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Bisphenol A in Baby Food Products in Glass Jars with Metal Lids from Canadian Markets

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A method based on solid phase extraction and derivatization with acetic anhydride followed by gas chromatography−mass spectrometry was validated for the determination of bisphenol A (BPA) in baby foods. The average method detection limit (MDL) was 0.18 ng/g for a 5 g sample. Method repeatability was demonstrated with the replicate analyses of various different types of baby foods; relative standard deviations (RSD) ranged from 1.2 to 16.1% with an average of 8.7%. Extraction recoveries ranged from 93.5 to 102.5% for different types of baby foods spiked at levels of 1−8 ng/g. This method was used to analyze 122 baby food products of 7 brands in glass jars with metal lids for BPA. The presence of BPA could not be confirmed and quantified for 23 of the 122 products due to interference from sample matrices. For the other 99 products, 15% had BPA levels of less than the average MDL, about 70% had BPA levels of less than 1 ng/g, and the average BPA levels in all 99 products was 1.1 ng/g. The average BPA level in the baby food products from brand E (3.9 ng/g) is higher than the average BPA levels in the products from the other brands (0.54−1.1 ng/g). The highest level of BPA, 7.2 ng/g, was found in two products from brand E as well. The average BPA level in the fruit products from all brands (0.60 ng/g) is lower than those in the mixed-dish products (1.1 ng/g) and the vegetable products (1.2 ng/g).

KEYWORDS: Bisphenol A; baby food; gas chromatography−mass spectrometry; solid phase extraction

INTRODUCTION

Bisphenol A (BPA) is used as a monomer in the production of epoxy resins, which are frequently used in the internal coating for food and beverage cans to protect the food and beverage from direct contact with metal and are also used in the internal coating on metal lids for foods in glass jars. Residues of BPA in these coatings can migrate into foods, especially at elevated temperatures. Because BPA is a potential endocrine disruptor that mimics the action of the hormone estrogen (*1*), the specific migration limit for BPA in food or food simulant was set at 0.6 μg/g by the EC Directive in an amending document relating to plastic materials and articles intended to come into contact with foodstuffs (*2*). The maximum acceptable dose and tolerable daily intake (TDI) for BPA were established at 50 μg/kg of body weight/day by the U.S. Environmental Protection Agency (*3*) and the European Food Safety Authority (*4*), respectively, whereas Health Canada established the provisional TDI for BPA at 25 μg/kg of body weight/day (*5*).

Although levels of BPA have been determined in various canned food products (*6*−*16*), information on BPA in foods, baby foods in particular, contained in glass jars with metal lids is simply not available. This may be due to the assumption that migration of BPA from the coating on metal lids into foods rarely happens compared to the canned foods and, thus, BPA levels in jarred foods are expected to be low. However, there are situations in which migration of BPA from coating on lids into foods will occur, such as during transportation due to shaking and accidental storage of the jarred foods in a nonvertical upright position. Although it is still not clear, the plasticized polyvinyl chloride (PVC) used in the gasket in the metal lids to seal against the glass rim could also be an additional source of BPA in jarred foods, as it is already known that BADGE is used as an additive for the elimination of surplus hydrochloric acid in the production of PVC organosols (*17*) and it is also reported that BPA is used as an additive in PVC films (*18*).

Because baby foods in glass jars with metal lids are an important part of the diets for children aged 6 months and older, the current exposure assessment for BPA for this age group conducted under the government of Canada's chemicals management plan (CMP), based on results from infant formula, human milk, and baby bottles only, is not complete and may be underestimated (*19*). In this work, the method used previously for the determination of BPA in liquid infant formula products (*15*) and soft drinks (*16*) was adapted and validated for the determination of BPA in baby foods; levels of BPA in various baby foods

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Table 1. Method Detection Limit, Repeatability, and Extraction Recoveries for Various Baby Food Samples

					recovery	
sample	*n*	av concn (ng/g)	RSD (%)	MDL (ng/g)	av ± SD[a] (%)	spiking level (ng/g)
apricot with mixed fruits, strained	6	0.37	14.5	0.18	99.2 ± 1.1	1
apple strawberry banana, strained	6	0.27	13.8	0.13	100.0 ± 5.7	1
mixed vegetables, strained	6	1.06	12.4		102.5 ± 5.0	1
vegetables and beef, strained	6	0.55	4.3	0.080	98.5 ± 3.0	1
wax beans	6	0.55	16.1	0.30	96.8 ± 3.3	1
mixed vegetables, strained	6	0.59	14.0	0.28	95.3 ± 2.5	1
mixed fruit with oatmeal cereal	6	<MDL			99.0 ± 1.1	1
creamed corn, organic	6	0.62	14.5	0.30	101.0 ± 1.0	1
vegetables and turkey, organic	6	0.66	3.1	0.068	101.1 ± 5.9	1
vegetables, organic	6	4.91	2.0		96.4 ± 2.9	5
apples and mango, strained, organic	6	2.68	1.2		99.3 ± 4.3	3
sweet potatoes and chicken, strained, organic	6	7.74	2.2		98.4 ± 5.2	8
sweet potatoes	6	0.76	4.1	0.10	94.5 ± 1.4	1
squash	6	0.64	10.9	0.23	93.5 ± 2.3	1
water	7	0.075	33.4	0.079		

[a] *n* = 3.

Table 2. Concentrations of BPA in Baby Foods Contained in Glass Jars with Metal Lids

		av concn (ng/g)	
brand	product name	by product	by brand
A	apricot with mixed fruits, strained	0.59	1.1 (range 0.40−1.6)
	pears, strained	0.40	
	peaches, strained	0.60	
	apple strawberry banana, strained	0.39	
	peas, strained	1.6	
	squash, strained	1.4	
	carrots, strained	1.6	
	sweet potatoes, strained	1.4	
	vegetable chicken dinner, strained	1.5	
	vegetable beef dinner, strained	1.6	
	chicken noodle dinner, strained	1.1	
B	strawberry dessert, strained	0.45	1.1 (range <MDL−1.7)
	apples and apricots, strained	<MDL	
	pears, strained	0.34	
	pears	<MDL	
	strawberry dessert	<MDL	
	vegetables, chicken, and rice	1.7	
	vegetables and turkey, strained	1.1	
	mixed vegetables, strained	1.3	
	green beans, strained	1.6	
	carrots and peas, strained	1.1	
	carrots, strained	0.95	
C	vegetables and beef, strained	0.71	0.77 (range <MDL−1.4)
	beef and beef broth, strained	1.4	
	chicken and chicken broth, strained	1.4	
	sweet potatoes, strained	1.4	
	sweet corn casserole, strained	0.52	
	apples and oatmeal, strained	<MDL	
	peaches, strained	0.19	
	apples and blueberries, strained	0.34	
	vanilla custard pudding with apples, strained	0.29	
	bananas, strained	<MDL	
D	wax beans	0.50	0.57 (nonorganic) (range <MDL−1.3)
	peas and carrots, strained	0.87	
	butternut squash	0.95	
	sweet potatoes	0.63	
	carrots	0.69	

Table 2. Continued

		av concn (ng/g)	
brand	product name	by product	by brand
	green beans	1.3	
	peas	0.90	
	creamed corn, strained	0.24	
	mixed vegetables, strained	1.1	
	peas	0.74	
	squash	0.76	
	apple juice from concentrate	<MDL	
	strawberry dessert, strained	0.26	
	custard, strained	0.83	
	apples and strawberries	<MDL	
	strawberry dessert	0.24	
	mixed fruit with oatmeal cereal	<MDL	
	sweet potato and turkey, strained	0.70	
	vegetables, rice and chicken, strained	0.62	
	chicken casserole with vegetables and rice, strained	0.40	
	vegetable and turkey, strained	0.75	
	chicken casserole with vegetables and rice	0.36	
	vegetable chicken and fruit medley	0.23	
	turkey stew	0.56	
	country casserole with chicken	0.30	
	turkey rice with vegetables	0.27	
	peas, organic	0.75	0.65 (organic) (range <MDL−1.1)
	butternut squash, organic	0.44	
	sweet potatoes, organic	0.54	
	carrots, organic	0.68	
	creamed corn, organic	0.42	
	apples and strawberries, organic	<MDL	
	green beans and apple blend, organic	0.84	
	vegetables and turkey, organic	0.69	
	vegetables and chicken, organic	1.1	
	vegetable beef, organic	0.85	
E	sweet potatoes, strained, organic	3.8	3.9 (range 1.7−7.2)
	carrots, strained, organic	7.2	
	vegetables, organic	4.5	
	apples, strained, organic	3.7	
	apples and apricots, strained, organic	1.9	
	apples and mango, strained, organic	2.7	
	apples and blueberries, strained, organic	2.3	
	apples and bananas, strained, organic	1.7	
	sweet potatoes and chicken, strained, organic	7.2	
F	carrots, organic	0.66	0.73 (range <MDL−1.2)
	green peas and brown rice, strained, organic	0.79	
	green beans and brown rice, strained, organic	1.2	
	apples and apricots, strained, organic	0.23	
	apples and blueberries, strained, organic	<MDL	
	oeach and apple oatmeal, strained, organic	<MDL	
	vegetable turkey casserole, organic	0.77	
	apples and bananas, organic	<MDL	
G	peas	0.48	0.54 (range <MDL−0.84)
	sweet potatoes	0.77	
	squash	0.73	
	carrots	0.71	
	mixed vegetables, strained	0.44	
	peas and carrots, strained	0.84	
	apples	0.22	
	peaches	<MDL	
	pears	<MDL	
	apples and raspberries, strained	<MDL	
	strawberry dessert, strained	0.25	
	vegetables and chicken, strained	0.59	
	vegetables and turkey, strained	0.62	
	vegetables, rice, and chicken, strained	0.32	

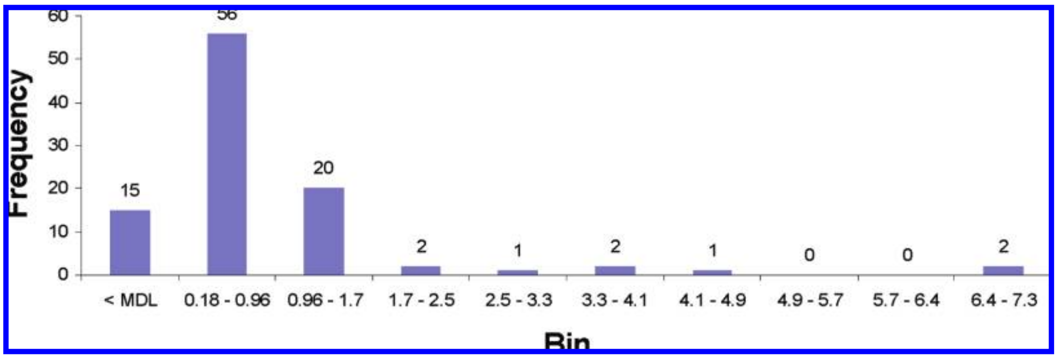

Figure 1. Histogram of BPA concentrations (ng/g) in 99 baby food products collected in 2008.

Table 3. Percentile Distribution of BPA Concentrations (Nanograms per Gram) in 99 Baby Food Products

min	10th	20th	30th	40th	50th	60th	70th	80th	85th	90th	95th	max
<MDL	0.28	0.40	0.54	0.66	0.74	0.84	1.1	1.4	1.6	1.7	3.5	7.2

contained in glass jars with metal lids sold in Canada were determined for the first time to provide data for updating the current exposure assessment.

Table 4. Ranges of Ion Ratios for the Baby Foods with Interferences Compared with the Corresponding Ion Ratios from the Standard

ion ratio (%)	standard	baby foods with interferences
m/*z* 228/213	25.6 ± 0.3	50.1−133
m/*z* 270/213	17.4 ± 0.4	0.3−12.9
m/*z* 312/213	5.2 ± 0.2	0.1−3.9

MATERIALS AND METHODS

Sample Collection. Samples of a total of 122 baby food products in glass jars with metal lids were collected in August 2008 from local stores in Ottawa. The size for most of the jars was 128 mL; a few jars of 105 or 213 mL were also included. These samples covered a wide variety of foods (fruits, vegetables, mixed dishes, organic, nonorganic, etc.) from seven brands with at least 80% market share of the baby food products sold in Canada. All samples were stored at room temperature before analysis.

Materials and Reagents. Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from J. T. Baker (Phillipsburg, NJ). Toluene (glass distilled), acetic anhydride (ACS grade), H_3PO_4 (85% HPLC grade), bisphenol A (99%), bisphenol A-d_{16} (98%), isooctane (pesticide-residue grade), methyl *tert*-butyl ether (MTBE, 99.9%), Na_2SO_4 (anhydrous, ACS grade), 1-pentanol (99%), and dodecane (99%) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Potassium carbonate (ACS grade) and Na_2HPO_4 (ACS grade) were purchased from Fisher (Ottawa, ON, Canada).

The 50-place stirring block was obtained from Barnstead (Dubuque, IA). The 13 × 100 mm, 20 × 150 mm, and 16 × 100 mm disposable glass tubes were purchased from VWR (Montréal, QC, Canada). The 15-mL centrifuge tubes were purchased from Fisher. The 22-mL vials and 6-mL glass columns were obtained from Supelco (Oakville, ON, Canada). The C18 SPE cartridges were purchased from Varian (Mississauga, ON, Canada).

BPA and BPA-d_{16} standard solutions were prepared in methanol and stored at 4 °C. The pH 7 phosphate buffer was prepared by dissolving 14.3 g of Na_2HPO_4 in 1 L of tap water, and the pH was adjusted to 7.0 ± 0.2 with H_3PO_4. The 1.0 M K_2CO_3 solution was prepared by dissolving 69 g of anhydrous K_2CO_3 in 500 mL of H_2O. The keeper solution, used to minimize the loss of derivatized BPA during the concentration process, was a 50:50 v/v mixture of 1-pentanol and dodecane.

Derivatized BPA calibration standard solutions (20−480 ng/mL) were prepared by adding standards of spiking solutions to 22-mL vials containing 12 mL of 1.0 M K_2CO_3 solution, and by repeating this derivatization procedure with the samples. The concentration of derivatized internal standard (BPA-d_{16}) in the calibration standard solutions was 200 ng/mL.

Sample Extraction and Derivatization. All glassware was conditioned in an oven at 200 °C for at least 2 h to eliminate environmental BPA that may be present.

About 5 g of baby food sample was weighed in a 15-mL polypropylene centrifuge tube. The sample was spiked with 10 μL of 5 ng/μL BPA-d_{16} internal standard solution and mixed. Seven milliliters of acetonitrile was added, and the sample was shaken for 30 s and vortexed for 30 s. The sample was then centrifuged at 3220 rcf or 4000 rpm and room temperature for 15 min. The liquid was decanted in a 70-mL glass tube. Fifty-five milliliters of pH 7.0 buffer solution was added to each tube, the tube was capped, and the contents were mixed.

The sample was then poured into the C18 SPE cartridge, which was conditioned with 13 mL of methanol and 13 mL of H_2O, and absorption was allowed to take place without vacuum. The C18 SPE cartridge was rinsed with 6.5 mL of H_2O and 13 mL of 30% MeOH/H_2O and the eluate was discarded. The C18 SPE cartridge was eluted with 6.5 mL of 50% acetonitrile in water; the eluate was collected in a 16 × 100 mm glass tube. The eluate was mixed using a vortexer and concentrated to about 3 mL using a N_2 evaporator.

The concentrated aqueous extract was transferred to a 22-mL amber vial for derivatization according to the same procedure used previously (*15*, *16*). Ten milliliters of 1.0 M K_2CO_3 solution and 200 μL of acetic anhydride were added to each vial. All sample vials were placed into the 50-place stirring block and stirred at low speed. Another 200 μL of acetic anhydride was added after 5 min and kept stirring for 10 min. Five milliliters of isooctane was added to the vial. The pH of the sample extracts was checked using a pH indicating strip and Pasteur pipet and adjusted so the pH was above 10. If pH adjustment was needed, an additional 0.5 mL of 3 M K_2CO_3 solution was added. One hundred microliters more acetic anhydride was then added and the extract stirred for another 10 min. The stirring was then stopped, and the two phases were allowed to separate, which required approximately 10 min. If there was still an emulsion, the sample was split into two vials and diluted with H_2O, more isooctane was added, and then the sample was re-extracted.

The isooctane phase from the 22-mL amber vial was transferred to a glass column packed with anhydrous Na_2SO_4, which was baked at 650 °C. The aqueous phase in the 22-mL vial was re-extracted with 5 mL of MTBE by stirring for at least 10 min at high speed. The MTBE phase was transferred to the Na_2SO_4 column. The dry organic extract was transferred to a 13 × 100 mm disposable glass tube, and 30 μL of keeper solution was added to the 13 × 100 mm glass tube.

The sample extract was evaporated to almost dryness at 40 °C for about 45 min, using the N_2 evaporator. The extract was reconstituted with 220 μL of toluene and vortexed for 30 s. The sample was transferred to a GC vial containing an insert for analysis.

GC-MS Analysis. The Agilent 6890 gas chromatograph (GC) coupled to a 5973 mass selective detector (MSD) was used for the analysis. The flow rate of the helium carrier gas was 1.2 mL/min. The injector temperature was 280 °C. One microliter of sample extract was injected into the GC system in splitless mode. The analytes were separated on a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The GC oven temperature program was set at an initial temperature of 100 °C for 1 min, raised to 225 °C for 5 min at 20 °C/min, then raised to 325 at 35 °C/min, and held for 1 min.

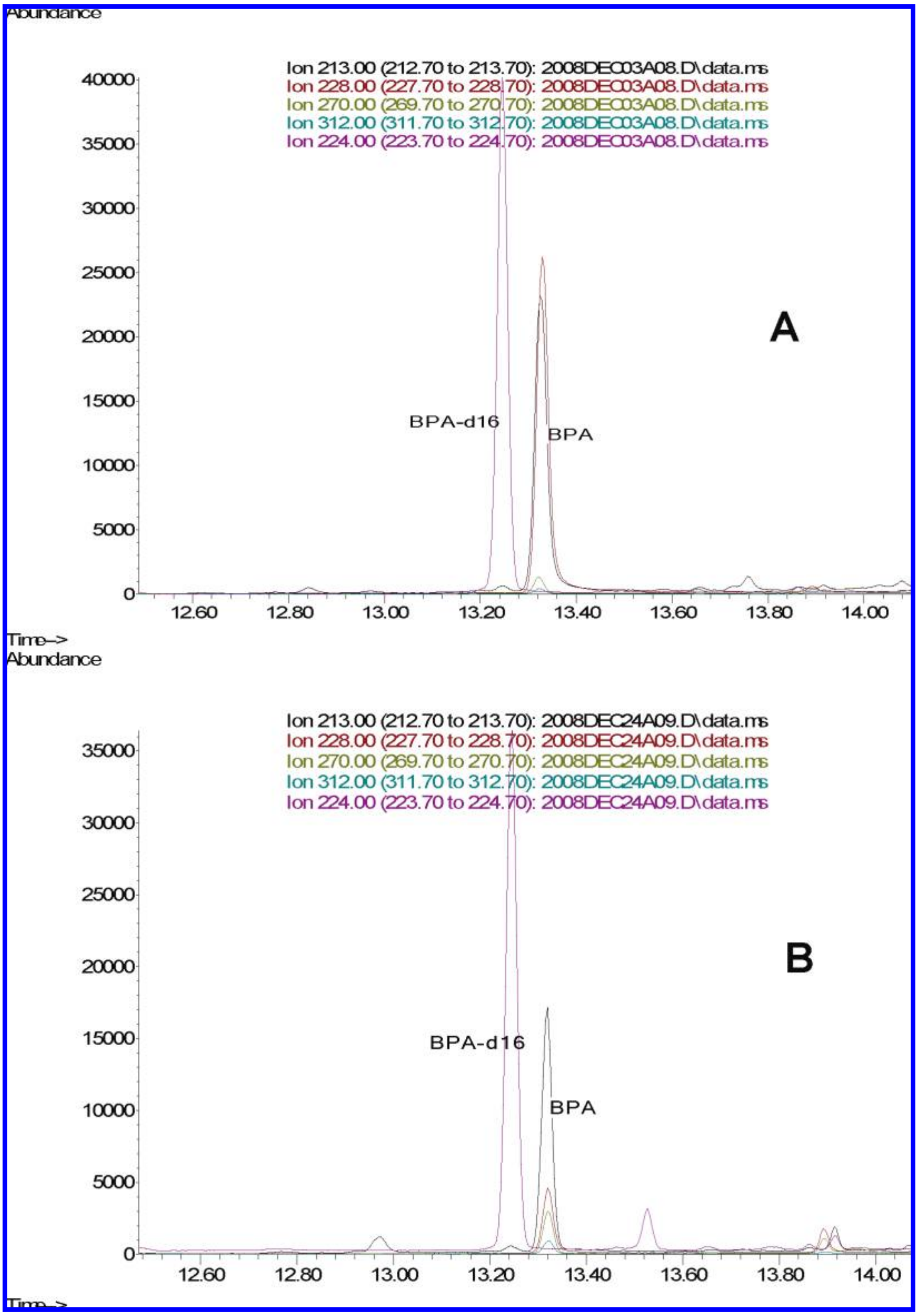

Figure 2. GC-MS ion chromatograms of baby food samples: (**A**) interference with ions *m*/*z* 213 and 228 from sample matrix; (**B**) no interference.

The MSD was operated with electron impact ionization in selected ion monitoring (SIM) mode. The following ions were selected for derivatized bisphenol A: *m*/*z* 213, 228, 270, 312. For bisphenol A-d_{16} *m*/*z* 224 was used. Dwell time was 35 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 and ion ratios. The calculation of BPA concentrations in samples was based on the calibration curves of peak area ratios of BPA (ion *m*/*z* 228) over the internal standard peak area plotted with the ratios of native BPA concentration over the internal standard concentration. Two method blanks (water) were analyzed in every extraction batch, and the average BPA level in the water blanks was subtracted from the results of all baby food samples.

RESULTS AND DISCUSSION

Method Performance. Linearity of the instrument and the method was demonstrated using five standard solutions with concentrations from 20 to 480 ng/mL. Linearity with a R^2 value of 0.9999 was observed for BPA's calibration plot with peak areas normalized to internal standard versus concentrations.

To cover the variety of the food types in this work, 14 baby food products were selected for method validation. These products included fruits, vegetables, mixed dishes, and organic and nonorganic foods. Six replicates of each of the 14 products were analyzed for BPA, and levels of BPA in these products ranged from below the detection limit to 7.2 ng/g. The results from the products with BPA levels above the detection limit but below 1 ng/g were used to calculate the method detection limits (MDLs) using the equation (*20*)

$$\mathrm{MDL} = t_{(n-1, 1-\alpha=0.99)} \times \mathrm{SD}$$

where $t_{(n-1,\ 1-\alpha=0.99)}$ is Student's t value at 99% confidence level and is 3.365 when six replicates are used. SD is the standard deviation of n replicates. The MDLs ranged from 0.068 to 0.30 ng/g (**Table 1**) with an average of 0.18 ng/g. A similar MDL (0.079 ng/g) was also obtained by analyzing seven replicates of water samples (method blank, $t_{(n-1,\ 1-\alpha=0.99)} = 3.143$) with an average blank BPA level of 0.075 ng/g. The method repeatability was demonstrated with the 6 replicate analyses of each of the 13 baby food products; relative standard deviations (RSD) ranged from 1.2 to 16.1% with an average of 8.7% (**Table 1**).

The extraction recoveries of the method were obtained from the analyses of samples of the 14 baby food products spiked with BPA standard solutions at 1−8 ng/g depending on the BPA levels in the nonspiked products; average recoveries ranged from 93.5 to 102.5% (**Table 1**).

BPA in Baby Food Products. For each of the 122 baby food products, 2 subsamples from the same jar were analyzed. BPA was detected positively in 99 of the 122 baby food products, and the average of the differences between the results of the two replicate analyses was 11.5%. Concentrations of BPA in each of the 99 products shown in **Table 2** were the average of the two. The percentile distribution and the histogram of BPA concentrations in the 99 baby food products are shown in **Table 3** and **Figure 1**, respectively.

Considerable interferences with the quantification ion m/z 213 and the qualification ion m/z 228 from the sample matrices were observed for the other 23 baby food products; none of the ion ratios (m/z 228/213, 270/213, 312/213) was within the acceptable criteria (25% of the corresponding ion ratios of the standard) as shown in **Table 4**. Typical GC-MS ion chromatograms for baby food samples with and without interferences are shown in **Figure 2**. Although BPA may be present in some or all of these 23 baby food products, it could not be quantified or confirmed due to this interference.

Concentrations of BPA in most of the baby food products were low in general; 15% of the products had BPA levels of less than the average MDL (0.18 ng/g), about 70% of the products had BPA levels of less than 1 ng/g, and the average BPA level in all products was 1.1 ng/g. The highest level of BPA, 7.2 ng/g, was detected in two brand E products, strained organic carrots and strained organic sweet potatoes and chicken, but was still well below the specific migration limit of 0.6 μg/g set by the EC Directive for BPA in food or food simulant (*2*).

The average BPA concentrations in brands A−D, F, and G baby food products are low in general, varying from 0.54 to 1.1 ng/g. However, the average BPA concentration in brand E baby food products (3.9 ng/g) is considerably higher than those in the baby food products of other brands.

Although BPA concentrations in baby food products within a brand varied slightly for brands A−D, F, and G, they varied considerably for brand E products (1.9−7.2 ng/g). The average BPA level in the nonorganic baby food products from brand D (0.57 ng/g) is very close to that in the organic products from the same brand (0.65 ng/g). The average BPA level in the 34 fruit products from all brands (0.60 ng/g) is lower than those in the 34 vegetable products (1.2 ng/g) and the 31 mixed-dish products (1.1 ng/g), but there is almost no difference between the average BPA levels in the 34 vegetable products and the 31 mixed-dish products.

Because this is the first time that BPA was determined in baby food products in glass jars with metal lids, information on BPA levels in similar products is not available in the literature for comparison with the results from this work. Compared with BPA levels in canned foods (*6*−*14*), however, BPA levels in jarred foods with metal lids are considerably lower. Although BPA levels in jarred foods with metal lids from most of the brands tested in this work were lower than the BPA levels in canned liquid infant formula products investigated earlier (*15*), BPA levels in baby food products from one of the brands (brand E), 1.7−7.2 ng/g, are very similar to the BPA levels found in canned liquid infant formulas (2.3−10.2 ng/g).

The exact reasons for the presence of BPA in baby food products, especially of brand E, are not known. Migration from the epoxy coatings, and possibly the PVC gaskets as well, on the metal lids due to occasional contact with foods is obviously one of the potential sources. However, if there are no considerable differences in the coatings and PVC gaskets in the metal lids used in baby foods of different brands, the relatively high BPA levels found in brand E products would suggest other sources in addition to migration from the lids. Because all baby food products are cooked and processed, some of these additional sources could be the processing equipment and storage containers used, which may have epoxy coatings and/or plastic parts (such as polycarbonate).

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