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Research Note

Sources of Low Concentrations of Bisphenol A in Canned Beverage Products

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ABSTRACT

Although migration from can coatings is likely the source of bisphenol A (BPA) for the canned soft drink products with relatively high BPA concentrations, questions have been raised concerning the exact sources of BPA for those canned soft drink products with low BPA concentrations. Information is also needed for BPA concentrations in canned beer products to conduct proper exposure assessment for BPA under the Government of Canada's Chemicals Management Plan. In this work, 22 soft drink samples and 16 beer samples in both cans and plastic and/or glass bottles were analyzed for BPA. BPA was not detected in any of the soft drink samples in either plastic or glass bottles except for one product with a BPA concentration (0.018 μg/liter) close to the limit of quantification (0.015 μg/liter). BPA was detected in all of the corresponding soft drink products in cans, indicating that migration from can coatings is the likely source for BPA in canned products. Because considerable interference with ions *m/z* 213 and *m/z* 228 from sample matrices was observed for all beer samples, BPA concentrations in beer samples were measured using the ion *m/z* 270 instead. BPA was detected in only one of the seven beer products in glass bottles (0.054 μg/liter) but was detected in all corresponding beer samples in cans at low concentrations ranging from 0.081 to 0.54 μg/liter, indicating that migration from can coatings is likely the source of BPA in canned beer products.

One of the applications of bisphenol A (BPA) is as a monomer in the production of epoxy resins that are frequently used in the internal coating for food and beverage cans to protect the contents from direct contact with metal. 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 *(6),* the specific migration limit for BPA in food or food simulators was set at 0.6 μg/g by a European Commission directive in an amending document related to plastic materials and articles intended to come into contact with foodstuffs *(2).* The maximum acceptable dose and tolerable daily intake for BPA were established at 50 μg/kg of body weight per day by the U.S. Environmental Protection Agency *(8)* and the European Food Safety Authority *(3),* respectively. Health Canada established the provisional tolerable daily intake for BPA at 25 μg/kg of body weight per day *(5).*

BPA is one of the chemical substances on the Canadian Environmental Protection Act domestic substance list identified for further evaluation under the Government of Canada's Chemicals Management Plan. As part of this evaluation process for BPA, a study on BPA in canned soft drinks was conducted recently *(1).* Samples of more than 70 products were analyzed for BPA, and BPA concentrations in the majority of the products (about 75%) were <0.5 μg/liter; only a few products contained BPA at concentrations above 1 μg/liter (as high as 4.5 μg/liter). Although migration from can coatings is likely the dominant source of BPA for canned soft drink products with relatively high BPA concentrations, questions have been raised concerning the exact sources of BPA for those canned soft drink products with low BPA concentrations. Because biodegradation of BPA during sewage treatment is not rapid or complete *(7),* the aquatic environment and water supply could become contaminated by discharges from BPA manufacturing or processing plants, and thus the low concentrations of BPA in canned soft drinks could come from the low background BPA concentrations in the water used for soft drink production rather than from migration from can coatings. BPA concentrations in canned beer products also must be determined to conduct a complete exposure assessment for BPA. Although BPA concentrations are expected to be low in canned beer products based on the results from canned soft drinks, this hypothesis has not been tested because of lack of information in this area. Thus, the objective of the present study was to investigate the sources of BPA in canned soft drink and beer products and to provide information on BPA concentrations in canned beer products for exposure assessment.

The ideal approach for investigating sources of BPA in canned beverages is to compare the BPA concentrations in a beverage before it is placed in the can with those in the same beverage after it is placed in the can and has completed the

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sterilization process. However, because of the difficulty of this approach, a more practical method was used to compare the BPA concentrations in the same beverage product contained in different types of containers (e.g., can, plastic, and glass). If environmental contamination were the major source of BPA, then BPA concentrations in the same beverage product contained in different types of containers would be about the same. If migration from can coatings were the dominant source of BPA, then BPA concentrations in the same beverage product contained in cans would be higher than those in the same beverage product in containers of other types (glass or plastic).

MATERIALS AND METHODS

Sample collection. A total of 38 beverage samples were collected in April 2009 in Ottawa (Ontario, Canada): 22 soft drink samples from a local grocery store and 16 beer samples from a local liquor store. Samples of the same product in different containers were all produced at the same location. Selection of the soft drink samples was based on the availability of the soft drink products with low BPA concentrations tested earlier *(1)* in both cans and other types of containers (glass or plastic). The 22 soft drink samples included 8 products (six brands) in both cans and PET (polyethylene terephthalate) bottles and 2 products (1 brand) in cans, PET bottles, and glass bottles. The 16 beer samples included 8 products (eight brands) with 4 to 5% alcohol in both cans and glass bottles. All soft drink samples were stored at room temperature before analysis, and beer samples were stored at 4°C.

Materials and reagents. Acetonitrile (high-performance liquid chromatography [HPLC] grade) and methanol (HPLC grade) were purchased from J. T. Baker (Phillipsburg, NJ). BPA-d14 (>99% D) was purchased from Polymer Source Inc. (Montréal, Québec, Canada). Toluene (glass distilled), potassium carbonate (American Chemical Society [ACS] grade), BPA (99%), isooctane (pesticide residue grade), MTBE (methyl *t*-butyl ether, 99.9%), K_2HPO_4 (ACS grade), Na_2SO_4 (anhydrous, ACS grade), 1-pentanol (99%), and dodecane (99%) were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Acetic anhydride (ACS grade) and H_3PO_4 (85%, HPLC grade) were purchased from Fisher (Ottawa, Ontario, Canada).

The 50-place stirring block was obtained from Barnstead (Dubuque, IA). The disposable glass tubes (13 by 100 mm, 20 by 150 mm, and 16 by 100 mm) and 15-ml centrifuge tubes were purchased from VWR (Montréal, Québec, Canada). The 22-ml vials and 6-ml glass columns were obtained from Supelco (Oakville, Ontario, Canada). The C_{18} SPE cartridges were purchased from Varian (Mississauga, Ontario, Canada).

BPA and BPA-d14 standard solutions were prepared in acetonitrile and stored at 4°C. The 1.0 M K_2CO_3 solution was prepared by dissolving 69 g of anhydrous K_2CO_3 in 500 ml of water. The keeper solution, used to minimize the loss of derivatized BPA during the concentration process, was a 50:50 (vol/vol) mixture of 1-pentanol and dodecane. Derivatized BPA calibration standard solutions (0 to 480 ng/ml) were prepared by adding BPA standard solution in acetonitrile to the 22-ml vials containing 12 ml of 1.0 M K_2CO_3 solution and following the same derivatization procedures as used for the samples. The concentration of derivatized internal standard (BPA-d14) in the calibration standard solutions was 200 ng/ml.

Sample extraction and derivatization. Details of the sample extraction and derivatization method have been described previously *(1)*. A 10-ml sample of soft drink or beer was weighed in a 15-ml polypropylene centrifuge tube, spiked with 10 μl of 5 ng/μl BPA-d14 solution, and mixed. The sample was then poured into the conditioned C_{18} SPE cartridge. After rinsing with 6.5 ml of water and 13 ml of 30% MeOH in water, the C_{18} cartridge was eluted with 6.5 ml of 50% acetonitrile in water, and the eluate was collected in a glass tube measuring 16 by 100 mm, mixed, and concentrated to about 3 ml with a nitrogen evaporator.

TABLE 1. *Concentrations of bisphenol A in soft drink products in different types of containers*

Brand	Product	Container type	Concn (μg/liter)
A	A-1 (cola)	PET	ND[a]
		Glass	ND
		Can	0.11
	A-2 (diet cola)	PET	ND
		Glass	ND
		Can	0.13
B	B-1 (cola)	PET	ND
		Can	0.021
	B-2 (diet cola)	PET	0.018
		Can	0.037
C	C-1 (soda)	PET	ND
		Can	0.042
	C-2 (diet soda)	PET	ND
		Can	0.21
D	D-1 (ginger ale)	PET	ND
		Can	0.024
E	E-1 (ginger ale)	PET	ND
		Can	0.13
F	F-1 (soda)	PET	ND
		Can	0.019
G	G-1 (soda)	PET	ND
		Can	0.20

[a] ND, not detected. Limit of detection was 0.0045 μg/liter.

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 stirring continued for another 10 min. Five milliliters of isooctane was added to the vial. The pH of the derivatized sample extracts was checked using a pH indication strip and a Pasteur pipette and was adjusted to be above 10. One hundred microliters of acetic anhydride was added, and the extract was stirred for another 10 min. 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 reextracted 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 disposable glass tube measuring 13 by 100 mm, and 30 μl of keeper solution was added to the tube. The sample extract was evaporated almost to dryness at 40°C for about 45 min with the nitrogen evaporator. The extract was reconstituted with 220 μl of toluene and vortex mixed for 30 s. The sample was then transferred to a gas chromatography (GC) vial containing an insert for analysis.

GC-MS analysis. An Agilent 6890 GC apparatus coupled to a 5973 mass selective detector (MSD) was used for the GC–mass spectrometry (MS) analysis. The flow rate of the helium carrier gas was 1.2 ml/min. The injector temperature was 280°C. One

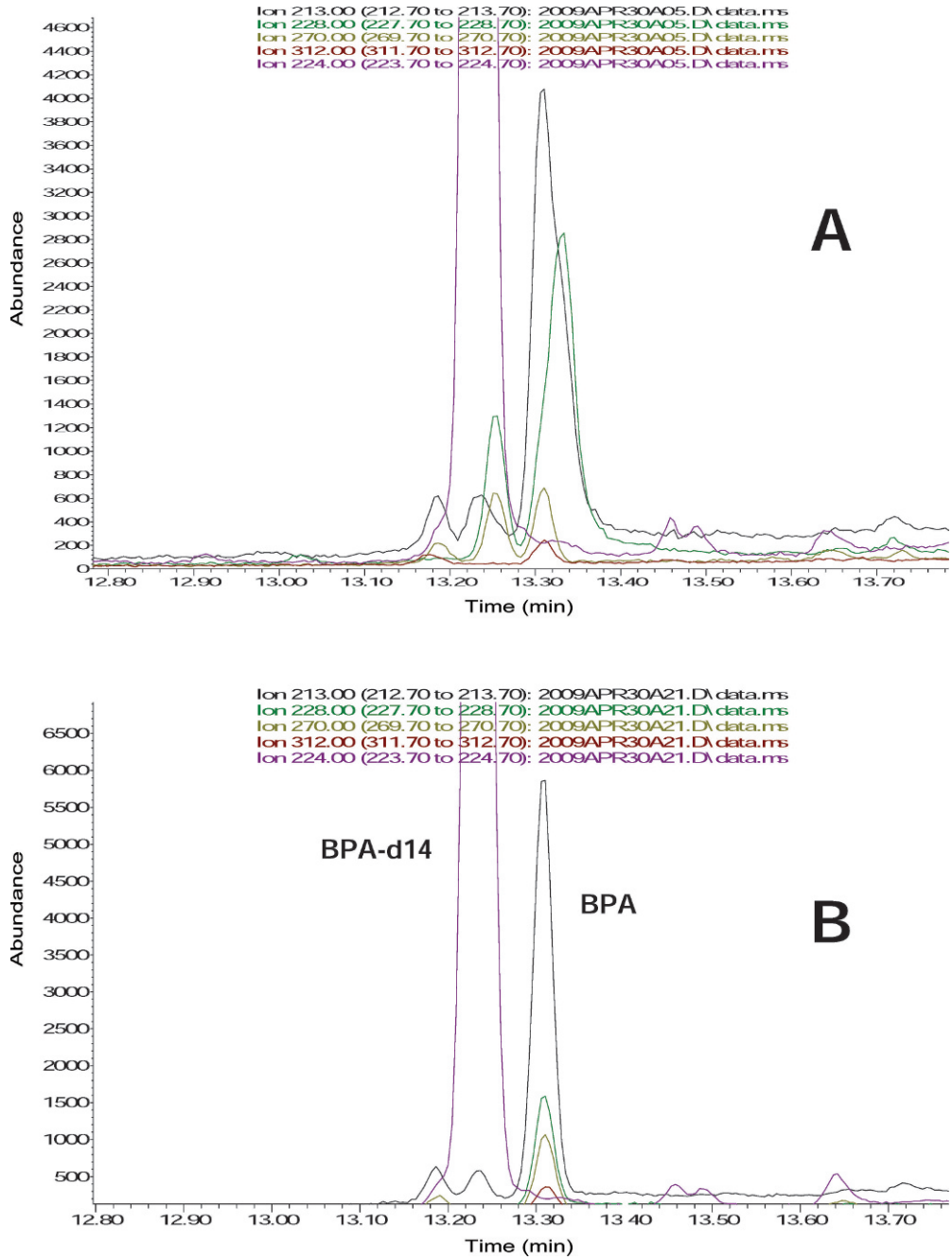

FIGURE 1. *GC-MS ion chromatograms. (A) Beer sample (interference with ions* m/z *213 and* m/z *228); (B) standard (no interference). BPA ion abundance in decreasing order:* m/z *213,* m/z *228,* m/z *270, and* m/z *312.*

microliter of sample extract was injected into the GC system in splitless mode. The analytes were separated on an HP-5MS capillary column (30 m by 0.25 mm by 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, raised to 325°C at 35°C/min, and held for 1 min. The MSD was operated with electron impact ionization in selected ion monitoring mode. The following ions were selected for derivatized bisphenol A: *m/z* 213, *m/z* 228, *m/z* 270, and *m/z* 312. For bisphenol A-d14, *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. Confirmation of BPA identity was based on the retention time and the ion ratios. Two reagent blanks were processed and analyzed with each set of samples for BPA, and the average blank BPA concentration was subtracted from all sample results. The calculation of BPA concentrations in samples was based on the isotope dilution method. The final results were expressed on a sample volume basis (micrograms per liter) by converting sample weight to volume based on the density of each product. The method limit of detection and limit of quantification, estimated as 3 and 10 times the signal-to-noise ratio, respectively, were 0.0045 and 0.015 μg/liter.

RESULTS AND DISCUSSION

Because BPA was not expected to be present in high concentrations in canned beer products based on the canned soft drink results, an extensive survey was not deemed necessary; thus, only the beer products available in both cans and glass bottles were collected to check BPA

TABLE 2. *Concentration of bisphenol A in beer products in different types of containers*

Brand	Product	Container type	Concn (μg/liter)
H	H-1	Glass bottle	0.054
	H-2	Can	0.19
I	I-1	Glass bottle	ND[a]
	I-2	Can	0.081
J	J-1	Glass bottle	ND
	J-2	Can	0.33
K	K-1	Glass bottle	ND
	K-2	Can	0.12
L	L-1	Glass bottle	ND
	L-2	Can	0.14
M	M-1	Glass bottle	ND
	M-2	Can	0.54
N	N-1	Glass bottle	ND
	N-2	Can	0.11
O	O-1	Glass bottle	ND
	O-2	Can	0.18

[a] ND, not detected. Limit of detection was 0.0045 μg/liter.

concentrations and to investigate the sources of BPA in canned beer products. For each of the 38 soft drink and beer samples, two subsamples from the same container were analyzed, and the mean of the two results was reported. The mean relative difference between the two replicates was 23%. Table 1 provides the results of BPA concentrations in the 22 soft drink samples. BPA was not detected in samples of almost all soft drink products in PET and glass bottles except for one soft drink product in a PET bottle. Sample B-2 (soft drink in a PET bottle) had a concentration of 0.018 μg/liter, i.e., above the method limit of detection (0.0045 μg/liter). In contrast, BPA was detected in samples of all soft drink products in cans, but the concentrations were low, from 0.019 to 0.21 μg/liter, similar to the BPA concentrations in the same products of different lots tested previously *(1)*. Because BPA was not detected in the soft drink products in PET and glass bottles, the trace concentrations of BPA detected in the corresponding canned products were very likely the result of migration from can coatings.

Considerable interference with the quantification ion *m/z* 213 and the qualification ion *m/z* 228 from sample matrices were observed for all 16 beer samples; none of the ion ratios (*m/z* 228/213, *m/z* 270/213, and *m/z* 312/213) were within the acceptable range (25% of the corresponding ion ratios of the standard). Figure 1 shows the typical GC-MS ion chromatograms of a beer sample (with interference) and a standard (without interference). The presence of BPA in these beer samples could not be determined accurately because of the interference. However, because the other two qualification ions, *m/z* 270 and *m/z* 312, did not show evidence of interference by the sample matrix for all beer samples (the ratio *m/z* 312/270 for all beer samples was 0.29 to 0.33 compared with 0.31 for the standard), BPA concentrations in these beer samples were estimated using ion *m/z* 270 as the quantification ion, and the results are summarized in Table 2. Based on the ion *m/z* 270, BPA was not detected any of the beer samples in glass bottles except for product H (0.054 μg/liter), but BPA was detected in all beer samples in cans at concentrations of 0.081 to 0.54 μg/liter. BPA presence in canned beer samples and absence (or lower concentration) in bottled beer samples indicates that migration from can coatings is the source of BPA in canned beer products. As demonstrated by Goodson et al. *(4)* for canned foods, migration of BPA from can coatings occurs during the can processing step, and BPA concentrations in canned foods did not change during extended storage at room temperature. Thus, the low BPA concentrations in the canned beer products are very likely due to the migration of BPA from can coatings during the can sterilization process, whereas migration from can coatings into beer at room temperature or below is extremely slow and considered negligible. Because the low BPA concentrations in beer products were similar to those in canned soft drinks, migration of BPA from can coatings may not be affected by the presence of alcohol in the beer products.

In summary, although concentrations of BPA in canned soft drink and beer products were low, the absence of BPA in plastic- and glass-bottled beverage products and its presence in the corresponding beverage products in cans indicate that migration from can coatings is very likely the source of BPA in canned beverage products. However, the low BPA concentrations in both canned soft drink and beer products indicate that migration of BPA from can coatings at room temperature or below is negligible. The interference with BPA analysis due to the sample matrices for the beer products suggests that MS detection is essential in analysis of foods for various contaminants to avoid obtaining false-positive results.

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