thus, is not encouraged.

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