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Levels of Bisphenol A in Canned Liquid Infant Formula Products in Canada and Dietary Intake Estimates

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A sensitive, efficient, and reproducible method, based on solid phase extraction and derivatization with acetic anhydride followed by gas chromatography−mass spectrometry in selected-ion monitoring mode, was developed for the determination of bisphenol A (BPA) in liquid infant formula. The method quantification limit was 0.5 ng g^{-1}. Extraction recoveries were 85−94% over the concentration range of 2.5−20 ng g^{-1}. Good reproducibility of the method was observed at levels of 0.54 and 10.4 ng g^{-1} with relative standard deviations of 5.0 and 2.8%, respectively. The method was used to analyze samples of 21 canned liquid infant formula products for BPA. BPA was detected in all samples at levels ranging from as low as 2.27 ng g^{-1} to as high as 10.2 ng g^{-1}. The probable daily intakes of BPA due to consumption of canned liquid infant formula were estimated for infants from premature to 12−18 months of age. The maximum probable daily intake of BPA was 1.35 μg kg^{-1} of body weight day^{-1} for 0−1-month-old infants with the maximum formula intake, which is below the provisional tolerable daily intake for BPA established by Health Canada, 25 μg kg^{-1} of body weight day^{-1}.

KEYWORDS: Bisphenol A; liquid infant formula; dietary intake

INTRODUCTION

Bisphenol A (BPA) is the common name for 2,2-(4,4′-dihydroxydiphenyl)propane, 4,4′-isopropyllidenediphenol, or 2,2′-bis(4-hydroxyphenyl)propane. It is used as an intermediate in the production of polycarbonate (PC) plastics and epoxy resins. PC is used in food storage containers such as water bottles and baby bottles, and epoxy resins are used in the internal coating for food and beverage cans to protect the food from direct contact with metal. BPA can migrate from PC plastic containers and cans with epoxy coating into foods, especially at elevated temperatures (for example, for hot-fill or heat-processed canned foods). Because BPA is a potential endocrine disruptor, which mimics the action of the hormone estrogen (*1*), an EC Directive has reduced the previous specific migration limit (SML) for BPA at 3 mg kg^{-1} in food or food simulant (*2*) to 0.6 mg kg^{-1} in an amending document relating to plastic materials and articles intended to come into contact with foodstuffs (*3*). The tolerable daily intake (TDI) for BPA was established at 50 μg kg^{-1} of body weight day^{-1} by the U.S. EPA and the European Food Safety Authority (*4*), whereas Health Canada established the provisional tolerable daily intake (pTDI) for BPA at 25 μg kg^{-1} of body weight day^{-1}.

BPA is one of the 23000 chemical substances on the Canadian Environmental Protection Act (CEPA) domestic substance list (DSL) identified for further evaluation under the government of Canada's chemical management plan (CMP). As part of this evaluation process for BPA, an exposure assessment needs to be conducted to determine if the infant exposures to BPA due to consumption of canned liquid infant formula products are within Health Canada's pTDI. Although surveys of BPA in canned foods were conducted in some countries (*5–13*), information on BPA in canned liquid infant formula is very limited (*8*) and is not available in Canada.

Gas chromatography−mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) with various detectors are commonly used in analytical methods for the determination of BPA in foods (*5–13*). Although method detection limits as low as 1 ng g^{-1} were reported (*8, 10*), methods with detection limits as high as 10 ng g^{-1} were frequently used in

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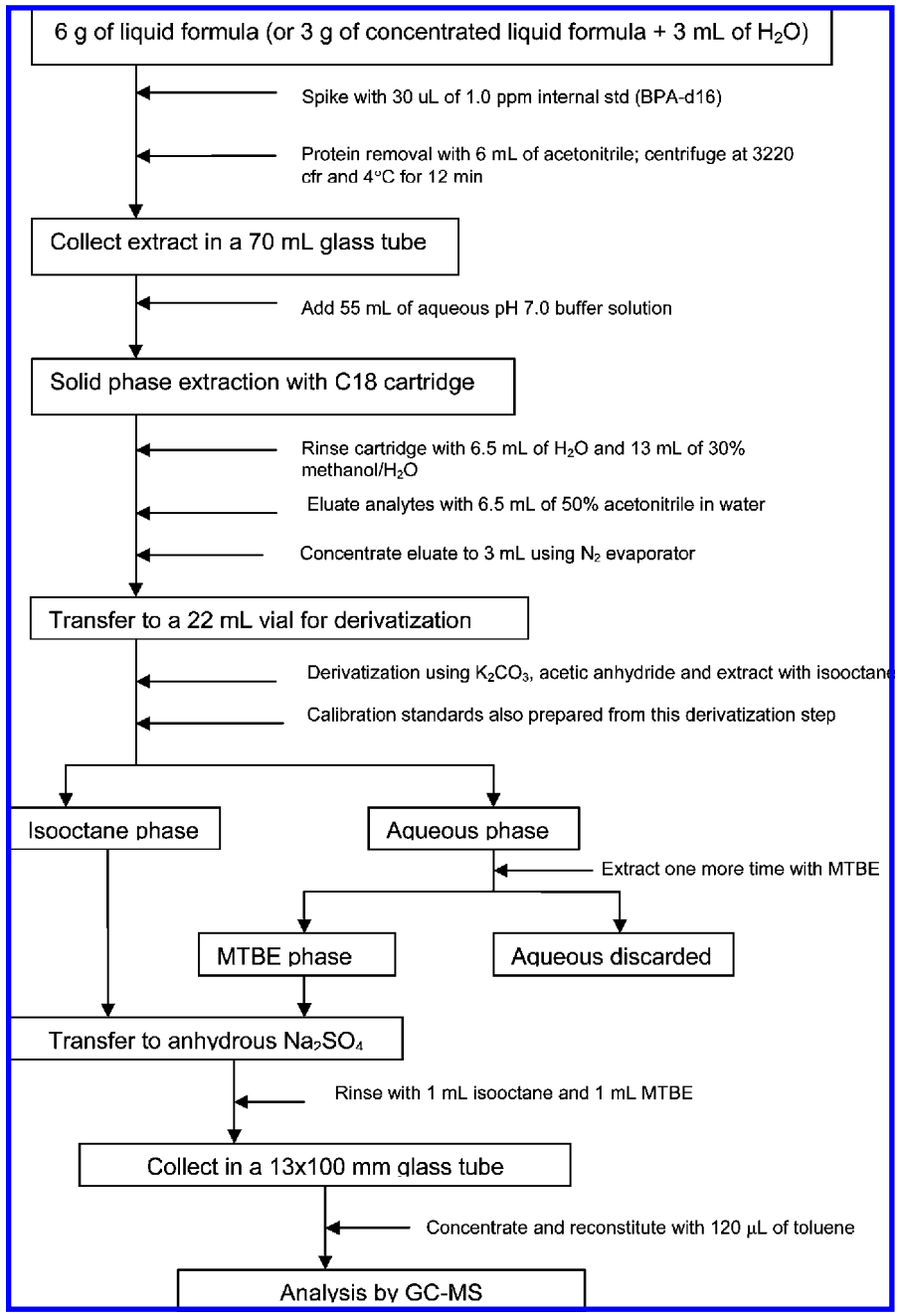

Figure 1. Flowchart of sample extraction procedure.

many of the surveys to determine BPA in foods. This will overestimate human exposure assessment if the detection limit (or half of the detection limit) is used for samples with BPA levels of less than the detection limits. Thus, sensitive methods with detection limits as low as possible should be developed and used for the determination of BPA in food samples to assess human exposure to BPA more accurately.

In this work, samples of 21 canned liquid infant formula products of various brands were analyzed for BPA using a method with a quantification limit as low as 0.5 ng g^{-1}, and the results were used to estimate dietary intakes of BPA for infants in different age groups.

MATERIALS AND METHODS

Sample Collection. A minimum of 2 cans (with the same lot number) of each of the 21 liquid infant formula products were purchased in October 2007 in a local grocery store in Ottawa. These products covered 8 brands from 4 companies. Among the 21 products, 17 products were milk based, and 4 were soy based; 18 were concentrated, and 3 were ready to use; 12 products were iron fortified, and 4 were calcium enriched. 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), potassium carbonate (ACS grade), bisphenol A (99%), bisphenol A-d_{16} (98%), isooctane (pesticide-residue grade), methyl *tert*-butyl ether (MTBE, 99.9%), K_2HPO_4 (ACS), Na_2SO_4 (anhydrous, ACS grade), 1-pentanol (99%), and dodecane (99%) were purchased from Sigma-Aldrich (Oakville, ON). Acetic anhydride (ACS grade) and H_3PO_4 (85% HPLC grade) were purchased from Fisher (Ottawa, ON). Deionized water was obtained from a Milli-Q system.

The 50 place stirring block was obtained from Barnstead (Dubuque, IA). The 13 × 100, 20 × 150, and 16 × 100 mm disposable glass tubes and 15 mL centrifuge tubes were purchased from VWR (Montréal, QC). The 22 mL vials and 6 cm^3 glass columns were obtained from Supelco (Oakville, ON). The C18 SPE cartridges were purchased from Varian (Mississauga, ON).

Table 1. Concentrations of BPA in Canned Liquid Infant Formula Products

company	brand	product	formula type	surface area (cm^2)	can type	country of origin	infant age (months)	BPA concn (ng g^{-1})	av BPA concn (ng g^{-1})
A	A1	A1-1	soy	296.7	two-piece	USA	0−12	8.94	8.05
		A1-2	soy	296.7	two-piece	USA	0−12	6.35	
	A2	A2-1	milk	229.6	easy open, two-piece	Canada	0−12	4.25	
		A2-2	milk	296.7	two-piece	USA	0−12	9.77	
		A2-3	milk	296.7	two-piece	USA	6−18	10.23	
		A2-3	milk	296.7	two-piece	USA	0−12	8.66	
		A2-4	milk	296.7	two-piece	USA	6−18	8.16	
B	B1	B1-1	milk	296.7	two-piece	USA	0−12	3.74	3.97
		B1-2	soy	296.7	two-piece	USA	0−12	5.00	
		B1-3	milk	296.7	two-piece	USA	0−12	3.19	
C	C1	C1-1	milk	296.7	two-piece	USA	6 and up	3.42	4.71
	C2	C2-1	milk	296.7	two-piece	USA	0−12	5.43	
		C2-2	milk	296.7	two-piece	USA	0−12	4.39	
		C2-3	milk	296.7	two-piece	USA	0−12	4.86	
	C3	C3-3	milk	235.5	easy open, three-piece	USA	12 and up	5.44	
D	D1	D1-1	soy	296.7	two-piece	Switzerland	0−12	2.69	2.37
		D1-2	milk	296.7	two-piece	Switzerland	6−18	2.27	
		D1-3	milk	296.7	two-piece	Switzerland	6−18	2.28	
		D1-4	milk	296.7	two-piece	Switzerland	0−12	2.30	
		D1-5	milk	296.7	two-piece	Switzerland	0−12	2.33	
E	E1	E1-1	milk	229.6	easy open, two-piece	Canada	12 and up	3.91	3.91

Stock (400 mg L^{-1}), intermediate (10 mg L^{-1}), and spiking solutions (0.1 and 1 mg L^{-1}) of BPA and BPA-d_{16} in acetonitrile were prepared in 25 mL volumetric flasks and stored at 4 °C. These standard solutions were stable for at least 6 months at 4 °C.

The pH 7 phosphate buffer was prepared by dissolving 35 g of K_2HPO_4 in 2 L of deionized water, and the pH was adjusted to 7.0 ± 0.1 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 (0, 10, 20, 60, 160, and 480 ng mL^{-1}) were prepared by adding standards of spiking

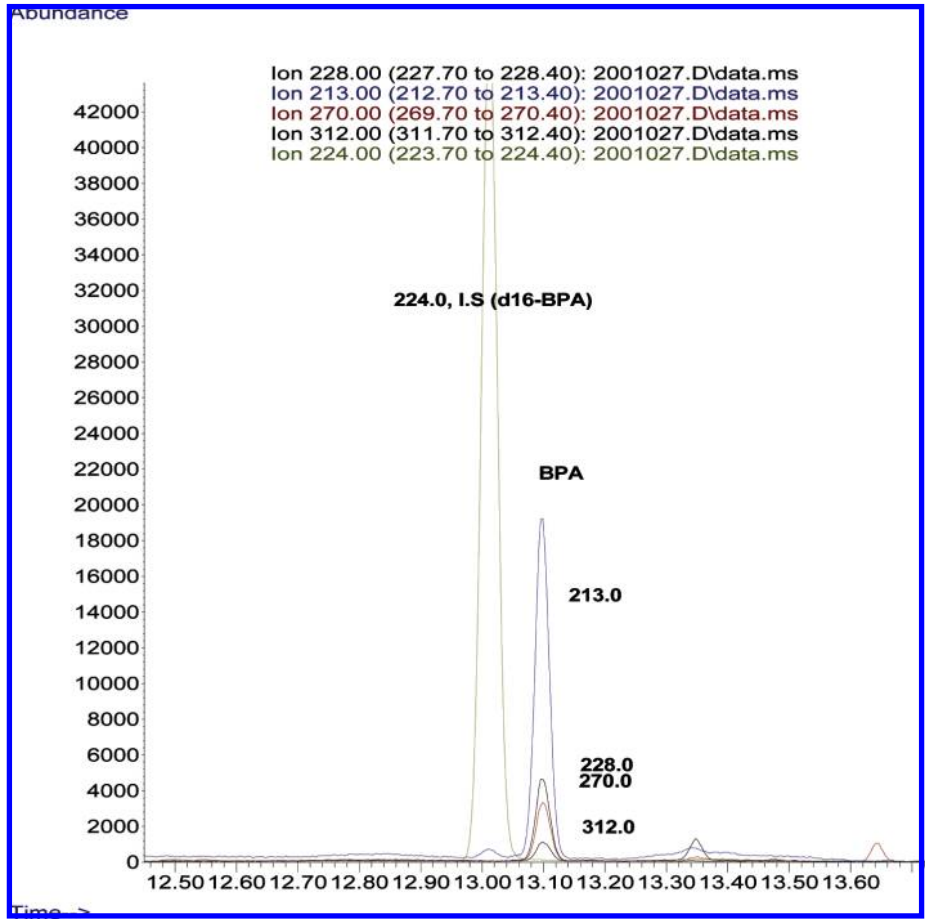

Figure 2. Typical GC-MS ion chromatograms for a canned liquid infant formula sample containing BPA at 2.7 ng g^{-1}.

Table 2. Formula Intake and Average Body Weight for Different Age Groups of Infants

age group (months)	av body wt[a] (kg)	formula intake[b] (g day^{-1}) av	formula intake[b] (g day^{-1}) max
premature	1.5	100	
0−1	3.9	644	1080
2−3	5.5	1080	1470
4−7	7.2	1050	1440
8−12	9.0	735	960
12−18	10.6	750	900

[a] The average weight of infants is based on the growth charts from the *Pediatricians Guide to your Children's Health and Safety* (www.keepkidshealthy.com/growthcharts/girlsbirth.html). [b] The formula intake by infants for the specific growth period is according to *A Practical Guide to Baby Care*, prepared by the Institute National de Santé Publique du Québec (2001).

Table 3. Average, Minimum, and Maximum Probable Daily Intakes (PDI) of BPA for Different Age Groups of Infants

age group (months)	formula intake (g day^{-1})	PDI (μg kg^{-1} of body wt day^{-1}) av	min	max
premature	100	0.17	0.08	0.33
0−1	644	0.45	0.19	0.81
	1080	0.75	0.32	1.35
2−3	1080	0.50	0.23	0.96
	1470	0.69	0.31	1.31
4−7	1050	0.38	0.17	0.75
	1440	0.52	0.23	1.02
8−12	735	0.21	0.09	0.42
	960	0.28	0.12	0.55
12−18	750	0.23	0.08	0.38
	900	0.27	0.10	0.46

solutions to 22 mL vials containing 12 mL of 1.0 M K_2CO_3 solution, and by going through the same derivatization procedures as the samples. The concentration of derivatized internal standard (BPA-d_{16}) in the calibration standard solutions was 200 ng mL^{-1}. Although these derivatized standards were stable for 5 weeks when recapped and stored at 4 °C, they were prepared new every week or for every other batch when two or more batches were processed in the same week.

Sample Extraction and Derivatization. All glassware was conditioned in an oven at 260 °C for at least 2 h to eliminate environmental BPA that may be present.

The overall sample extraction procedure was demonstrated in a flowchart as shown in **Figure 1**. Approximately 6 g of liquid formula was weighed in a 15 mL polypropylene centrifuge tube. For concentrated liquid infant formula, 3 g was used instead with 3.0 mL of H_2O. The sample was spiked with 30 μL of 1.0 ppm BPA-d_{16} internal standard and then mixed. Six milliliters of acetonitrile was added, and the samples were shaken for 30 s and vortexed for 30 s. The sample was then centrifuged at 3220 rcf or 4000 rpm and 4 °C for 12 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 C18 SPE cartridge was conditioned with 13 mL of methanol and 13 mL of H_2O. The aqueous extract was poured into a reservoir fitted on top of the cartridge, and absorption was allowed to take place without vacuum. The 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 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 vial, and a small stirring bar was added. 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 a 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 was stirred for another 10 min. The stirring was then stopped, and the two phases were allowed to separate; this may take 10 min or more. 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 vial was transferred to a glass column packed with anhydrous Na_2SO_4. 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 (50:50 mixture of 1-pentanol and dodecane) was added to the 13 × 100 mm glass tube. The Na_2SO_4 column and tube were rinsed with 1 mL of isooctane and 1 mL of MTBE, and the eluates were combined in a 13 × 100 mm glass tube.

The sample extract was evaporated to almost dryness at 35 °C for about 30 min., using the Speedvac evaporator. If there was any water residue, 1 mL of acetone was added and the mixture re-evaporated. The extract was reconstituted with 120 μL of toluene, vortexed for 30 s, and placed in an ultrasonic bath for 5 min. The sample was transferred to a GC vial containing an insert for analysis.

GC-MS Analysis. An Agilent 6890 gas chromatograph (GC) coupled to a 5975 mass selective detector (MSD) was used for the analysis. The flow rate of the helium carrier gas was 1.1 mL min^{-1}. 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 ZB-5 ms capillary column (5% diphenyl−95% dimethylsilicone, 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^{-1}, then raised to 325 °C at 35 °C min^{-1}, and held for 1 min.

The MSD was operated with electron impact ionization in selected ion monitoring (SIM) mode. The following ions were selected for bisphenol A: 213, 228, 270, and 312. For bisphenol A-d_{16} ion 224 was selected. The 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 the 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. Every extraction batch contained the following control samples: (1) one method blank (6 mL of water); (2) one method blank spiked with BPA at 20 ng g^{-1}; (3) one or two in-house reference materials; and (4) one unknown sample spiked with BPA at 20 ng g^{-1}.

RESULTS AND DISCUSSION

Method Performance. Although methods based on HPLC have been developed for the determination of BPA in milk and various food samples (*5, 9*), we were unable to reduce the method detection limit below 1 ng g^{-1} with fluorescence detection due to chromatographic interferences. Thus, a sensitive method based on BPA derivatization followed by GC-MS analysis was developed instead. The trimethylsilyl (TMS) derivative of BPA was initially investigated. However, interferences from the GC septum or column bleed with the only abundant ion, *m/z* 357, of the BPA−TMS derivative made the

confirmation difficult. Therefore, BPA was derivatized with acetic anhydride instead to form the diester derivative.

Linearity of the instrument and the method was demonstrated using five standard solutions with concentrations from 10 to 480 ng mL^{-1}. Linearity with an R^2 value of better than 0.99 was observed for BPA's calibration plot with peak areas normalized to internal standard versus concentrations.

The instrument detection limit (IDL), defined as 3 times the signal-to-noise ratio, was better than 0.1 ng g^{-1} for BPA. The method quantification limit was established at 0.5 ng g^{-1} because this was equivalent to the lowest calibration standard of 10 ng mL^{-1} for a 3 g sample, and it is similar to the limit of detection (0.9 ng g^{-1}) reported by Biles et al. (*8*).

The extraction recoveries of the method were obtained from the analyses of five to eight replicates of liquid formula samples spiked with BPA standard solutions at 2.5, 8.0, and 20.0 ng g^{-1}; recoveries were 85−94% with relative standard deviations (RSD) from 2.7 to 3.9%. The reproducibility of the method was demonstrated by analysis of six replicates of two in-house reference materials (0.54 and 10.4 ng g^{-1}); RSDs were 5.0 and 2.8%, respectively.

Levels of BPA in Canned Liquid Infant Formula Products. For each of the 21 infant formula products, samples from two cans were analyzed for BPA. The average of the differences between the results of the two cans for all 21 products was 7.5%. Two subsamples in the same can were also analyzed for 5 of the 21 products, and the average of the differences between the results of the two replicate analyses was 1.7%. Concentrations of BPA in each of the 21 products, as shown in **Table 1**, were the average of the two to four analyses. The typical GC-MS ion chromatogram for a sample with BPA at 2.7 ng g^{-1} is shown in **Figure 2**.

BPA was detected in all 21 products. Concentrations of BPA ranged from 2.27 to 10.2 ng g^{-1} with an average of 5.12 ng g^{-1}, well below the specific migration limit of 0.6 μg g^{-1} set by the EC Directive for BPA in food or food stimulant (*3*). This is consistent with the results from Biles et al. (*8*); levels of BPA in 14 canned liquid infant formula products were from 0.1 to 13.2 ng g^{-1} with an average of 5.0 ng g^{-1}. Although BPA was detected in five powdered infant formula products at levels of 45−113 ng g^{-1} by Kuo and Ding (*10*), this is likely due to the contamination introduced at different stages of production of the powdered infant formula products as the authors discussed. Migration of BPA from cans into powdered infant formula is unlikely because, unlike the cans for liquid infant formula products, cans for powdered infant formula products are not coated in general, and even if there is a coating inside the cans for powdered infant formula products, migration of BPA from can coating into powder infant formula will be extremely slow compared to that for the liquid product. BPA was not detected in our previous survey of 56 canned powdered infant formula products using a method with detection limit of 1.0 ng g^{-1}.

Levels of BPA in products of the same company agreed well, and they do not vary in general with different brands of the same company or with formula type (milk or soy based). The average BPA levels in products from different companies varied from as low as 2.37 ng g^{-1} for company D to as high as 8.05 ng g^{-1} for company A. This is likely because the same cans were used in a company for all products and the thickness of the epoxy resin coatings and residual levels of BPA were different in cans used by different companies. Higher residual levels of BPA in can coatings and thicker can coatings will result in higher levels of BPA in the products.

Exposure to BPA from Formula for Infants. The probable daily intake (PDI) was estimated using the equation

$$\mathrm{PDI} = C \times \mathrm{intake} \times \mathrm{DF}/(\mathrm{BW} \times 1000) \quad (1)$$

where PDI is the probable daily intake (μg kg^{-1} of body weight day^{-1}), C is the concentration of BPA (ng g^{-1}), intake is the average formula intake during the specific growth period (g day^{-1}), DF is the dilution factor (1 for ready-to-use formula and 0.5 for concentrated formula), and BW is the average body weight of infants during the specific growth period (kg).

The average weight of infants from keepkidshealthy.com (*14*) and the average and maximum formula intakes by infants from the Institute National de Santé Publique du Québec (*15*), shown in **Table 2**, were used in PDI calculations.

The PDI of BPA due to exposure to BPA from canned liquid formula was estimated for infants of the following age groups: premature infants, 0−1 month, 2−3 months, 4−7 months, 8−12 months, and 12−18 months. The average PDI for premature infants was based on their average body weight of 1.50 kg and the intake of 100 g of formula per day. For infants of the other age groups, both the average and the maximum PDIs were estimated according to the average infant body weight, and the average and maximum formula intakes listed in **Table 2**. Because the 21 infant formula products were intended to be used for different ages of infants, only the results from relevant products were used to estimate the PDI for infants of a specific age group. For example, only the results from products intended for 0−12 months infants were used to estimate the PDIs for premature, 0−1 month, and 2−3 month infants, whereas for 12−18 month infants, results from products intended for 6−18 months, for 6 months and older, and for 12 months and older were used to estimate PDIs.

Table 3 shows the average, minimum, and maximum PDIs of BPA for infants of different age groups. The PDIs of BPA varied from as low as 0.08 μg kg^{-1} of body weight day^{-1} for premature and 12−18 months infants to as high as 1.35 μg kg^{-1} of body weight day^{-1} for 0−1 month infants with the maximum formula intake and are below Health Canada's pTDI of 25 μg kg^{-1} of body weight day^{-1} for BPA. The average, minimum, and maximum PDIs for 0−1 and 2−3 month infants are very close and start to decrease for the older age groups due to higher body weights and/or lower formula intakes. The average PDI for premature infants was low due to their low formula intake.

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