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Determination of Bisphenol A in U.S. Infant Formulas: Updated Methods and Concentrations

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An updated survey of U.S. infant formula was conducted to determine the concentrations of bisphenol A (BPA). The purpose was to accurately assess BPA concentrations across the infant formula market, accounting for lot variability, and determine if geographic location or can age influences BPA concentrations. A method was developed to measure BPA in formula utilizing isotope dilution, solid-phase extraction, and liquid chromatography−tandem mass spectrometry (HPLC-MS/MS). The method was tested and found to be reproducible (10% relative standard deviation), reliable (47 ± 1% recovery), and sensitive (0.15 ng g^{-1} method detection limit). Over 160 analyses were conducted using 104 formula containers representing 36 products. Samples from U.S. east and west coast markets demonstrated no significant difference, and concentrations in older cans were not higher. BPA concentrations in liquid formula (0.48−11 ng g^{-1}) were consistent with previous studies, and BPA was detected in only 1 of 14 powder formula products analyzed.

KEYWORDS: BPA; MS/MS; infant formula; can

INTRODUCTION

Low-acid canned foods are at substantial risk of life-threatening growth of *Clostridium botulinum* or other microbes and thus are required to undergo a heat and/or pressure treatment (retort) to destroy any potential spores or microbes (*1*). During retort and storage, if the metal can is not coated, canned food and the metal can may undergo substantial chemical interaction, including leaching of metals, corrosion of the can, food breakdown, and production of off-flavors (*2*). To protect the can and food from these interactions, epoxy resins are frequently used to coat the metal surfaces of food cans (*3*). These epoxy coatings are frequently composed of phenolic polymers of the commonly named molecule bisphenol A (BPA) or 2,2-(4,4′-dihydroxydiphenyl)propane (i.e., 4,4′-isopropylidenediphenol) (*3*). Because a small residual amount of the monomer often remains after the polymerization process, a low-level migration of BPA is expected from a retorted, epoxy-coated can to the food (*4*).

Previous work has demonstrated that U.S. infant formula samples from epoxy-phenolic coated cans contained 0.1−11.6 ng g^{-1} of BPA, likely migrating from the coating (*4*). More recent studies of U.K. formula samples suggested levels were <2 ng g^{-1} (*5*), whereas most recently Canadian canned formula samples were shown to range from 2.2 to 10.3 ng g^{-1} (*6*). A recent study from Canada demonstrated slightly lower concentrations in baby food samples from glass jars with metal lids (*7*). Other studies have documented similar or slightly higher, but more variable, concentrations in numerous noninfant food products (*5*, *8*−*13*).

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Recently, the Australian, Canadian, European, German, Japanese, New Zealand, U.K., and U.S. food safety authorities have reviewed the safety of BPA in food-contact materials. Most authorities did not initiate new regulatory action as a result. The U.S. FDA began an effort to update and expand the exposure scenarios in its draft health assessment for BPA (*14*). Goals of the updated exposure analysis included assessing and accounting for variability in infant consumption of BPA, in particular any possible geographic, temporal, or retail variance, and the use of updated methodology to address issues of sensitivity, laboratory contamination, and specificity.

No studies to date have specifically assessed variability of BPA concentrations in infant formula. Additionally, only two studies to date have measured BPA in powder formula, and the reported concentrations in Taiwanese powder formula appear to be higher than previously reported in Canadian powder formula and North American and U.K. liquid formula (*15*). Whereas the Taiwanese results suggest further measurements of BPA levels in powder formulas are warranted, the unexplained difference from liquid formula concentrations could be due in part to the samples coming from a different market and/or differences in analytical methods.

Previous methods for the analysis of BPA in foods have utilized a wide range of sample preparations and analysis techniques (*16*). The most common sample preparations have been solid-phase or liquid extraction, and the most common detection techniques were LC−fluorescence or derivatization GC-MS (*4*, *16*, *17*). Previous work has suggested that fluorescence and perhaps even LC-MS may be subject to interferences from comigrants (*18*). Numerous studies have stated that low-level laboratory contamination

is often a challenge for accurately determining concentrations below nanogram per gram levels (*19*−*21*). This is exactly the lower end of most previous measurements of BPA in formula. Extensive sample handling, improperly cleaned glassware, precontaminated SPE columns, and syringe needle cements have been suggested sources of laboratory contamination (*19*−*21*). Methods to assess variability of BPA formula should minimize contributions from variable blank contamination, variable recovery, and variable comigrants. Although there has been a more specific derivatization GC-MS/MS procedure recently employed in evaluating migration of BPA into bottles (*22*), there is reason to believe LC-MS/MS should provide adequate sensitivity and good specificity (*16*, *23*) while minimizing sample manipulation required to avoid frequent GC maintenance, thus avoiding contamination and variability.

This study sought to accurately assess BPA concentrations across the U.S. infant formula market, including ready-to-feed (RTF), concentrates, and powder formula accounting for a variety of products consumed and to determine if purchase location or formula age influenced BPA concentrations. To this end a highly specific, sensitive, and robust method to measure BPA in infant formula products was developed and validated within this laboratory.

MATERIALS AND METHODS

Samples. Containers of infant formula (293) were purchased from retail locations in and around the U.S. District of Columbia (DC) (161 containers) and southern California and Arizona (LA) (132 containers) in December 2008 and January 2009. These samples comprised at least one container of 36 unique combinations of manufacturer, commercial product, and container type and size. Two products were obtained from a local hospital in the DC area. Care was taken to select multiple containers from multiple lots for several product types. Upon receipt, containers were assigned a unique code, and expiration dates, lot numbers, container type, size, surface area, manufacturer, and product type were recorded; the samples were stored at room temperature (or according to package label). Of these, 46 containers from LA and 57 containers from DC were subselected for analysis and the remainder archived. The subselection represented at least one of the 36 unique products/containers collected. Thirty of the unique products/containers analyzed were collected from both geographic locations. Multiple lots of six product/container combinations were analyzed, with three of these lots originating from both LA and DC. The infant formulas purchased and analyzed represented products from all U.S. manufacturers. Products acquired were either liquid ready-to-feed (RTF), concentrated liquid (concentrate) requiring 1:1 dilution with water, or dry powder requiring ∼8:1 dilution (water/powder). All dilutions made were per instructions listed on the formula containers. Ready-to-feed products were acquired in ∼2 fl oz (59 mL) metal-lidded polymeric bottles, 3 fl oz (89 mL) metal-lidded glass jars, 8 or 8.45 fl oz (237 or 250 mL) three-piece cans with easy-open tops, or 32 fl oz (946 mL) three-piece cans. All liquid concentrate products acquired were 13 fl oz (384 mL) two-piece cans. Powder formula products were acquired in a much wider variety of three-piece cans and metal end-caped cans with polymeric-coated paperboard walls.

Reagents. Standards of BPA (InChI = 1S/C15H16O2/c1-15(2,11-3-7-13(16)8-4-11)12-5−9-14(17)10-6-12/h3-10,16-17H,1-2H3) (>99% Sigma Aldrich, St. Louis, MO), d_6-BPA, and d_{16}-BPA (>99.9/98%, Cambridge Isotope Laboratories, Cambridge, MA) were tested for purity and suitability by LC-MS upon receipt. The chloroform, water, acetonitrile (ACN), and methanol used were 99.9% Fischer Optima LC-MS grade (Fair Lawn, NJ) or EMD OmniSolv (Gibstown, NJ), whereas the hexane (Burdick & Jackson, Muskegon, MI) was of UV grade. Solid-phase extraction cartridges were Supelco's 0.5 g styrene−divinylbenzene Env-Chrom-P cartridges (St. Louis, MO). Grade 5 argon for the MS/MS collision cell, as well as liquid N_2 for the electrospray ion source (ESI), and grade 5 N_2 for sample reduction were from Roberts Oxygen (Laurel, MD).

Equipment. Sample preparation used disposable, highly crystalline polypropylene (PP) labware, including positive displacement pipettors, centrifuge tubes, 2 mL syringes, 0.2 μm nylon membrane syringe filters, and autosampler vials. PTFE inserts were used in the vacuum manifolds, and sample reductions were conducted in a Techne drybath DB-3A with grade 5 N_2 and stainless steel tips. Aliquots were centrifuged on a Fischer Marathon 2100R centrifuge. HPLC-MS/MS analyses were performed using an Agilent 1100 series HPLC with micro volume mixing chamber, a Varian Pursuit XRs C18 column (2 × 150 mm, 3 μm particles), and a Sciex/API-5000 triple-quadrupole ESI-MS/MS. Data analysis was performed using Analyst (Applied Biosystems) and Excel software (Microsoft). FTIR analysis of selected can coatings and container surfaces was conducted with a Nicolet Magna 550 series II (Nicolet Analytical Instruments, Madison, WI) spectrometer equipped with a 30° horizontal specular reflectance/transmittance attachment (Janos Optical Corp., Townshend, VT) and processed by Omnic (Nicolet Analytical Instruments) software with comparison to Hummel (Thermo Inc.) spectral libraries for polymers and additives.

Analytical Methods. Once opened, samples were analyzed immediately, and 50 mL was archived at 5 °C. Empty cans were cleaned with water, wiped, and air-dried overnight. Metal coupons measuring ca. 5 cm by 2.5 cm were cut from the walls of the can, flattened with a hydraulic press, and placed flat on the horizontal specular reflectance/transmittance table, ensuring the IR beam was incident (30°) to the coating. FTIR spectra of the can coupons were analyzed and compared to infrared spectra of known epoxies and other polymers.

Sample formula aliquots were 5.0 g of formula as fed. For ready-to-feed products, 5.0 g was weighed out. Powder formulas and concentrated liquid formulas were first prepared as directed on the packaging by dilution with LC-MS grade water and shaken for ∼30 s prior to weighing a 5.0 g aliquot. Method blanks were performed with every sample set (≤7 samples) using 5.0 g of LC-MS grade water. To each sample was added a 50 μL aliquot of 1.0 μg mL^{-1} d_6-BPA as a recovery surrogate. Subsequently, 5.0 mL of ACN was added, and the samples were capped and vortexed for 30 s prior to being centrifuged for 20 min at 4000 rcf. The supernatant was poured into a 50 mL centrifuge tube, taking care not to disturb the precipitate. The supernatant was diluted to ∼50 mL with LC-MS grade water before SPE processing on a vacuum manifold. SPE columns were conditioned with 10 mL each of chloroform, ACN, or methanol and then 20 mL of LC-MS grade water. Care was taken not to let the cartridges dry. Samples were loaded onto the SPE cartridges and washed with 10 mL of water before being pulled dry (5 min). The cartridges were washed with 10 mL of hexane and suctioned dry again before extracts were eluted with 12−14 mL of chloroform. Chloroform extracts were immediately reduced to near dryness under N_2 at 50 °C. Methanol/water (100 μL of 50:50) was added when the chloroform extract was at or below ∼1 mL. When a chloroform layer was no longer visible, the extract was brought to ∼1 mL with 50:50 methanol/water and syringe-filtered into an autosampler vial. The internal standard, d_{16}-BPA (50 ng), was added to each vial, and the vial was capped, shaken, and stored at 5 °C until analysis.

Instrumental. Separations were performed at a flow rate of 0.4 mL min^{-1} and a column temperature of 60 °C. A water−methanol gradient between 40 and 100% methanol during the first 6 min eluted BPA around 5.0 min. Following BPA elution the analytical column was methanol washed for ∼1 min and returned to 40% methanol for a 9 min equilibration time. Negative ion electrospray at −4.5 kV with 50 psi of N_2 nebulizer gas (35 psi of curtain gas) was used, and two MS/MS transitions (6 psi of collision gas) were monitored for each of the three analytes (BPA, m/z 227.1 to 133.1 and 212; d_6-BPA, m/z 233.1 to 138.1 and 215.1; d_{16}-BPA, m/z 241.1 to 142.1 and 223.1). Analyte confirmation required both mass transitions to peak at >3:1 signal-to-noise (S/N) within ±0.02 min of expected relative retention time (t_R) (according to standards and d_6-BPA t_R) and the relative intensity of the two transitions to match their ratio in the standard to within 20%. Calibration solutions ranged from 0.01 to 100 ng mL^{-1} BPA, with 50 ng mL^{-1} of d_6-BPA and d_{16}-BPA in each calibration solution. Three sets of calibration standards were prepared by different analysts. Calibration solutions were analyzed prior to and throughout daily sets of sample extracts, and BPA was quantified as the area ratio of one BPA transition (m/z 227−133) to the d_6-BPA transition (m/z 233−138). The area ratio of one d_6-BPA transition (m/z 233−138) to the d_{16}-BPA transition (m/z 241−142) was used to quantify the recovery of d_6-BPA in every sample. The area ratio of laboratory blanks was subtracted from corresponding sample area ratios prior to quantitation by the calibration curve. All resulting calibration curves were linear

between 0.03 and 100 ng mL^{-1} ($r^2 > 0.99$). A set of 12 calibration solutions was prepared to quantify d_6-BPA recovery in every sample, using d_6-BPA concentrations (2.5−75 ng mL^{-1}) equivalent to 5−150% recovery of the surrogate and d_{16}-BPA as the corresponding internal standard. All d_6-BPA calibration solutions included the internal standard (d_{16}-BPA) present at 50 ng mL^{-1}.

Statistical. Analysis of the numerical data was performed using Excel 2003 software with care taken to understand software limitations. Infant formula characteristics tested for potential differences in BPA concentrations included manufacturer, brand, product, protein base (soy or milk), container size, metal surface area-to-formula mass ratio, days until expiration, and location purchased. Whenever possible, each characteristic was tested in isolation by selecting sets of formula samples for which all other characteristics were held constant. Results were tested for normal and log-normal distributions. Two-tailed Student's t tests (assuming heteroscedatic variance) were used to test for differences in BPA concentrations between different categorical characteristics and lack-of-fit F tests for regressions of highly numeric characteristics (expirations, surface area/mass ratios).

RESULTS AND DISCUSSION

Can Coating FTIR Spectra. Selected two- and three-piece cans and containers were analyzed by Thermo Nicolet 6700 FTIR to confirm the use of epoxy-phenolic coatings and determine if different manufacturer, can, or coating type could be distinguished by FTIR analysis of the coupons. Comparisons of spectra from six liquid formula cans with the spectral library confirmed the use of epoxy-phenolic coatings in both two- and three-piece cans, whereas the coatings of two powder formula cans did not match. Comparisons between can coatings demonstrated no distinguishing features unique to any manufacturer or formula characteristic, but small peaks at 700 and 1726 cm^{-1} were present in the spectra of all two-piece cans and none of the three-piece cans, consistent with a different can coating formulation being used on most two-piece cans.

Method Validation. Figure 1 illustrates typical HPLC-MS/MS chromatograms for the analysis of BPA in infant formula samples. It is important that high chromatographic resolution of bisphenolic substances is achieved because BPA-similar, but unidentified, peaks were observed adjacent to BPA in HPLC-MS/MS chromatograms of canned infant formula extracts (**Figure 1B**). These unidentified peaks were not observed in any standards or blanks and did not increase in area with subsequent BPA fortification of formula samples, eliminating analytical artifacts as a source of these peaks. Because these three unidentified peaks exhibited both BPA MS/MS transitions (m/z 227.1 to 133.1 and 212) in relative intensities consistent with BPA standards, it appears that they contain some BPA moiety. The exact identities of these components are under investigation.

In the analysis of BPA, the method limit of detection (LOD) was determined in accordance with U.S. Code of Federal Regulations (40.CFR-1.136 App.B v.1.11). A powder sample, in which analyses consistently demonstrated no detectable levels of BPA, was split into two aliquots and fortified at concentrations of 0.30 and 0.16 ng g^{-1}. Three analysts processed three aliquots each of both fortification levels, yielding estimated method detection limits (EMDL) of 0.17 and 0.14 ng g^{-1}. No significant difference was found between the variance at the two fortification levels ($p < 0.05$); therefore, pooling the results yielded a method LOD of 0.15 ng g^{-1}. The method limit of quantitation (LOQ), defined as 10 times the standard deviation of the blanks, was set at 0.5 ng g^{-1}. Method blanks averaged an estimated 0.07 ng g^{-1} and ranged from nondetectable to 0.2 ng g^{-1}. All samples reported were method blank subtracted and d_6-BPA recovery corrected.

Absolute analyte recovery (before isotope dilution recovery correction) was calculated for a low-level-fortified sample (0.5 ng g^{-1}, **Figure 1**) and for the d_6-BPA recovery surrogate in every aliquot analyzed. After isotope dilution recovery correction, quantified BPA concentrations corresponded to 104−107% recovery of estimated fortifications. The fortified recovery sample averaged an absolute recovery of 47%, closely matching the d_6-BPA recovery in the same sample (50%) and similar to the d_6-BPA recoveries in all unknown samples (47%). Additional experiments identified sample reduction under N_2 as a source of analyte loss, but method alterations to reduce loss resulted in increased sample handling, blank contamination, and recovery variability.

Due to the lack of certified reference materials (CRM), method accuracy was assessed using the low-level-fortified powder sample and four blind, fortified samples prepared for an interlaboratory study by the Grocery Manufacturers Association (GMA). The within-laboratory fortified sample was determined to within 7.8% (less than 1 standard deviation of the triplicate analysis) of the 0.5 ng g^{-1} fortification level (0.539 ± 0.050 ng g^{-1}), assuming no error in the sample fortification. BPA concentrations determined for two of three blind round-robin samples (5.2 and 10.7 ng g^{-1}) were within 1 standard deviation of the reported fortification levels (5 and 10 ng g^{-1}), whereas the third sample was within 1.5 standard deviations of the reported fortification (1.4 vs 1.0 ng g^{-1}). The three samples averaged 17% deviation from the reported fortification levels. The result for a fourth round-robin sample (< 0.15 ng g^{-1}) was consistent with the reported fortification (0.1 ng g^{-1}).

Within-laboratory method validation procedures to estimate precision included multiday replicate instrumental quantification of select high- and low-concentration unknowns (using separate calibration solutions prepared by three analysts), single-analyst within-day triplicate preparation of unknown samples, between-day triplicate preparation of unknown samples, and between-analyst/day triplicate preparation of unknown samples. For three unknown samples (1.7−9.8 ng g^{-1}, 1.4−5.5% RSD), the within-day reproducibility yielded a relative mean standard error (RMSE) of 0.2%. For eight unknown samples (2.3−10.6 ng g^{-1}, 2.9−18% RSD), the between-day analyses yielded a RMSE of 11.8%, whereas the between-analyst/day repeatability of two unknown samples (0.48−0.68 ng g^{-1}, 13−16% RSD) yielded a RMSE of 0.5%. Within-day variabilities of instrumental analysis of high- and low-level extracts were 4.7 and 5.3% RSD ($n = 3$), whereas between-day variances ($n = 10$) were 6.8 and 12.3% RSD, respectively.

Method performance was more than sufficient for the purpose of this study, and because sensitivity, recovery, precision, and accuracy were measured with samples from the sample set, it is clear that these metrics accurately describe this method's performance across the range of samples in this study. Reproducibility remained sufficiently high across the entire sample set and concentration range (average 10% RSD), allowing potential differences in BPA concentrations between lots to be distinguished. The method LOD (< 0.15 ng g^{-1}) was low enough to measure BPA in all liquid samples and was comparable to that of a previous study investigating BPA in powder formula (*15*). The method accurately measured fortified samples to within 1 standard deviation of their fortified concentrations (< 14% deviation), which covered the concentration range of commercial infant formulas (0.5−10 ng g^{-1}). The use of two isotope-labeled BPA surrogates and the confirmation that their recoveries closely matched the unlabeled BPA (47 vs 50%) gave the method a high degree of robustness and confidence and prevented potentially low or variable analyte recoveries from limiting method performance.

Concentrations. Concentrations of BPA are reported undiluted (i.e., as received in the can) for ready-to-feed (RTF) and

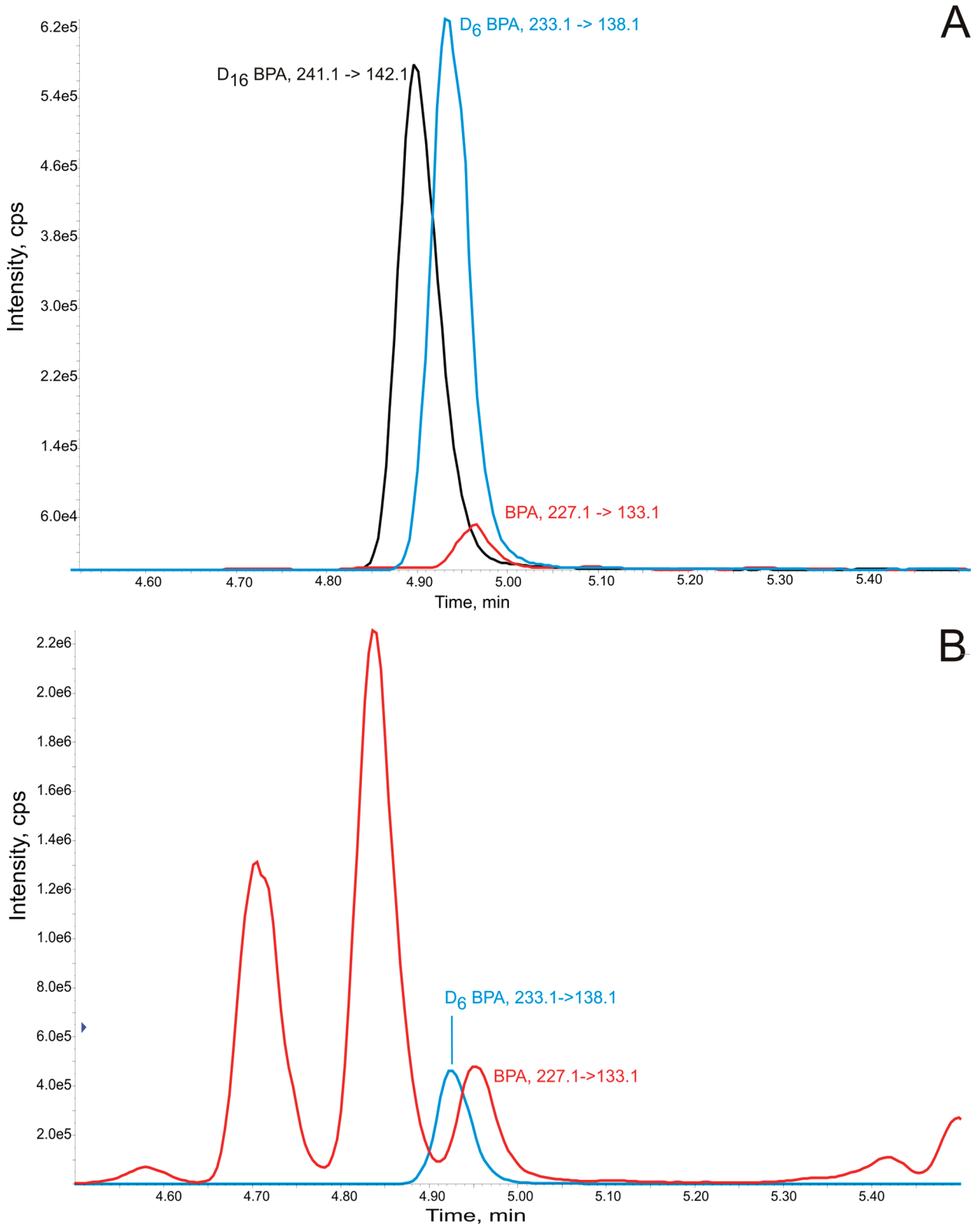

Figure 1. (**A**) Typical powder formula HPLC-MS/MS chromatogram (BPA fortified at 0.5 ng g^{-1}). (**B**) Typical canned liquid infant formula HPLC-MS/MS chromatogram.

concentrated liquid formulas and as-fed (diluted) for powder formula. This is done to facilitate analysis and comparisons of BPA migration. Any BPA consumption analysis should correct for the dilution of the concentrated formula. Concentrations in powder infant formula were below the method LOD in all but one sample (**Table 1**). Concentrations in liquid formula ranged from 0.48 to 11 ng g^{-1}. Lot-to-lot variability averaged 16% RSD (2.2−49% RSD), and this variability was not significantly different between manufacturers, cans with different surface area-to-formula mass ratios, or geographical locations. Although BPA concentrations in liquid formula varied largely by manufacturer, they were generally lowest in the smallest (glass or polymeric) containers of RTF liquid formula (0.48−2.1 ng g^{-1}). BPA concentrations were higher in the large, 32 fluid ounce (fl oz;

Table 1. U.S. Infant Formula and Container Characteristics and BPA Concentrations

infant formula				container					DC samples, BPA[a] (ng g^{-1})				LA samples, BPA[a] (ng g^{-1})			
				volume												
manufacturer	product	base	type[b]	fl oz	mL	wall	no. of metal pieces	area/mass (cm^{2}g^{-1})	container 1	container 2	container 3	container 4	container 1	container 2	container 3	container 4
A	1	milk	R	2.0	59	HDPE[c]	1	0.21	0.48							
B	1	milk	R	3.0	89	glass	1	0.12					1.4	1.9		
C	1	milk	R	2.0	59	HDPE	1	0.21	1.9	2.1	1.5[d]	1.6[d]				
A	1	milk	R	8.0	237	metal[e]	3	1.01	9.6	9.5[d]	9.9[d]		9.3	6.6[d]	8.1[d]	
A	1	soy	R	8.0	237	metal[e]	3	1.01	9.5	10	9.0	10	10			
B	1	milk	R	8.5	250	metal[e]	3	0.85	1.5				1.6			
A	1	milk	R	32.0	946	metal	3	0.66	4.7				4.3			
A	1	soy	R	32.0	946	metal	3	0.66	4.7				5.8			
A	2	milk	R	32.0	946	metal	3	0.66	6.1				6.2			
B	1	milk	R	32.0	946	metal	3	0.66	0.56				0.60			
B	1	soy	R	32.0	946	metal	3	0.66	0.67				0.88			
D	1	milk	R	32.0	946	metal	3	0.66	4.9				5.0			
D	1	soy	R	32.0	946	metal	3	0.66	5.0	5.0	5.5	5.3	4.9	5.5	5.3	5.4
A	1	milk	C	13.0	384	metal	2	0.77	6.8				4.6	4.2	4.2	5.1
A	1	soy	C	13.0	384	metal	2	0.77	5.3	5.2[d]	4.1[d]		5.8	5.4[d]	5.6[d]	
A	2	milk	C	13.0	384	metal	2	0.77	4.4				5.8			
B	1	milk	C	13.0	384	metal	2	0.77	2.3				1.1	3.0[d]	3.4[d]	
B	1	soy	C	13.0	384	metal	2	0.77	3.6	3.4	3.5	3.4	3.6			
C	2	milk	C	13.0	384	metal	2	0.77	9.1	7.4[d]	7.7[d]		9.1	7.0[d]	10[d]	
C	2	soy	C	13.0	384	metal	2	0.77	7.4				6.6			
C	3	milk	C	13.0	384	metal	2	0.77	8.0				9.0	11	8.7	9.2
D	1	milk	C	13.0	384	metal	2	0.77	4.0	5.1			3.7			
A	3	milk	P	42.9	1267	multi[f]	2	0.35	<0.15				<0.15			
A	1	milk	P	25.8	762	multi	2	0.42	<0.15							
A	2	soy	P	25.8	762	multi	2	0.42	<0.15				<0.15			
A	3	milk	P	25.8	762	multi	2	0.45	<0.15							
B	1	milk	P	25.8	762	multi	2	0.45	<0.15				<0.15			
C	2	milk	P	25.8	762	multi	2	0.42	<0.15				<0.15			
C	3	milk	P	25.8	762	multi	2	0.42	<0.15				<0.15			
C	4	soy	P	25.8	762	multi	2	0.42	<0.15				<0.15			
D	1	soy	P	25.8	762	metal	3	1.27	<0.15	<0.15[d]			<0.15			
D	1	soy	P	42.9	1267	metal	3	0.89					<0.15	<0.15[d]	<0.15[d]	
D	2	milk	P	42.9	1267	metal	3	0.89	<0.15							
D	2	milk	P	32.1	950	metal	3	1.44					<0.15	<0.15[d]		
D	2	soy	P	32.1	950	metal	3	1.44					<0.15	<0.15[d]		
E	1	soy	P	30.0	887	metal	3	1.37	0.40[h]							

[a] Undiluted. [b] R, ready-to-feed, C, concentrate; P, powder. [c] High-density polyethylene. [d] Different lots. [e] Easy-open lid. [f] Polymer-coated paperboard. [h] $n = 4$.

946 mL) cans of RTF formula (0.56−6.2 ng g^{-1}), which in turn were comparable to concentrations in concentrated liquid formula (1.1−10 ng g^{-1}). BPA concentrations were highest in small cans of RTF liquid formula (1.6−11 ng g^{-1}). Because few replicate samples of liquid formula from small glass or polymeric containers were acquired, few definitive comparisons can be drawn between formula from cans versus glass or plastic, but, in general, these noncanned samples contained lower BPA concentrations and the lowest surface area-to-formula mass ratios.

The concentrations reported in this survey fall entirely within the range of previously published measurements of BPA in liquid infant formula from the United States as well as Canada (*4*,*6*). All powder formulas analyzed, except one, yielded BPA concentrations below the LOD (**Table 1**). This is consistent with publicly available results of Canadian powder formula samples (<0.13 ng g^{-1}) (*24*). Nondetectable concentrations of BPA in powders are consistent with several characteristics of powder formula, including infrequent use of all-metal cans, a limited contact area with epoxy-phenolic coatings, resistance to mass transfer between packaging and solid food, and the absence of in-can retort for powder formula. A study of Taiwanese powder infant formulas did report BPA concentrations (≥5.5 ng g^{-1}) higher than those reported here (*15*), but the Canadian powder formula samples and the results herein would suggest the Taiwanese results are likely not applicable to the North American powder formula market. Direct comparisons between the concentrations listed in **Table 1** and most previously published infant formula analyses are hampered by different manufacturers, containers, types of formula samples collected, and different analytical methods. Still, the values reported here (0.48−11 ng g^{-1}) fall entirely within the range of previously reported liquid U.S. infant formula BPA concentrations (0.1−13.2 ng g^{-1}) (*4*) and range slightly lower than previously reported Canadian infant formula concentrations (2.27−10.2 ng g^{-1}) (*6*).

Container Characteristics. Statistical tests for differences in BPA formula concentrations demonstrated that liquid infant formula from different manufacturers and formula in containers with different surface area-to-formula mass ratios yielded the most significant differences [$p = 0.05-4 \times 10^{-13}$]. Samples from different retail products, formula bases, or geographic locations or with different days to expiration did not yield significantly

different BPA concentrations or correlations ($p \geq 0.06$). No other correlations or significant differences were observed in this sample set. Whereas the observational nature of this study precludes firmly identifying can coatings as the primary or sole source of BPA measured, its absence in most powders and the correlations with can coating surface area-to-formula mass ratios suggest can coatings are a likely source of BPA.

Several other characteristics (formula type, container volume, wall material, number of metal pieces) of the samples collected were nearly coincident, and thus tests for differences were inappropriate. For example, all 32 fl oz (946 mL) cans contained RTF formula, all 13 fl oz (384 mL) cans contained concentrated formula, all 8 and 32 fl oz cans were made with three pieces of metal, and all 13 fl oz cans were made with two pieces. Therefore, the relative BPA concentrations cannot be attributed to one of these characteristics over another.

Controlled Comparisons. Concentrations of BPA in formula were significantly different between manufacturers and between cans with different surface area-to-formula mass ratios. To ensure subtle effects were not masked by these differences, other formula characteristics were tested within groups of the same manufacturer and surface area-to-formula mass ratio. For example, there was a significant correlation between BPA concentration and the surface area-to-formula mass ratio of all samples, but when this correlation was tested within samples from each manufacturer, the relationship was only significant for one manufacturer. This could be due to manufacturers using different coatings in different-sized cans. Also, two of the four manufacturers sold products in only two of the four can sizes, precluding regression.

BPA formula concentrations remained significantly different between manufacturers for 9 of 12 comparisons made within groups of cans with a single surface area-to-formula mass ratio. BPA concentrations were significantly different between samples of different surface area/formula mass ratios (when grouped by manufacturer) in three of the six comparisons.

When manufacturer and/or surface area/mass ratio were controlled, there was no significant difference between samples purchased in the southern California and Arizona area (LA) and those purchased in the U.S. District of Columbia (DC) area ($p > 0.06$). Similarly, when samples of the same surface area-to-formula mass ratio were collected in three or more unique lots from the same manufacturer, tests demonstrated there was no significant difference in BPA concentration between milk- and soy-based liquid formulas or between different retail products or formulations.

Geographical/Temporal Comparisons. Another question we sought to answer was whether formulas sold and/or manufactured in different geographic locations contained significantly different concentrations of BPA. Because it was established that samples from different manufacturers and surface area-to-formula mass ratios yielded different concentrations, the first geographic tests were concentration comparisons of samples within a manufacturer/surface area-to-formula mass ratio group. Tests comparing multiple lots of cans of a single product formulation (same manufacturer, size, type, formula base, and product name) at both locations demonstrated no significant difference ($p \geq 0.17$) in BPA concentrations between samples purchased from LA and DC areas. This is the strongest evidence to suggest the absence of a geographic effect.

However, samples were not collected from three or more lots for every formulation (manufacturer, size, type, formula base), so the analysis was widened to group both milk and soy bases of a particular product together because previous tests showed no significant difference in soy and milk formula BPA concentrations. These product-specific geographic tests also showed no significant differences ($p \geq 0.1$) in BPA concentrations. Finally, a single average BPA concentration was calculated for every formula product tested in both locations, and a paired t test was conducted to see if the differences between the DC and LA area samples were significantly different from 0. This, too, suggested no significant difference between the locations across this population of formula samples ($p = 0.88$). In total, formula samples from three of four can sizes and four of the five manufacturers were tested for geographical differences, and all showed no significant difference (i.e., within experimental error). This is consistent with the fact that there are only a small number of can manufacturers and licensed infant formula manufacturing facilities in the United States and a large product distribution chain.

Finally, we sought to determine if the formula age was related to the concentration of BPA in the formula. It has been suggested that older formulas may receive additional BPA migration after the retort and during storage. If we assume that all canned formula is assigned a similar shelf life (days to expiration) or at least varies by a small degree relative to the overall shelf life (~1 year), then the days to expiration at the time of analysis may be a reasonable inverse measure of the time since packaging, or inverse age. Lack-of-fit F tests demonstrated no significant relationship between BPA formula concentrations and the inverse age (days to expiration) when all of the liquid samples were included in the regression. Similarly, when samples only from the same surface area-to-formula mass ratio groups were regressed, all four groups demonstrated no significant relationship between BPA and inverse age. Finally, when samples from the same manufacturer and surface area-to-formula mass ratio group were regressed, there was a slight significant positive relationship ($p = 0.044$) in only 1 of 12 manufacturer/size groups tested. The positive correlation meant BPA concentration increased as the number of days until expiration increased, suggesting a negative correlation if plotted instead as days since packaging, or age. A weak negative correlation of BPA concentration with can age has also been reported previously (*25*), but the weak significance and small change in concentration caution against overinterpretation. The absence of significant relationships in the vast majority of the sample set and the weak slope and significance of the one relationship would suggest that, on the whole, the samples tested here likely did not undergo significant change in BPA concentration with time, which is consistent with previous experimental work (*25*). This supports the idea that the BPA migration process is essentially complete at the retort step.

Because the manufacturer and the surface area-to-formula mass ratio were the only factors yielding significantly different BPA concentrations, and because both factors remained significant in a majority of comparisons when the other factor was held constant, this would suggest that the two characteristics are fairly independent factors. This independence would be consistent with different formula manufacturers (9 of 12 comparisons) tending to use different can manufacturers but only some can manufacturers tending to use different coatings for different can sizes (3 of 6 comparisons). Previous work has demonstrated that BPA migration from similar epoxy-phenolic coatings is primarily controlled by the initial migrant concentration in the coating and that 80−100% of the extractable BPA migrates during the retort (*25*). It is possible that the initial BPA concentration in the epoxy coatings varied primarily with the can manufacturer and can size (generally coincident with surface area-to-formula mass ratio). Also, because the final concentration of BPA in packaged food is the mass of BPA per unit of food, then the amount of food per unit coating (i.e., surface area-to-food mass ratio) was likely a geometric factor in the final BPA concentration for food from identical coatings.

BPA concentrations across the U.S. infant formula market were accurately measured with an updated, more specific method and potential interferences noted. The developed isotope dilution SPE-LC-MS/MS method was tested and found to be reproducible (10% RSD), reliable (47% recovery), and sensitive (LOD of 0.15 ng g^{-1}). Lot variability was typically $<15\%$ RSD, and neither geographic location nor can age influenced BPA concentrations. BPA was detected in only one powder formula sample, and all liquid formula concentrations (0.48−11 ng g^{-1}) were within the range of previous studies. The infant formula manufacturer and can surface area-to-formula mass ratio or can size were the primary factors correlated with different BPA concentrations in infant formula.

ABBREVIATIONS USED

BPA, bisphenol A; DC, District of Columbia; LA, Los Angeles, CA, and southern California and Arizona; RTF, ready-to-feed; HPLC, high-performance liquid chromatography; MS, mass spectrometry; ESI, electrospray ionization; PTFE, polytetrafluoroethylene; EMDL, estimated method detection limit; S/N, signal to noise ratio; SE, standard error; RMSE, relative mean standard error; rcf, relative centrifugal force.

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