Potential Exposure to Bisphenol A from Food-Contact Use of Epoxy Coated Cans

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The potential dietary exposure to bisphenol A (BPA) from the use of food and beverage cans coated with bisphenol A-based epoxies was determined. The calculation was based on migration data from extraction studies using food-simulating solvents and time and temperature conditions recommended by U.S. Food and Drug Administration (FDA). The study demonstrates that no detectable BPA was found in the extracts from beverage cans using a method sensitive to five parts per billion (5 ppb) in the food simulant; the average migration of BPA from food cans was determined to be 37 ppb. Using these data, along with the use patterns for food and beverage cans, the maximum potential dietary exposure to bisphenol A was estimated to be approximately 2.2 ppb. Because the conditions of the migration tests exaggerate actual use conditions, this value overstates the reasonably anticipated actual potential exposure.

INTRODUCTION

Epoxy resins have been used as components of coatings for food and beverage cans for more than 50 years. Epoxies based on bisphenol A (BPA)(4,4'-isopropylidenediphenol, 2,2-bis(4-hydroxyphenyl)propane) are cleared by the United States Food and Drug Administration (FDA) for use as the food-contact surface of coated cans,[1] in addition, bisphenol A-based epoxies are permitted for the same use throughout Europe[2] and the Pacific Rim. While the European Union (EU) has established a specific migration limit in food of 3 mg/kg [i.e., 3 parts per million (3 ppm)], FDA has not imposed a comparable limit on BPA.

While analyses to determine the potential migration of BPA from coatings to food simulants have been published, these have not employed collaboratively studied methods nor were they developed to have the necessary performance characteristics for suitable analyses in regulatory food simulants.[3,4]

The present study was conducted to determine the potential migration of BPA from cans coated with BPA-based epoxies. The study, conducted in accordance with the procedures developed by FDA,[5] was performed using food-simulating solvents and time and temperature conditions recommended by FDA. Using the results of the migration study, the potential dietary exposure to BPA was estimated.

Specifically, the postulated dietary consumption of a substance depends on the potential level in food (e.g., a value derived from migration studies) and on the fraction of an individual's diet likely to contact packaging materials containing the substance. FDA employs the term Consumption Factor (CF) to describe the portion of the diet likely to contact specific packaging materials.[5] In addition, to account for the variable nature of food contacting each packaging material, FDA has developed Food-Type Distribution Factors (f_T) for each packaging material to indicate the fraction of the food contacting each material that is aqueous, acidic, alcoholic, and fatty. Using these parameters, along with the experimentally determined potential migration levels, the possible dietary exposure to BPA from the use of epoxy can coatings was determined.

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MATERIALS AND METHODS

Preparation of the Samples

Test specimens consisted of commercial cans collected at two different points in time. In both cases, the samples consisted of unused cans obtained from the major can manufacturers in the United States. The types of cans fall into three categories: two-piece beverage/beer cans, two-piece food cans, and three-piece food cans. In all

Table 1—Instrumental Conditions for High Performance Liquid Chromatography (HPLC) Analyses of Bisphenol A (Phase 1, Initial Conditions)

Pump	Hewlett Packard 1050
Autosampler	Hewlett Packard 10
Detector	Hewlett Packard 1046 programmable fluorescence detector
Wavelength	Excitation 229 nm Emission 311 nm
Columns	Two Supelco C18 5μ (250 x 4.6 mm) in series
Mobile phase	13.0% methyl alcohol, 44.2% water, and 42.8% acetonitrile
Flow rate	1.0 mL/min isocratic
Column oven temperature	40 C
Injection volume	20 μL

cases, the can samples were selected to maximize the level of BPA-based epoxy present in the coatings

Two-Piece Beverage/Beer Cans

Coated aluminum beer and beverage cans from three major suppliers were used. While can coatings for beverages and beer are identical from a given supplier, beverage cans were used since the coating weight of beverage cans is slightly greater than that of beer cans. These samples were all "standard" 12-ounce cans supplied by three individual can manufacturers using coating formulations supplied by three different suppliers.

Two-Piece Food Cans

Four types of steel two-piece food cans/coatings were used. The four represent primarily aqueous types of food, a fifth sample, virtually identical to one of the previous four, was used to accomplish two purposes. First, it represents cans used to pack meat products, which are considered fatty foods and, second, it permits the examination of potential hydrolysis of the epoxy coating, since 95% ethanol, a potentially severely aggressive solvent for this application, was used as the food simulant (as described in the following). Thus, a total of five two-piece food can samples was tested. The can sizes were 300 × 407, which may be considered "one-pound" cans, and one 211 × 315 sample. The can samples for fatty foods and one of the can samples for aqueous foods were supplied by the same can manufacturer and used coatings, supplied by the same coating formulator, that were virtually identical, with the exception that the can sample for fatty foods had an approximately 30% greater coating weight than the can sample for aqueous foods. The other three samples were provided by three other can manufacturers using coating formulations supplied by three different suppliers.

Table 2—Instrumental Conditions for High Performance Liquid Chromatography (HPLC) Analyses of Bisphenol A (Phase 1, Addition of Flush Step)

Pump	Hewlett Packard 1050
Autosampler	Hewlett Packard 10
Detector	Hewlett Packard 1046 programmable fluorescence detector
Wavelength	Excitation 229 nm Emission 311 nm
Columns	Two Supelco C18 5μ (250 x 4.6 mm) in series
Mobile phase	13.0% methyl alcohol, 44.2% water, and 42.8% acetonitrile
Flow rate	1.0 mL/min isocratic, with a 12 min acetonitrile "flush" to remove low polarity materials from the columns between injections
Column oven temperature	40 C
Injection volume	20 μL

Three-Piece Food Cans

A total of 10 types of steel three-piece cans/coatings was used. The can sizes were either 300 × 407 or 300 × 404, which may be considered "one-pound" cans. The coatings/cans typify their use with the following classes of packed foods: infant formula (liquid), vegetables, meat, fruit juice, and tomato products. Five of the samples were collected for the initial phase of the study. Four of these five were available for the second phase of the study, five additional samples were collected for the second phase. The cans were supplied by four individual can manufacturers using coating formulations supplied by five different suppliers.

Food-Simulating Liquids. FDA accepts the use of food-simulating liquids to determine the migration of components from polymers into foods because analyzing for trace levels of migrants in complex food matrices is technically challenging. In accordance with FDA's recommendations regarding food simulants, the food simulants used in the present study were 10% ethanol and 95% ethanol. 10% ethanol is an appropriate simulant for aqueous, acidic, and low alcohol content (up to 15% ethanol) foods, including beer, while 95% ethanol is an appropriate, albeit exaggerative, alternative fatty food simulant. The water was processed by deionization and distillation. The 10% ethanol was prepared by dilution of 95% ethanol (Quantum Chemical Corporation) with deionized, distilled water.

EXPERIMENTAL PROCEDURES

Testing was conducted under conditions that simulate or exaggerate the most severe conditions of actual use, which vary depending on the type of food packed in the cans.

Two-Piece Beverage/Beer Cans

Carbonated soft drinks, by far the predominant type of beverage packed in cans, are typically filled at room temperature and stored at or below room temperature. This corresponds to FDA's Condition of Use E* Some of

*Room temperature filled and stored—no thermal treatment in the container.

the beer manufactured in the U S is pasteurized after it is filled into the can This corresponds to FDA's Condition of Use D† Therefore, the testing of the representative beverage cans (higher coating weight) was performed under the more severe of the two conditions, i e , Condition of Use D (described in the following), so that the testing would serve as a simulation of beer use and, at the same time, an exaggeration of beverage use All of these samples were tested using 10% ethanol as the food simulant

Two-Piece Food Cans

The types of food packed in two-piece cans generally require retort sterilization, followed by room temperature storage This corresponds to FDA's Condition of Use A** Four of the samples were tested using 10% ethanol, while a fifth sample, used for meat products, was tested using 95% ethanol

Three-Piece Food Cans

The thermal processing conditions for the foods packed in three-piece cans vary with each type of food

Vegetables retort (Condition of Use A),

Meat retort (Condition of Use A),

Infant formula retort (Condition of Use A),

Tomato products retort (Condition of Use A),

Fruit juice hot filled at temperatures as high as approximately 190°F, which corresponds to FDA's Condition of Use C††

Eight of the samples were tested using 10% ethanol, while two (used for meat products) were tested using 95% ethanol

In accordance with FDA's "Recommendations for Chemistry Data for Indirect Food Additive Petitions",[5] Condition of Use A testing was performed at 250°F for 2 hr followed by 120°F for 10 days, Condition of Use C testing was performed by filling the container with "hot" solvent, holding at 212°F for 30 min, followed by 120°F for 10 days, and Condition of Use D testing was performed by filling the container with solvent at 150°F, holding at 150°F for 30 min, followed by 120°F for 10 days

Each exposure test was performed in duplicate Duplicate solvent blanks were similarly extracted The extracts obtained in the first phase were analyzed by high performance liquid chromatography (HPLC) direct injection, using the HPLC parameters in either *Tables* 1, 2, or 3 (see Discussion Section) The extracts obtained in the second phase were analyzed by HPLC using the parameters in *Table* 4 and by gas chromatography with mass selective detector (GC/MS) using the parameters in *Table* 5 The GC/MS is operated in the selective ion monitoring (SIM) mode and the method is based on the derivatization of the phenols to their silyl ethers using N,O-bis(trimethylsilyl)trifluoroacetamide (Pierce),

†Hot filled or pasteurized below 190°F
**High temperature heat-sterilized e g , over 212°F
††Hot filled or pasteurized above 190°F

Table 3—Instrumental Conditions for High Performance Liquid Chromatography (HPLC) Analyses of Bisphenol A (Phase 1, Final Conditions)

Pump	Hewlett Packard 1050
Autosampler	Hewlett Packard 1 0
Detector	Hewlett Packard 1046 programmable fluorescence detector
Wavelength	Excitation 229 nm Emission 311 nm
Columns	Two Supelco C18 5µ (250 x 4 6 mm) in series
Mobile phase	14 3% methyl alcohol, 48 6% water, and 37 1% acetonitrile
Flow rate	0 8 mL/min isocratic, with a 12 min acetonitrile "flush" to remove low polarity materials from the columns between injections
Column oven temperature	40 C
Injection volume	20 µL

bisphenol F (bis(4-hydroxylphenyl)methane) is employed as an internal standard A full description of the methods used in the second phase is discussed in the following article [6]

For the analyses in both the first and second phases, quantitation was based on a standard curve generated by external BPA standards, prepared in either 10% ethanol or 95% ethanol, at concentrations of 5, 10, 20, 40, 80, and 160 ng/mL (parts per billion (ppb)) The detector response was measured by peak area The method of analysis has a correlation coefficient of greater than 0 990 when a plot was generated of known standard concentrations versus detector response The limit of detection (defined as not quantifiable) was set at 5 ppb

For each food simulating liquid in the first phase of the study, validation of the results was performed by spiking the extract with BPA at either 5, 10, 20, 40, or 50 ppb prior to work up of the extract, the spiked extract was analyzed as described for each food simulant The average of the recoveries was 101±19%

Several of the extracts in the second phase of the study were likewise spiked with either 10 or 20 ppb BPA to validate both the HPLC and the GC/MS analyses The average of the recoveries for HPLC was 86±1% and that for GC/MS was 110±0% Further validation of the results was achieved by concomitant analyses of each of the second phase extracts by both HPLC and GC/MS

RESULTS AND DISCUSSION

Migration Results

The study was conducted in two phases at different points in time In the first phase, the 13 different can samples used in the study were selected to cover the major U S can manufacturers and their coating suppliers The extracts obtained from these samples were analyzed using HPLC with a fluorescence detector For the three beverage/beer can samples, BPA was not detectable (defined as not quantifiable below 5 ppb) in any of

Table 4—Instrumental Conditions for High Performance Liquid Chromatography (HPLC) Analyses of Bisphenol A (Phase 2)

Pump	Hewlett Packard 1050
Autosampler	Hewlett Packard 10
Detector	Hewlett Packard 1046 programmable fluorescence detector
Wavelength	Excitation 229 nm Emission 311 nm
Columns	Two Supelco C18 5µ (250 x 4.6 mm) in series, plus a Supelco LC-18 guard column
Mobile phase	43.0% acetonitrile (ACN) and 57.0% water
Flow rate	0.8 mL/min isocratic

Inter-injection gradient

Time	Mobile Phase	Flow Rate
30 min	81.6/18.4 ACN/water	1.0 mL/min
35 min	0/100 ACN/water	1.0 mL/min
40 min	0/100 ACN/water	1.0 mL/min
45 min	100/0 ACN/water	1.2 mL/min
50 mim	100/0 ACN/water	1.0 mL/min

Column oven temperature	40 C

the extracts. On the other hand, the two-piece and three-piece food cans gave results ranging from no detectable levels of BPA in the extracts (method sensitivity of 5 ppb) to 121 ppb, the two-piece food cans and three-piece food cans have been grouped together, since the results do not indicate a migration behavior that would distinguish one from the other.

In performing these analyses, the initial instrumental conditions, described in *Table* 1, were derived from the beverage container experiments. As the extraction experiments proceeded to the food cans, where the extraction conditions were more aggressive, a flush step was added to the instrumental conditions (described in *Table* 2) to remove materials trapped on the column which could interfere with subsequent determinations. A final set of instrumental conditions was developed (described in *Table* 3) when an interfering chromatographic peak was coeluting, the final set of instrumental conditions, which employed a lower acetonitrile content than the first two sets of conditions, resulted in extending the elution of BPA from approximately 11 min to approximately 17 min.

In reviewing these data, authors came to believe that some of the migration values reported, which ranged from none detected (using an analytical method having a limit of detection of 5 ppb) to 121 ppb, appeared to be higher than expected. This was based, in part, upon the unusual or unsymmetrical shape of the chromatographic signal attributed to BPA in several of the extracts, not all of which may have been due to the presence of bisphenol A. Upon consideration of the chromatograms, it was postulated that there may have been overlap between BPA and one or more substances exhibiting the same or similar retention time, i.e., analytical interferences may have been present that contribute to the BPA response.

For this reason, a series of round-robin tests was undertaken to generate methodology so that suitably equipped laboratories could confidently perform valid analyses of extracts for BPA. These efforts, a complete description of which is presented elsewhere in this issue,[6] resulted in the development of both HPLC and GC/MS methods.

Having obtained methodology to ensure, to the extent possible, that analytical interferences would be minimized, a second phase of the study was undertaken. The objectives were to repeat, and expand upon, the analyses obtained in the first phase on extracts of two-piece and three-piece food cans, i.e., those types of test samples that, as a group, showed detectable levels of BPA.

As noted previously, the first phase consisted of 13 different can samples selected to cover the major U.S. can manufacturers and their coating suppliers. Three of the 13 samples were two-piece beverage/beer cans, which showed no detectable BPA in the first phase, using methodology sensitive to 5 ppb. Because of the non-detected results in these samples, the second study focused entirely on the two-piece and three-piece food cans, i.e., the repeat study did not include beverage/beer cans. Of the 10 food can samples tested previously, nine were available for the current study. In addition to these samples, five additional samples, all three-piece cans, representing major types manufactured by the principal can manufacturers, were included in the second phase of the study.

The results from phases one and two are presented in *Table* 6. It should first be noted that the HPLC and GC/MS results in phase two give virtually identical results for any individual sample. These sets of comparable results validate the methodology employed in the second phase of the study. For this reason, the discussion following focuses on the averages of the HPLC and GC/MS results.

The two-piece and three-piece food cans that were duplicated from the first phase gave results ranging from no detectable levels of BPA in the extracts (method sensitivity of 5 ppb) to 77 ppb, as noted, the two-piece food cans and three-piece food cans have been grouped together since the results do not indicate a migration behavior that would distinguish one from the other. The five "new" cans gave results ranging from 12 ppb to 94 ppb. A review of the data indicates that five of the samples that were duplicated from the previous study showed essentially no change. Apparently, the use of the refined analytical methods demonstrated that chromatographic interferences were insignificant for these

Table 5—Instrumental Conditions for Gas Chromatography with Mass Selective Detector (GC/MS) Analyses of Bisphenol A (Phase 2)

Instrument	Hewlett Packard GCMS model 5970 Selective Ion Monitoring mode Masses 329 and 344 for internal standard (bisphenol F), and masses 357 and 372 for bisphenol A
Column	0.25 mm x 15 mm RTX-5
Oven temperature	250 C
Injector temperature	325 C
Transfer line temperature	300 C
Injection mode	Split, 50/1
Column head pressure	2 psi

samples However, the remaining four repeated samples showed substantial *reductions* in the measured BPA levels It is important to recognize that these reductions are not a consequence of changes in the coatings or the manufacturing procedures for the cans Instead, these results illustrate that several of the first phase results were artificially high because these particular coatings produced one or more interfering signals in the chromatographic analyses In fact, the average of the duplicated food can samples dropped from 63 ppb (in the first phase) to 36 ppb

The average of the "new" samples (i e , second phase only) is 39 ppb, and the average of all samples is 37 ppb This average value, as well as the results from the beverage and beer cans (not detectable at 5 ppb), have been used to derive an estimate of potential exposure to BPA from the use of epoxy-coated cans

Table 6—Summary of Extraction Results

Can Sample	Simulant	Test Condition	BPA Level (ppb)[a] 1st Phase	2nd Phase HPLC	2nd Phase GC/MS
Two-piece beer/beverage cans					
	10% ethanol	D	ND	—	—
	10% ethanol	D	ND	—	—
	10% ethanol	D	ND	—	—
Two-piece food cans					
non-fatty food	10% ethanol	A	71	65	68
non-fatty food	10% ethanol	A	120	72	67
non-fatty food	10% ethanol	A	71	78	77
non-fatty food	10% ethanol	A	8	7	9
fatty food	95% ethanol	A	81	47	49
Three-piece food cans					
infant formula	10% ethanol	A	121	7	8
vegetable	10% ethanol	A	40	—	—
meat	95% ethanol	A	ND	ND	6
fruit juice	10% ethanol	C	86	26	26
tomato product	10% ethanol	A	25	22	18
vegetable	10% ethanol	A	—	21	19
meat	95% ethanol	A	—	47	53
vegetable	10% ethanol	A	—	14	9
vegetable	10% ethanol	A	—	95	94
vegetable	10% ethanol	A	—	20	16

[a] ND = non-detectable or not quantifiable below limit of detection (5 ppb)

Estimate of Exposure

The evaluation of a component of a food packaging material depends on the postulated consumption of the substance (or any compound resulting from the use of the substance) Such consumption of the substance depends on the potential level in food (e g , a value derived from migration studies) and on the fraction of an individuals diet likely to contact packaging materials containing the substance As noted previously, FDA employs the term consumption factor (CF) to describe the portion of the diet likely to contact specific packaging materials, and has developed Food-Type Distribution Factors (f_T) for each packaging material or application to indicate the fraction of the food contacting each material that is aqueous, acidic, alcoholic, and fatty

FDA uses CF and f_T values, along with estimates of the potential concentration of substances that may migrate from food packaging to the contacted food products, to estimate potential human exposure to the substances The "average" concentration of the migrant in food contacting the packaging material, <M>, is derived by summing the products of the appropriate f_T values and the migration values relevant to the types of food, M_i The concentration of the migrant in the diet is obtained by multiplying <M> by the appropriate CF

Dietary Concentration = CF x <M>

$$= CF[f_{aq}M_{aq}+f_{ac}M_{ac}+f_{al}M_{al}+f_fM_f]$$

where the subscripts aq, ac, al, and f refer to aqueous, acidic, alcoholic, and fatty foods, respectively

FDA's current CF for polymer-coated metal, which includes cans, is 0 17, and the corresponding aqueous, acidic, alcoholic, and fatty food f_T values are at 0 16, 0 35, 0 40, and 0 09, respectively [5]

We have used these CF and f_T values, in conjunction with the migration results summarized earlier, to estimate the potential exposure to BPA We have used the "worst case" assumption that the potential migration of BPA to acidic and alcoholic foods (i e , beverages and beer, respectively) is 5 ppb (based on the non-detectable levels from the beverage/beer cans using the methodology which is sensitive to 5 ppb) and to all other food is 37 ppb, the average value expressed Therefore, we have estimated the potential dietary exposure to BPA from use of epoxy can coatings to be

Dietary Concentration = CF x <M>

$$= CF[f_{aq}M_{aq}+f_{ac}M_{ac}+f_{al}M_{al}+f_fM_f]$$

$$= 0\ 17[0\ 16(37\ ppb) + 0\ 35(5\ ppb) + 0\ 40(5\ ppb) + 0\ 09(37\ ppb)]$$

$$= 0\ 17\ (13\ ppb)$$

$$= 2\ 2\ ppb$$

CONCLUSIONS

Great care must be taken in analyzing extracts of can coatings for potential migrants, especially when using a non-specific analytical methodology such as liquid chromatography with fluorescence, ultraviolet, or other non-specific detection As has been demonstrated by this

study, approximately one-half of the duplicated samples showed substantial *reductions* in the measured BPA levels Furthermore, the fact that the other one-half of the can samples showed results virtually identical to those obtained in the first phase indicates that each coating is unique and its potential migration characteristics must be examined on a case-by-case basis These results amply demonstrate that determined migration values may be artificially high unless the chromatograms are examined carefully so as to preclude the presence of interfering chromatographic signals However, our observations are that artificially high results may be obtained even in instances where symmetrical peaks are obtained For this reason, it is important to perform dual confirmatory analyses (e g , HPLC as well as GC/MS) to eliminate the possibility of erroneously identifying an interference as BPA

The study demonstrates that no detectable BPA is found in the extracts from beverage cans using a method sensitive to 5 ppb in the food simulant The migration of BPA from food cans ranged from non-detectable (<5 ppb) to 94 ppb, with an average of 37 ppb Using these data, along with information regarding the use patterns for food and beverage cans, the potential dietary exposure to BPA from use of epoxy can coatings was estimated to be approximately 2 2 ppb

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References

(1) Title 21 Code of Federal Regulations (C F R) § 175 300
(2) Directive 90/128/EEC Bisphenol A is cleared by several European countries that maintain "positive lists" of permissible components of food-contact articles For example, it is permitted for use in food-contact materials in Germany, Spain, Italy, Belgium, and the Netherlands In addition, BPA has been included on the European Union's (EU) so-called "Monomers Directive" (Directive 90/128/EEC) for use as a monomer in food-contact plastics
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