Levels of Bisphenol A in Canned Soft Drink Products in Canadian Markets

Xu-Liang Cao,* Jeannette Corriveau, and Svetlana Popovic

Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Canada, 251 Frederick Banting Driveway, AL: 2203D, Ottawa, Ontario, Canada K1A 0K9

The method developed previously for the determination of bisphenol A (BPA) in liquid infant formula was adapted and validated for determination of BPA in soft drink products. This method was based on solid phase extraction and derivatization with acetic anhydride followed by gas chromatography–mass spectrometry in selected-ion monitoring mode. The average method detection limit was 0.045 μg/L for a 10 mL sample. The average extraction recoveries were 101 and 99.9% obtained with seven different soft drink products spiked with BPA at 0.5 and 2.5 μg/L, respectively. Good repeatability of the method was observed with replicate analyses of seven different soft drinks; relative standard deviations ranged from 1.3 to 6.6%. This method was used to analyze samples of 72 canned soft drink products for BPA. Except for three products from which BPA-d16 could not be recovered at all due to interference of product compositions (e.g., quinine hydrochloride in tonic water), BPA was detected in samples of all the other products at levels ranging from 0.032 to 4.5 μg/L. About 75% of the products had BPA levels of <0.5 μg/L, and 85% of the products had BPA levels of <1 μg/L. Exposure to BPA through consumption of canned soft drink products is low; dietary intake of BPA was estimated at 0.027 μg/kg of body weight/day on the basis of the consumption of one canned soft drink with the highest BPA level (4.5 μg/L) for an adult with a 60 kg body weight, well below the provisional tolerable daily intake of 25 μg/kg of body weight/day established by Health Canada.

KEYWORDS: Bisphenol A; canned soft drinks; gas chromatography–mass spectrometry; solid phase extraction

INTRODUCTION

Bisphenol A diglycidyl ether (BADGE) is used in the production of epoxyphenolic resins and is also used as an additive for the elimination of surplus hydrochloric acid in the production of PVC organosols. Both epoxy resins and PVC organosols are frequently used in the internal coating for food and beverage cans to protect the food and beverage from direct contact with metal. Residues of non-cross-linked BADGE in these coatings can migrate into foods, especially at elevated temperatures. Because BADGE is the reaction product of bisphenol A (BPA) with epichlorohydrin, residual amounts of BPA may be present in BADGE due to incomplete reaction, and migration of BPA into foods is also expected to occur together with BADGE.

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.

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, exposure data from various canned food products are needed to conduct exposure assessment for both infant and general populations.

Surveys of BPA in canned foods were conducted in some countries (*5–13*), and BPA was detected at levels as high as 422 ng/g in ham (*7*), 842 ng/g in sauces (*11*), 102.7 ng/g in fish (*10*), and 95.3 ng/g in vegetables (*6*). However, the available information on BPA in canned soft drinks is very limited and varies considerably. Due to the relatively high detection limits of the methods (0.5–10 ng/g), BPA was not detected in canned soft drinks by Horie et al. (*14*), Kawamura et al. (*15*), Goodson et al. (*7*), and Thomson and Grounds (*8*). BPA was detected in only one of the six canned soft drinks (3.4 ng/g) with a detection limit of 0.9 ng/g by Braunrath et al. (*9*). BPA was also not detected in the limited number of canned soft drinks (<13) tested

* Author to whom correspondence should be addressed (e-mail xu-liang_cao@hc-sc.gc.ca).

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Table 1. Method Detection Limit (MDL) and Repeatability

sample	n^a	av concn (μg/L)	SD	RSD (%)	MDL (μg/L)
cola, carbonated	7	0.18	0.008	4.7	0.027
cola, diet, carbonated	7	0.36	0.024	6.6	0.074
iced tea, lemon flavored, diet, noncarbonated	7	0.63	0.010	1.7	0.033
iced tea, lemon flavored, noncarbonated	7	0.27	0.018	6.6	0.057
soda, orange flavored, diet, carbonated	7	0.47	0.008	1.6	0.024
soda, orange flavored, carbonated	7	0.51	0.016	3.0	0.049
energy drink, carbonated	7	1.2	0.016	1.3	0.050

^{a} Number of replicates.

Table 2. Extraction Recoveries of BPA from Various Soft Drink Products at Two Spiking Levels

sample	av recovery $\pm$ SD,^{a} %	
	0.5 μg/L	2.5 μg/L
cola, carbonated	103 $\pm$ 2.30	101 $\pm$ 0.926
cola, diet, carbonated	95.4 $\pm$ 7.76	99.4 $\pm$ 1.07
iced tea, lemon flavored, diet, noncarbonated	103 $\pm$ 3.40	101 $\pm$ 0.317
iced tea, lemon flavored, noncarbonated	96.8 $\pm$ 4.47	98.3 $\pm$ 1.59
soda, orange flavored, diet, carbonated	96.5 $\pm$ 0.88	99.5 $\pm$ 1.53
soda, orange flavored, carbonated	101 $\pm$ 3.53	101 $\pm$ 1.51
energy drink	109 $\pm$ 3.30	99.5 $\pm$ 1.14

^{a} $n = 3$.

by Shao et al. (*16*), even though they used a method with an extremely low detection limit (0.6 ng/L).

Sensitive methods with detection limits as low as possible should be developed and used for the determination of chemical contaminants in foods to generate data for more accurate human exposure assessment. In this work, the method used previously for the determination of BPA in liquid infant formula products (*13*) was adapted and validated for the determination of BPA in soft drinks, and this method, with a detection limit of 0.045 μg/L, was used to determine levels of BPA in 72 canned soft drink products sold in Canada.

MATERIALS AND METHODS

Sample Collection. Samples of 72 canned soft drink products were collected in April 2007 in local stores in Ottawa. All products were carbonated except for four tea drink products. These samples covered a wide variety of products, diet, nondiet, fruit-flavored, energy drinks, etc. These products covered at least 84% market share of soft drink 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), potassium carbonate (ACS grade), bisphenol A (99%), bisphenol A-d16 (98%), isooctane (pesticide-residue grade), MTBE (methyl *tert*-butyl ether, 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).

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 and 15-mL centrifuge tubes were purchased from VWR (Montréal, PQ). The 22-mL vials and 6-mL glass columns were obtained from Supelco (Oakville, ON). The C18 SPE cartridges were purchased from Varian (Mississauga, ON).

BPA and BPA-d16 standard solutions were prepared in methanol and stored at 4 °C. The pH 7 phosphate buffer was prepared by dissolving 35 g of K_2HPO_4 in a 2 L of deionized water, and the pH was adjusted to 7.0 $\pm$ 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

Table 3. Concentrations of BPA in the 72 Canned Soft Drink Products

soft drink product	BPA concn (μg/L)
cola A	0.18
cola B	0.12
cola, diet, A	0.56
cola, diet, B	0.35
cola, diet, C	0.49
cola, diet, low sodium, caffeine-free, A	1.6
cola, diet, low sodium, caffeine-free, B	2.2
energy drink A	0.40
energy drink B	1.0
energy drink C	4.2
energy drink D	1.3
energy drink E	0.24
energy drink F	0.29
energy drink G	0.60
energy drink H	0.10
energy drink I	4.5
energy drink J	0.18
energy drink K	0.072
energy drink L	n/a^a
ginger ale A	0.22
ginger ale B	0.23
ginger ale C	1.0
ginger ale D	0.048
ginger ale, diet, A	1.7
ginger ale, diet, B	0.065
ginger ale, lemon	0.16
green tea, citrus, noncarbonated	0.075
iced tea, lemon, diet, noncarbonated	0.63
iced tea, lemon, noncarbonated, A	0.45
iced tea, lemon, noncarbonated, B	0.21
root beer A	0.21
root beer B	0.22
root beer C	0.30
root beer D	0.44
root beer, caffeine-free	0.16
root beer, diet	0.41
soda A	0.10
soda B	1.0
soda C	0.17
soda D	0.048
soda E	0.045
soda, caffeine-free	0.47
soda, cherry citrus flavored, diet	0.75
soda, citrus	2.3
soda, cranberry	0.15
soda, cranberry, diet	0.23
soda, cranberry/raspberry, A	0.25
soda, cranberry/raspberry, B	0.12
soda, cranberry/raspberry, diet	0.21
soda, diet A	0.032
soda, diet B	0.047
soda, diet C	0.32
soda, fruit fusion	0.13
soda, ginger	0.071
soda, ginger, diet	0.061
soda, grape	0.16
soda, grapefuit flavored, diet	1.1
soda, green apple	0.21
soda, green apple, diet	0.27
soda, lemonade	0.23
soda, lemonade, diet	1.5
soda, lemon-lime	0.33
soda, lemon-lime, diet	0.27
soda, orange, A	1.1
soda, orange, B	0.95
soda, orange citrus	0.48
soda, orange citrus	0.48
soda, orange citrus	0.45
soda, orange, diet	0.46
soda, strawberry	0.088
tonic water A	n/a
tonic water B	n/a

^{a} n/a, BPA could not be recovered from the product.

BPA in Canned Canadian Soft Drinks

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Table 4. Percentile Distribution of BPA Concentrations (Micrograms per Liter) in 69 of the 72 Soft Drink Products

min	10th	20th	30th	40th	50th	60th	70th	80th	85th	90th	95th	99th	max
0.032	0.069	0.13	0.18	0.22	0.27	0.40	0.47	0.87	1.0	1.4	2.0	4.3	4.5

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-d16) in the calibration standard solutions was 200 ng/mL.

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.

Ten milliliters of soft drink sample was weighed in a 15-mL polypropylene centrifuge tube. The sample was spiked with 10 μL of 5 ng/μL BPA-d16 internal standard solution and 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 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 an 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 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 of 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 for 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 the sample was then re-extracted.

The isooctane phase from the 22-mL vial was transferred to a glass column packed with anhydrous Na_2SO_4 conditioned 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 (50:50 mixture of 1-pentanol and dodecane) was added to the 13 × 100 mm glass tube.

The sample extract was evaporated to almost dryness at 40 °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 220 μL of toluene, vortexed for 30 s, and placed in 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 1.2 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^{-1}, then raised to 325 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. The ion selected for bisphenol A-d16 was 224. 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. Two method blanks (10 mL of water) were analyzed in every extraction batch.

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 soft drink products in this work, seven soft drink products were selected for method validation. These products included both carbonated and noncarbonated, diet and nondiet, fruit-flavored, and energy drinks. Levels of BPA in these products ranged from 0.18 to 1.2 μg/L. To determine the method detection limits (MDLs) and repeatability, seven replicates of each of the seven soft drink products were analyzed, and the MDLs were calculated using the equation

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

where $t_{(n-1, \ 1-\alpha=0.99)}$ is the Student *t* value at 99% confidence level and is 3.143 when seven replicates are used. SD is the standard deviation of *n* replicates. The MDLs ranged from 0.024 μg/L for the diet orange-flavored soda to 0.074 μg/L for the diet cola (**Table 1**) with an average of 0.045 μg/L. Because levels of BPA in method blanks were very low (<0.01 μg/L), the MDL could be easily improved further by extracting a larger sample size. The method repeatability was observed with the seven replicate analyses of each of the seven soft drink products; relative standard deviations (RSD) ranged from 1.3% for the energy drink to 6.6% for the lemon-flavored iced tea and the diet cola with an average of 3.7% (**Table 1**).

The extraction recoveries of the method were obtained from the analyses of samples of the seven soft drink products spiked with BPA standard solutions at 0.5 and 2.5 μg/L; average recoveries were 101 and 99.9% (**Table 2**) with average standard deviations (SD) of 3.6 and 1.2% at 0.5 and 2.5 μg/L, respectively.

BPA in Canned Soft Drink Products. For each of the 72 soft drink products, 2 subsamples from the same can were analyzed, and the average of the differences between the results of the two replicate analyses was 4.7%. Concentrations of BPA in each of the 72 products, as shown in **Table 3**, were the average of the two. The percentile distribution and the histogram of BPA concentrations in 69 of the 72 soft drink products are shown in **Table 4** and **Figure 1**, respectively.

BPA was detected in samples of almost all soft drink products except for the two tonic water soda products and one energy drink product from which BPA-d16 simply could not be recovered. It is noted that one of the ingredients for tonic water drink is quinine hydrochloride, which is commonly used as a bittering agent in tonic type drinks and may interfere with BPA extraction.

Concentrations of BPA in most of the soft drink products were low in general; about 75% of the products had BPA levels of <0.5 μg/L, 85% of the products had BPA levels of <1 μg/L, and the average BPA level in all products was 0.57 μg/L.
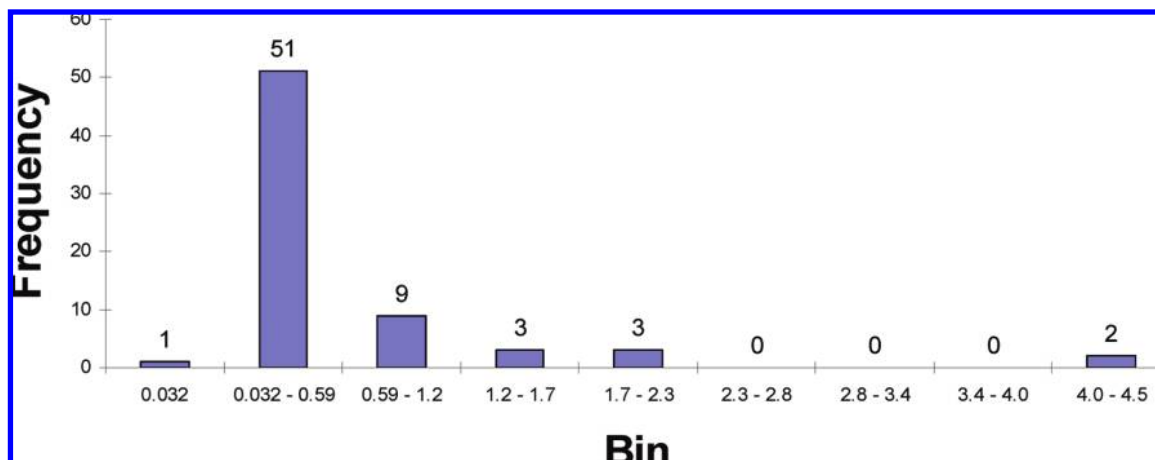
Figure 1. Histogram of BPA concentrations (μg/L) in 69 of the 72 canned soft drink products collected in 2007.

This explains why BPA was frequently not detected in canned soft drink products as reported in the literature due to the relatively high detection limits of the methods used (*7, 8, 14, 15*). One of the diet soda products had the lowest level of BPA, 0.032 μg/L, less than the average MDL (0.045 μg/L). The highest levels of BPA, 4.2 and 4.5 μg/L, were detected in two of the energy drink products; this is similar to the BPA level (3.4 μg/L) in the energy drink sample observed by Braunrath et al. (*9*), and they are all well below the specific migration limit of 0.6 μg/g set by the EC Directive for BPA in food or food simulant (*3*).

It should be mentioned that samples of the 72 soft drink products were collected in April 2007 and stored at room temperature before they were analyzed in July—August 2008. The fact that 85% of the 72 soft drink products had BPA levels of <1 μg/L demonstrates that migration of BPA from can coatings into the drink at room temperature or below is extremely slow and is negligible. Goodson et al. (*17*) also demonstrated that migration of BPA from can coatings occurs during the can processing step (sterilization at 121 °C for 90 min), and BPA levels in canned foods were not changed by extended storage. Thus, the few soft drink products with relatively high BPA levels are very likely due to the migration of BPA from their can coatings during the can sterilization process. To confirm this even further, 10 of the 13 soft drink products collected in 2007 that had BPA levels of >1 μg/L were collected in August 2008 and analyzed for BPA. Nine of these 13 products collected in April 2006 and stored at room temperature were also analyzed for BPA. The results are shown in **Table 5**. It should be mentioned that the samples collected in 2006, 2007, and 2008 were all analyzed in August 2008 to observe the trend (if any) of migration of BPA from can coatings in the same product during storage at room temperature. It should also be mentioned that the 9 products collected in 2006, the 10 products collected in 2008, and the 13 collected products in 2007 had different lot numbers (from different batches); thus, there could have been changes in coating type or weight, curing conditions, or processing conditions among them. Although BPA levels in the samples collected in 2008 were slightly lower in general, one of the energy drink products had a BPA level even higher than the same product collected in 2007. Despite being stored for >2 years at room temperature, levels of BPA in the samples collected in 2006 were even lower than or similar to those collected in 2007.

Variation of BPA levels in different canned soft drink products (0.032—4.5 μg/L) could be due to the differences in can coatings (type, amount, etc.) and can sterilization conditions

Table 5. Concentrations of BPA in Selected Canned Soft Drink Products Collected at Different Times (2006, 2007, and 2008) and Analyzed in August 2008

sample	BPA concn (μg/L)		
	2006	2007	2008
soda B	0.11	1.0	0.24
soda, orange, A	1.3	1.1	0.44
soda, grapefuit flavored, diet	0.79	1.1	0.62
ginger ale, diet, A	0.43	1.7	n/a[a]
ginger ale C	0.48	1.0	0.13
cola, diet, low sodium, caffeine-free, A	1.2	1.6	0.26
soda, lemonade, diet	1.5	1.5	0.12
cola, diet, low sodium, caffeine-free, B	0.29	2.2	n/a
soda, citrus	1.5	2.3	n/a
energy drink B	n/a	1.0	0.39
energy drink C	n/a	4.2	1.8
energy drink D	n/a	1.3	2.4
energy drink I	n/a	4.5	0.47

[a] n/a, products were not available during collection.

(temperature and duration) used by different soft drink product companies. Accidental or careless exposure of the soft drink products to heat (e.g., sunlight) during storage and transportation could also be a factor.

Compared to the BPA levels in other canned food products (e.g., fruit, vegetables, meat, etc.), BPA levels in canned soft drink products are much lower. This could be because a different coating may be used in the two-piece easy open cans for soft drinks, and the amount of the coatings applied in cans for soft drinks could also be less than that in cans for other foods. Thus, exposure to BPA through consumption of canned soft drink products will be low. If an adult (60 kg body weight) consumes one canned drink (355 mL) per day, the dietary intake of BPA will be 0.0034 μg/kg of body weight/day based on the average BPA level in soft drinks (0.57 μg/L) or 0.027 μg/kg of body weight/day based on the highest BPA level (4.5 μg/L) in one of the drinks, much lower than the provisional TDI of 25 μg/kg of body weight/day established by Health Canada.

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