Determination of Bisphenol A Migrating from Epoxy Can Coatings to Infant Formula Liquid Concentrates

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Migration of bisphenol A (BPA) from epoxy-coated can surfaces to infant formula concentrates is reported. Levels of BPA in the undiluted concentrates surveyed in this study range from 0.1 to 13 parts per billion (ppb) as determined by solid phase extraction/high-pressure liquid chromatography with fluorescence detection and confirmation by gas chromatography with mass selective detection. Fourier transform infrared spectroscopy with 30° specular reflectance/transmittance was used to screen formula cans for epoxy coatings.

Keywords: *Bisphenol A; epoxy; can coating; migration; infant formula*

INTRODUCTION

Epoxy polymers are resistant to solvents and can bond to a variety of substrates, especially metals (Gannon, 1990). These properties make epoxy resins a popular choice for use in enamel coatings on the food contact surfaces (FCS) of metal food and beverage cans. Whenever a polymeric material is in contact with food, the residues and additives in the polymer may migrate to the food. This is especially true for polymers exposed to food at elevated temperatures, i.e. heat-processed canned foods. Bisphenol A (4,4′-isopropylidenediphenol, CAS Registry No. 80-05-7, more commonly known as BPA) is a principal reactant in the preparation of many epoxy polymer resins, including those used in food contact can enamels. Recently, BPA has been associated with estrogen-like behavior in *in-vitro* cell culture studies (Krishnan et al., 1993). The effects of BPA *in vivo* are still unclear.

We have developed a protocol to screen for BPA-based epoxy can coatings on the FCS and determine BPA in infant formula concentrates. If a can coating is screened using Fourier transform infrared spectroscopy (FT-IR) and identified as an epoxy, migrated BPA in the formula is concentrated by solid phase extraction (SPE) and determined in the extract by high-pressure liquid chromatography (HPLC) with fluorescence detection. BPA is then confirmed in selected formula extracts by gas chromatography with mass selective detection (GC-MS).

To determine BPA in infant formulas, a protocol was needed to determine low parts per billion (ppb) levels. Methods reported in the literature for determination of BPA, bisphenol F (BPF), the diglycidyl ether of BPA (DGEBA or BADGE in Europe), and the diglycidyl ether of BPF (DGEBF) (Brotons et al., 1995; Begley et al., 1991; Losada et al., 1991; Crathorne et al., 1986; Gandara et al., 1990; Henriks-Eckermanand and Laijoki, 1988) were not suitable for our goals. The protocol described in this paper provides greater specificity and sensitivity for BPA than the methods reported in the literature.

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Table 1. Amount of BPA in Undiluted Canned Infant Formula Concentrates

formula brand[a]	can type[b]	BPA determined[c] ppb	BPA determined[c] ng/cm^2	% recov
A	1	13.2	11.6	75
A	1	12.1	10.6	90
E	3	9.5	8.5	90
B	1	8.3	7.4	79
A	4	5.1	4.5	83
B	4	4.8	4.2	67
C[d]	5	4.5	4.0	104
D[d]	4	3.9	3.5	109
B	4	3.6	3.2	95
C	2	1.5	10.1	80
C	2	1.3	8.8	89
C[d]	2	1.3	8.8	100
D	4	0.7	0.6	106
D	4	0.1	0.1	97

[a] At least three examples of each *major* manufacturer's formula were purchased. An example of one other brand is included (E). [b] Can types: 1, two piece, 384 mL volume, epoxy-coated lid, modified epoxy-coated body; 2, two piece, 384 mL volume, epoxy-coated lid, PVC-coated body; 3, three piece, 384 mL volume, epoxy-coated ends, modified epoxy side (body); 4, three piece, 384 mL volume, epoxy-coated ends and side (body); 5, three piece, 945 mL volume, epoxy-coated ends, modified epoxy side (body) (this can contained ready-to-feed formula, ca. 3 years old at time of analysis). [c] Area (cm^2) is calculated for epoxy-coated surfaces only. [d] Soy-based products; all others are milk-based.

EXPERIMENTAL PROCEDURES

Reagents. All solvents were of HPLC grade and purchased from Burdick and Jackson, Inc. (Muskegon, MI). BPA was of 99+% purity purchased from Aldrich Chemical Co. (St. Louis, MO). Water was distilled and then purified by a Milli-Q water purification system (Millipore Corp., Milford, MA). A powdered infant formula concentrate (Gerber Products Co., Freemont, MI) was purchased from a local supermarket and prepared daily as needed (for an analytical blank) according to instructions on the label.

Infant Formula Concentrates. At least three cans from each of four major manufacturers of infant formula in the United States were surveyed using the protocol presented here. All of the canned formula concentrates were purchased in suburban Maryland and Virginia near Washington, DC, during January and February 1996. One exception was a single 945 mL can of ready-to-feed formula, *ca.* 3 years old at the time of testing (as noted in Table 1.). Each manufacturer in the test group has several formulations of milk- and soy-based products. The variety of formulations ranges from those

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fortified with nutrients such as iron to products intended for consumption by different age groups. All of the formula concentrates contained between 7% and 8% (w/v) fat and were intended for a 1:1 dilution (v/v) with water. Except as noted, all of the cans in the survey were made to contain 384 mL and were of two- or three-piece design (two-piece having a top and seamless body and three-piece having two ends joined by a seamed body).

FT-IR Apparatus and Operating Conditions. A Nicolet Magna 550 Series II (Nicolet Analytical Instruments, Madison, WI) FT-IR equipped with a 30° horizontal specular reflectance/transmittance attachment (Janos Optical Corp., Townshend, VT) and interfaced with a personal computer running Omnic (Nicolet Analytical Instruments) software was used to prescreen all of the cans surveyed in this study. The FT-IR was set to average 32 scans.

Qualitative Analysis of Can Coatings by FT-IR. The lot number and any relevant description of the can such as design, volume, formula type (soy-based or milk-based, iron fortified, etc.), and manufacturers were recorded. (All were analyzed prior to the expiration date except as noted in Table 1.) The formula concentrate was transferred from the can to a clean Erlenmeyer flask and reserved for later analysis. The inside of the empty can was cleaned with soap and water to remove any food residues and rinsed. The inside surface of the can was dried with a clean towel and then air-dried for several hours. When the can had been thoroughly dried, a coupon measuring *ca.* 5 cm × 1.5 cm was removed from each side (body and lid or ends) of the can with tin snips. One coupon at a time was then placed flat on the horizontal specular reflectance/transmittance attachment mounted in the FT-IR so the infrared radiation was incident on the food contact surface of the can coupon. The can coupons were analyzed and the spectra compared to infrared spectra of known epoxies and other polymers.

SPE. SPE tubes were 6 mL styrene/divinylbenzene porous polymer packing available as Supelclean Envi-chrom P (Supelco, Inc., Bellefonte, PA).

HPLC Apparatus and Operating Conditions. The HPLC System consisted of a Spectra Physics Model 8800 pump (Spectra-Physics, San Jose, CA), a Rheodyne Model 7125 injector valve (Rheodyne, Inc., Cotati, CA) equipped with a 50-μL injection loop, a second Rheodyne 7125 injector valve equipped with a Shandon Hypercarb S 7 μm guard column (Keystone Scientific, Inc., Bellefonte, PA) as the injection loop, a Spectra-Physics SP8792 column heater set at 40 °C, a 7 μm particle size, 150 mm × 4.6 mm Shandon Hypercarb S graphitized carbon column (Keystone Scientific, Inc.), a Waters Model 470 fluorescence detector (Waters Division of Millipore Corp., Milford, MA) operated at 235 nm excitation and 317 nm emission, and a Spectra-Physics Chromstation/2 data system interfaced to an IBM PS/2 Model 80 personal computer. The HPLC mobile phases were as follows: solvent A, water/methanol/acetonitrile (90:5:5); solvent B, water/methanol/acetonitrile (5:90:5); and solvent C, water/methanol/acetonitrile (5:5:90). A mobile phase program was run as follows at a flow of 0.5 mL/min: isocratic 30% A, 30% B, and 40% C to 12 min; linear gradient to 10% A, 10% B, and 90% C in 1 min; isocratic for 3 min; linear gradient to 30% A, 30% B, and 40% C in 1 min; isocratic for 10 min to re-equilibrate the column.

HPLC Quantitation. External calibration was performed using chromatographic responses of at least five standard concentrations of BPA ranging from 1 to 50 ng/mL. The standard curve of the concentrations versus chromatographic peak areas was calculated from a linear regression program.

Quantitative Analysis by HPLC. A 10.0−30.0 mL aliquot (optimum volume of aliquot will vary depending on the concentration of BPA in the test formula) of the test formula was removed and the remainder reserved for additional testing in an Erlenmeyer flask in a refrigerator at 4 °C. The aliquot was diluted to 100.0 mL with distilled deionized water (DDW) and transferred to a 100 mL buret. An SPE cartridge was rinsed and prewetted with a total of 20 mL of DDW. Enough DDW was retained on the cartridge so that the bed remained completely covered. The buret was placed above the cartridge, and a flow from the buret to the cartridge was adjusted to be compatible with a flow of *ca.* 10 mL/min through the cartridge produced from a vacuum source. The total volume of diluted formula was passed through the cartridge. The buret was washed with 15 mL of DDW and the wash allowed to pass completely through the cartridge. The total aqueous eluate was drained and discarded, and the residual water was removed from the cartridge by air-drying with vacuum for 5 min. The buret was then washed with 15 mL of hexane and the wash allowed to pass through the cartridge. BPA was eluted from the cartridge into a calibrated 15 mL conical tube with three 5 mL volumes of chloroform. The cartridge was drained between each volume. The chloroform was evaporated (by gentle heating over a steam bath with the addition of two or three carborundum boiling chips to ease bumping) to *ca.* 50 μL. The concentrated extract was then diluted with up to 4.0 mL (amount of dilution depends on the concentration of BPA in the formula) of mobile phase (30% A, 30% B, and 40% C) and analyzed for BPA by HPLC. This extraction/concentration was performed in triplicate for each canned formula tested. Recoveries were calculated by fortifying the formula (in duplicate) with an amount of BPA equal to the average amount quantitated in the same formula. The average BPA concentration in the unfortified formula was subtracted from the total BPA concentration in the fortified formula to determine the net amount of BPA recovered. An analytical blank was analyzed as described above using formula prepared from dry powdered mix and HPLC grade water.

GC-MS Apparatus and Operating Conditions. The GC-MS system consisted of a Hewlett-Packard (HP) 5890B gas chromatograph (GC) with an HP 7673 automated liquid sampler, a capillary split−splitless injector, a 30 m × 0.25 mm i.d. Rt_x-5 (cross-linked 95% dimethyl−5% diphenyl polysiloxane) FSOT capillary column with 1.0 μm film thickness (Restek Corp., Bellefonte, PA), and an HP 5970B mass selective detector (MSD) with capillary direct interface to GC. The GC operating parameters were as follows: UHP helium carrier gas at *ca.* 10 psi column head pressure (1 mL/min); injection volume, 2 μL; split vent open after 1 min; injector temperature, 280 °C; interface temperature, 290 °C; oven program, initial temperature 100 °C, program at 10 °C/min to 280 °C, and hold 3 min (BPA t_r, *ca.* 19.1 min). The MSD was operated in the selected ion monitoring mode; ions *m*/*z* 119, 213, and 228 were monitored at 2.6 scans/s from 18.8 to 19.3 min, dwell 100 ms. The GC-MS system was controlled with a Pascal Chemstation data system.

A 2 μL injection of concentrated infant formula extract in chloroform was made, and confirmation of BPA was based on retention time and relative response ratios of the ions listed above.

RESULTS

Infrared analysis showed all cans to have at least one of the FCSs coated with an epoxy or "modified" epoxy enamel based on BPA. In three cases, only the lids of two-piece cans were found to be coated with an epoxy. All spectra were compared (visually and by a matching algorithm) with standards in the High Resolution Hummel Polymer Library and spectra acquired from the FCS of epoxy-coated "customer ready" (unused) cans obtained from a major supplier (Figure 1). The matching algorithm identified some epoxy coatings as modified with fatty acid esters and oils (Figure 2 and Table 1). The incorporation of flexibility-enhancing modifiers in epoxy resins based on BPA is common (Savla, 1977).

The presence of BPA was confirmed in selected formula extracts by GC-MS. The ion ratios of the extracts agreed with those of authentic BPA standards to within ±20%.

The highest amount of BPA measured in any of the infant formula concentrates was 13.2 ppb and the lowest was 0.1 ppb. Levels of BPA consumed in prepared formula would be lower because directions on the labels call for a 1:1 dilution (v/v) of formula concentrate with

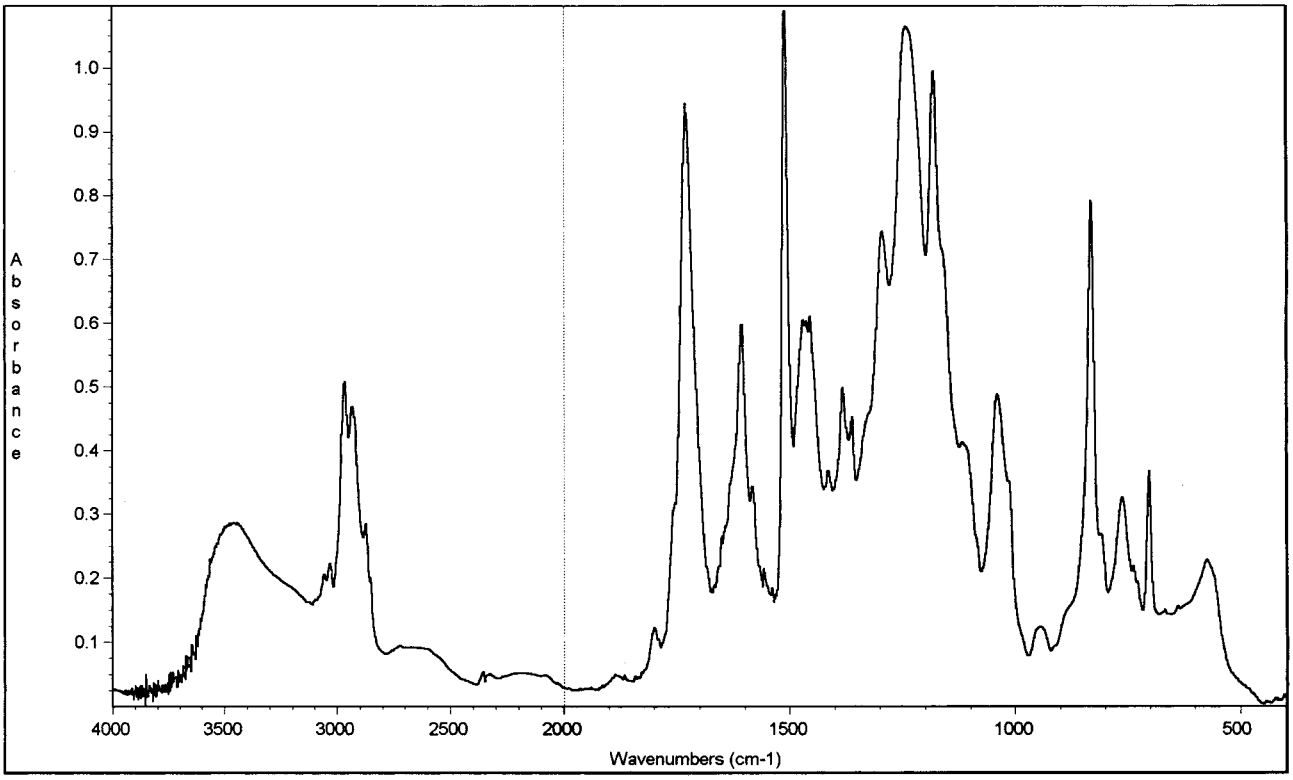

Figure 1. Infrared spectrum of unused epoxy-coated can food contact surface.

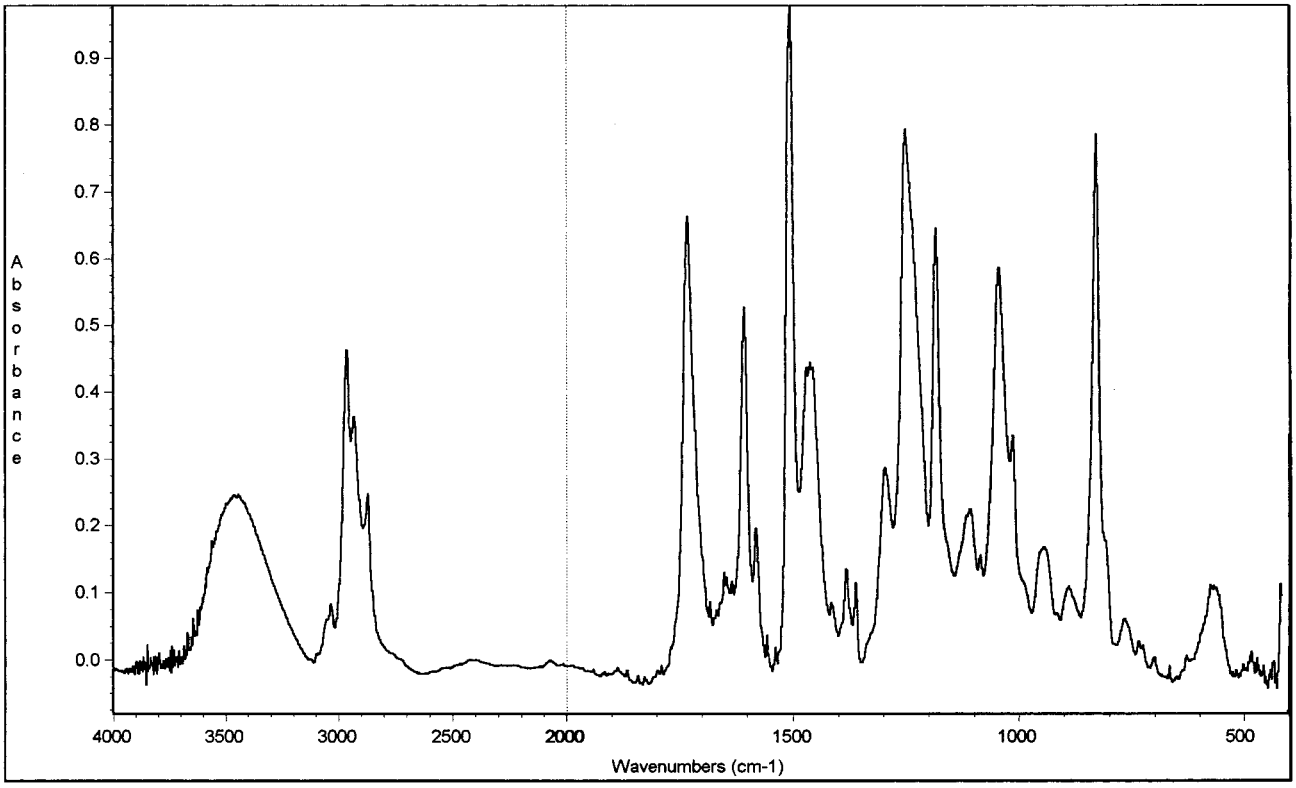

Figure 2. Infrared reflectance/transmittance spectrum of BPA-based epoxy can coating, typical of those encountered in this investigation.

water. Recoveries for milk-based formulas averaged 86%, while recoveries for soy-based formulas averaged 104%. Coefficients of variation for the triplicate runs ranged from 2% to 27% for milk-based products and from 9% to 27% for soy-based products. The limit of detection, defined as 3 times the standard deviation of the baseline produced by the detector when measuring a blank prepared from powdered formula after concentration by a factor of 30, was 0.9 ppb. BPA was extracted from formula and concentrated by as much as 30-fold by using SPE before HPLC analysis. An obvious correlation between the product's expiration date and the level of BPA determined in the formula was not observed.

The data in Table 1 are reported in terms of both concentration (ppb) and mass of BPA per unit area of

epoxy-coated FCS (ng/cm^2); the latter accounts for differences in can construction.

DISCUSSION

The qualitative FT-IR data presented here show that epoxy is used as an enamel coating on at least one of the FCSs of all of the infant formula cans in the test group. Specular reflectance/transmittance FT-IR provides a quick and easy means to screen can coatings. This information is essential in conjunction with quantitative techniques to determine the total amount of BPA migration to the formula on a surface area basis.

The protocol for HPLC determination of BPA described here is preferred to those for BPA, BPF, DGEBA, and DGEBF in aqueous-based food simulants (Gandara et al., 1990; Crathorne et al., 1986) and organic solvents (Losada et al., 1991; Henriks-Eckerman and Laijoki, 1988). The food matrix and migration levels of DGEBA investigated by Begley et al. (1991) differed significantly from those investigated here for BPA. Brotons et al. (1995) reported BPA in canned vegetables at levels from 1 to 6 times greater than the highest level reported here in infant formula and with higher coefficients of variation. The sensitivity reported here for BPA in formula concentrates is comparable to that reported in less complicated organic and aqueous matrices in the earlier studies.

This survey of canned infant formula liquid concentrates has shown that low parts per billion levels of BPA can be extracted, concentrated, determined, and confirmed using the combined techniques of SPE, HPLC with fluorescence detection, and GC-MS.

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