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Survey Results of Benzene in Soft Drinks and Other Beverages by Headspace Gas Chromatography/Mass Spectrometry

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Benzene, a carcinogen that can cause cancer in humans, may form at nanogram per gram levels in some beverages containing both benzoate salts and ascorbic or erythorbic acids. Through a series of reactions, a hydroxyl radical forms that can decarboxylate benzoate to form benzene. Elevated temperatures and light stimulate these reactions, while sugar and ethylenediaminetetraacetic acid (EDTA) can inhibit them. A headspace gas chromatography/mass spectrometry method for the determination of benzene in beverages was developed and validated. The method was used to conduct a survey of 199 soft drinks and other beverages. The vast majority of beverages sampled contained either no detectable benzene or levels below the U.S. Environmental Protection Agency's drinking water limit of 5 ng/g. Beverages found to contain 5 ng/g benzene or more were reformulated by the manufacturers. The amount of benzene found in the reformulated beverages ranged from none detected to 1.1 ng/g.

KEYWORDS: Benzene; beverages; headspace; gas chromatography/mass spectrometry

INTRODUCTION

Benzene is a carcinogen that can cause cancer in humans and has been reported to cause leukemia and other blood-related disorders (*1*). In the early 1990s, benzene was found to form in beverages and other foods containing added benzoate salts (an antimicrobial) and ascorbic acid (vitamin C) or erythorbic acid (vitamin C's isomer). Beverages with naturally occurring benzoic and ascorbic acids, such as cranberry juice, also could potentially form benzene. In 2005, the U.S. Food and Drug Administration (FDA) began to investigate the reoccurrence of benzene being found in beverages containing benzoate salts and ascorbic or erythorbic acids. It had been postulated that low levels of benzene may form in certain beverages as a result of decarboxylation of benzoate by a hydroxyl radical. The reactions are catalyzed by trace levels of metal ions that reduce oxygen via reactions involving ascorbic acid to form hydroxyl radicals (*2*). Heat and ultraviolet (UV) light accelerate these reactions. Chelating agents and nutritive sweeteners inhibit them. EDTA and sodium hexametaphosphate (SHMP) are two chelating agents commonly used by the beverage industry to sequester trace levels of metal ions. However, the effectiveness of chelating compounds in beverages can be reduced significantly by the addition of calcium and other minerals. Product formulation, shelf life, and storage conditions were determined to be important factors that affect whether or not benzene formed in beverages (*3*).

In the 1990s, the U.S. beverage industry responded to the benzene problem by voluntarily reformulating products found to contain benzene at or above the 5 ng/g maximum contaminant level (MCL) established for drinking water by the U.S. Environmental Protection Agency (EPA). Since that time, many new beverage manufacturers emerged into the marketplace and may have been unaware of the potential for benzene formation from the precursor compounds. In addition, some manufacturers began fortifying their products with ascorbic acid and other nutrients in response to consumers' requests for potentially healthier products. These factors likely contributed to the 2005 reoccurrence of benzene being found in beverages above the EPA MCL (*4*).

Static and dynamic purge and trap (P&T) headspace (HS) sampling followed by gas chromatography/mass spectrometry (GC/MS) and GC/flame ionization detection (FID) were frequently cited as methods for determining benzene and other VOCs in food (*5, 6*). Cryogenic trapping and oven cooling often were required to focus the VOCs at the head of the GC column. In 1992, static HS GC/MS was used by Page et al. to conduct a survey of fruit juices and beverages. The GC was equipped

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with an on-column injector and a cryogenically cooled oven. Seventy-three bottled juices and beverages were analyzed. The highest level found was 3.8 ng/g benzene (*7*). In 1993, McNeal et al. used P&T GC/FID and HS GC/MS with a cryogenically cooled GC oven to conduct a survey of over 50 foods. The highest level found in the 27 beverages analyzed was 3 ng/g (*8*). Fabietti et al. used P&T GC/MS with cryogenic trapping to determine benzene and toluene in 60 beverages. The highest benzene level found was 3.2 ng/g (*9*).

In 2005 and 2006, the FDA and several other food safety organizations conducted surveys to determine the amounts of benzene found in retail beverages (*10*). Health Canada conducted a survey of 118 products labeled to contain benzoate and ascorbic acid (*11*). Four of the products were found to contain benzene at or above the 5 ng/mL Canadian drinking water guideline. These samples were analyzed by using an automated HS GC/MS method. Cryogenic cooling was avoided by sampling a smaller headspace volume (*12*). The U.K.'s Food Standards Agency (FSA) conducted a survey of 150 beverages, most of which were labeled to contain benzoate and ascorbic acid. Four of the products analyzed were found to contain benzene above the World Health Organization's (WHO) 10 ng/g level for safe drinking water (*13*). A private laboratory used a proprietary HS solid-phase microextraction GC/MS method to analyze the survey samples. A survey conducted by the Food Safety Authority of Ireland (FSAI) found 2 of 76 beverages with benzene above the 10 ng/g WHO guideline (*14*). Food Standards Australia New Zealand (FSANZ) reported that 90% of the 86 beverages analyzed contained benzene below the 10 ng/g WHO guideline (*15*). Both of the FSAI and FSANZ surveys were conducted by independent laboratories using proprietary methods.

Two HS GC/MS methods were used to conduct the FDA survey. Initially, benzene was cryogenically focused at the head of a cyanopropyl-phenyl-dimethyl-polysiloxane (ZB-624) capillary column, but it was necessary to manually turn on the cryogenic unit during each analysis. To avoid this, the method was modified to incorporate a porous layer open tubular capillary column with a polystyrene−divinylbenzene stationary phase (PLOT-Q). The PLOT-Q column eliminated the need to cryogenically focus benzene at the head of the GC column and permitted complete automation of the HS GC/MS system.

This paper reports the FDA survey results of 199 beverage samples and the in-house validation of the FDA-modified HS GC/MS method. The method validation included determination of the method detection limit (MDL) and minimum level of quantitation (ML), linearity, repeatability, intermediate precision, and HS oven temperature and thermal equilibration time studies. A limited interlaboratory study was conducted to compare the amount of benzene found in retail beverages by using the modified HS GC/MS method with EPA SW-846 Method 8261A (*16, 17*). The EPA method has been used to determine benzene in food and environmental samples by vacuum distillation (VD) GC/MS (*18, 19*).

MATERIALS AND METHODS

Supplies and Reagents. Beverage samples were purchased from retail stores in Maryland, Virginia, Michigan, Massachusetts, and Maine. The samples included carbonated beverages (soft drinks), flavored waters, and other noncarbonated juices and drinks and included regular calorie, low calorie, and calorie-free products. Most of the beverage samples were packaged in plastic bottles [polyethylene terephthalate (PET) and high-density polyethylene]; a few samples were in cans. The majority of the plastic bottles were PET. No attempt was made to determine if the plastic bottles contained a UV stabilizer to protect the beverages from light. High-purity water (>18 MΩ resistance) was obtained in-house from a water purification system. HPLC grade methanol was purchased from J. T. Baker (Phillipsburg, NJ). Benzene (99.0%) was purchased from Fisher Scientific (Pittsburgh, PA) and stored in a 6 °C refrigerator. Benzene-d_6 (99.96%) was purchased from Aldrich (St. Louis, MO) and stored in a 6 °C refrigerator. All standards and test portions were prepared in 22 mL headspace vials (Shamrock Glass Co., Seaford, DE). The vials were precleaned by heating in a 90 °C forced air oven for >2 h and cooled to room temperature prior to use. Gas-tight syringes (Fisher Scientific, Pittsburgh, PA) were used to prepare the standard solutions and to transfer working standards to test portions.

Analytical Method. *Concentration of Stock and Intermediate Standards.* Benzene and benzene-d_6 stock standards (ca. 2.20 mg/mL) were prepared by transferring with a syringe 50 μL of neat benzene and benzene-d_6 into separate sealed, headspace vials containing 20 mL of methanol. Benzene and benzene-d_6 intermediate standards (ca. 54 μg/mL) were prepared by transferring 0.5 mL of the stock standard into separate sealed headspace vials containing 20 mL of methanol. The septa and aluminum seals on the standards were replaced once the septa were pierced. The standards were stored at room temperature and were stable for at least 2 weeks.

Concentration of Working Standards. Benzene and benzene-d_6 working standards (ca. 0.5 μg/mL) were prepared by transferring the appropriate volume of the intermediate standards into sealed headspace vials containing 20 mL of water. Working standards were prepared daily.

HS GC/MS with Cryogenic Focusing (Method 1). Ten gram test portions were analyzed using an Agilent 6890N capillary gas chromatograph equipped with an Agilent 5973N MSD detector and a Perkin-Elmer HS 40 automated headspace sampler (Boston, MA). A ZB-624 capillary column (30 m × 0.25 mm i.d. with a 1.7 μm film; Phenomenex, Torrance, CA) was used. The GC injector was 175 °C. The cryo trap was cooled with liquid nitrogen to −50 °C for 1 min and then ballistically heated to 210 °C. The GC split vent was closed 45 s prior to injection and then opened 45 s after injection with a 20:1 split ratio and a constant flow of 1 mL/min (UHP helium). The gas chromatographic conditions were initial temperature 60 °C, temperature program of 7.5 °C/min to 230 °C and hold for 7.3 min. The HS conditions were 75 °C oven, 125 °C needle, and 125 °C transfer line; 15 min thermal equilibration; 0.5 min pressurization, 0.2 min injection with vial pressurized to 30 psi, 0.2 min withdrawal; 20 psi column head pressure. The MSD parameters were electron impact in full scan mode from *m/z* 25 to *m/z* 250, 6.1 scans/s, 230 °C transfer line, 230 °C source, and 150 °C quadrupole.

Modified HS GC/MS without Cryogenic Focusing (Method 2). Ten gram test portions were analyzed using an Agilent 6890N capillary gas chromatograph equipped with an Agilent 5973N MSD detector and a Perkin-Elmer TurboMatrix 40 automated headspace sampler. An HP-PLOT-Q capillary column (30 m × 0.32 mm i.d. with a 20 μm film thickness; J&W Scientific, Folsom, CA) was used. The gas chromatographic conditions used were initial temperature 100 °C, temperature program of 10 °C/min to 225 °C and hold for 12.5 min. The injector temperature was 200 °C with a 2:1 split ratio and a constant flow rate of 1.7 mL/min (UHP helium). The HS conditions were 60 °C oven, 100 °C needle, and 120 °C transfer line; 15 min thermal equilibration; 0.5 min pressurization, 0.2 min injection with vial pressurized to 20 psi, 0.2 min withdrawal; 10 psi column head pressure. The MSD parameters were electron impact in select ion monitoring mode, 2.2 Hz, 225 °C transfer line, 230 °C source, and 150 °C quadrupole. Ions monitored were *m/z* 51, 77, and 78 for benzene and *m/z* 52 and 84 for benzene-d_6.

Preparation of Samples with 10 ng/g Benzene-d_6. All samples were analyzed within the manufacturer's established shelf life except the black cherry beverage (see Survey Results). Samples were stored and prepared at room temperature. Ten gram test portions of each sample were transferred to tared HS vials and fortified with 25 μL of 4 μg/mL benzene-d_6 working standard. Two quality control standards fortified with 10 ng/g of both benzene and benzene-d_6 were analyzed at the beginning and end of each batch of samples. The amount of benzene in the samples was determined by isotope dilution using benzene-d_6.

Table 1. Internal Standard Corrected Recoveries and RSDs from Analysis of Strawberry-Flavored Water, Diet Cola, and a Light Orange Beverage

fortification level, ng/g	av recovery, % ($n = 9$)	RSD, %
strawberry-flavored water		
1.0	97.3	2.3
2.5	96.9	2.1
5.0	98.9	1.4
10	98.4	1.9
diet cola (calorie free)		
1.0	96.4	2.3
2.5	96.9	1.4
5.0	99.0	1.0
10	99.1	1.8
light orange beverage (low calorie)		
1.0	96.0	2.5
2.5	98.1	2.0
5.0	99.9	2.9
10	101	3.4

Calibration Curve Solutions. The GC/MS linear range was established by analyzing a six-point internal standard calibration curve. Calibration solutions equivalent to ca. 0.5, 1, 2.5, 5, 10, and 20 ng/g benzene and 10 ng/g benzene-d_6 were prepared in HS vials containing 10 g of deionized water.

Determination of Internal Standard Corrected Recovery. The internal standard corrected recoveries from fortified beverages were determined in a diet cola, fruit flavored water, and light calorie orange beverage and were selected to represent a range of matrices at a target level of 5 ng/g benzene. The beverages were fortified in triplicate with 1, 2.5, 5, and 10 ng/g benzene and 5 ng/g benzene-d_6 and analyzed on each of 3 days. Prior to fortification, incurred benzene was removed by pouring the sample into a beaker and sparging for 1 h with a stream of nitrogen.

Benzene Confirmation. Benzene produces three diagnostic ions, *m/z* 51, 77, and 78. The integrated areas for *m/z* 51, 77, and 78 were determined for all test portions. Confirmation was performed by dividing the integrated areas for *m/z* 51 by *m/z* 78 and for *m/z* 77 by *m/z* 78. The response ratios for each test portion should agree with the average response ratio for the quality control standards by ±10%. The retention time (RT) for the test portions should agree with the average RTs for the quality control standards by ±2%.

RESULTS AND DISCUSSION

Method Validation. The modified HS GC/MS method was subjected to an in-house validation. The validation studies showed that the MSD response for benzene was linear over a range of 0.5−42 ng/g. The correlation coefficient from the regression analysis was 0.9999. All residuals from the calibration curve were less than 10%. The residuals are the differences between the obtained and regression predicted values for the *y*-ordinate. The PLOT-Q column showed good retention and peak resolution for benzene/benzene-d_6.

The estimated MDL of 0.05 ng/g and ML of 0.2 ng/g were determined on the basis of the standard deviation of five replicates multiplied by the Student *t*-value ($n - 1$) equal to a 99% confidence interval (*20*). The ability to confirm benzene in beverages was matrix dependent and may be as low as 0.5 ng/g or as high as 1.4 ng/g. The reportable ML with confirmation is estimated to be approximately 1 ng/g for benzene in beverages.

Table 1 shows the average percent recoveries for test portions fortified at 1, 2.5, 5, and 10 ng/g. The data show that the internal standard corrected recoveries range from 96% to 101% in three beverages. The relative standard deviations (RSDs) range from 1.0% to 3.4%, showing good repeatability over 3 days. **Table 2** shows the within-laboratory precision determined by comparing the amount of benzene found in 16 samples by two analysts on different days with different instruments and methods (method 1, HS GC/MS with cryofocusing; method 2, modified HS GC/MS method). For the majority of these beverages, the amount of benzene found was less than 5 ppb, and the average difference between the amounts found by each analyst was 19%. The difference observed can be attributed to the precision of each analyst and the use of two different HS GC/MS instruments and methods.

Table 2. Within-Laboratory Precision Determined by Two Analysts on Different Days with Different Instruments and Methods

		found, ng/g		
sample	product	method 1	method 2	% difference
51	raspberry-flavored sparkling water	nd[c]	<1	
6-A	diet[a] orange soda, brand 1, lot 1	15.2	10.7	35
6-B	diet[a] orange soda, brand 1, lot 2	13.2	11.4	15
1-B	citrus punch beverage	nd	nd	
41-1	strawberry-flavored water	10.4	9.2	12
41-A-1	peach mango flavored water	4.0	3.4	16
41-B	concord grape flavored water	4.6	4.0	14
48	carbonated energy beverage	<1	<1	
50-A	black cherry beverage	2.3	2.0	14
50-B	berry beverage	2.0	1.6	22
34	light[b] orange beverage, brand 1, lot 1	76.6	87.9	14
34-1	light[b] orange beverage, brand 1, lot 2	1.4	1.1	24
49	tropical punch beverage	2.9	2.3	23
49-A	kiwi-strawberry beverage	2.0	1.5	28
35-A	cranberry juice cocktail, brand 1	3.0	2.5	18
35-B	light[b] cranberry juice cocktail, brand 2	10.7	9.1	16

[a] Calorie free. [b] Low calorie. [c] Not detected.

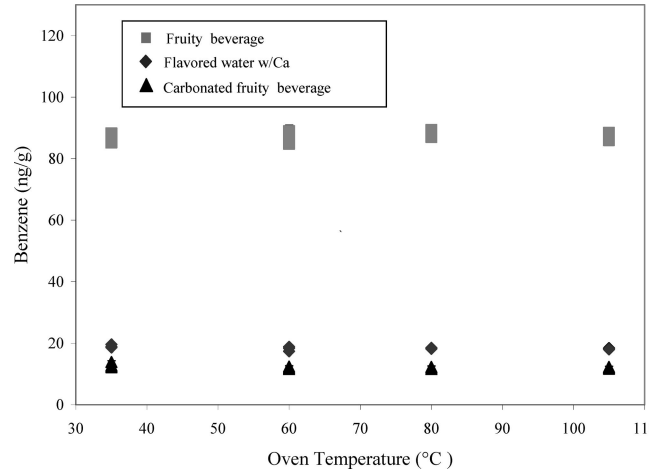

Figure 1. HS oven temperature study in three beverages with a 15 min thermal equilibration.

HS oven temperature and thermal equilibration time studies were conducted in three carbonated and noncarbonated beverages. The studies were conducted to evaluate whether or not benzene forms as a result of the HS conditions. All of the beverages evaluated contained benzoate salts, ascorbic acid, and greater than 5 ppb benzene and had potential for benzene formation during analysis. For the oven temperature study, test portions of the products were heated for 15 min in a HS oven at 35, 60, 80, and 105 °C. For the thermal equilibration time studies, test portions were heated in a HS oven at 60 °C for 5, 15, 30, and 45 min. **Figure 1**, which plots the amount of benzene found vs HS oven temperature, illustrates that the amount of benzene found did not change with increasing oven temperatures. **Figure 2**, which plots the amount of benzene found vs

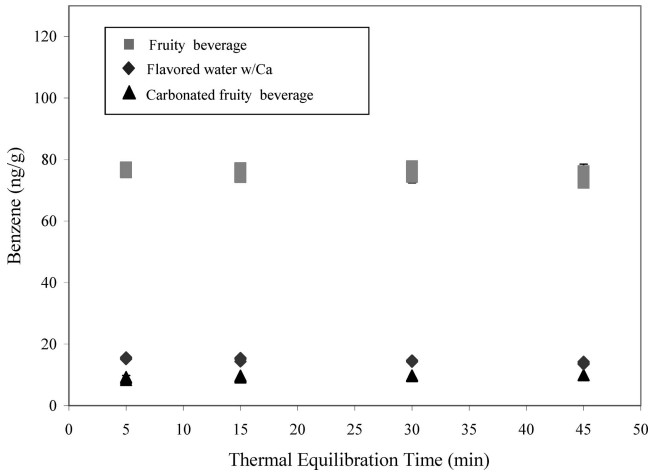

Figure 2. Thermal equilibration study in three beverages in a 60 °C HS oven.

Table 3. Benzene Found in Beverages by VD GC/MS and HS GC/MS

sample	EPA, ng/g ($n = 2$)[a]	FDA, ng/g (% RSD, $n = 3$)
diet[b] orange soda	10.0 ± 0.7 10.1 ± 0.7	12.2 (2.1)
diet[b] cola	0.7 ± 0.04 0.8 ± 0.02	0.5 (1.1)
light[c] orange beverage	1.4 ± 0.1 1.4 ± 0.1	1.4 (3.3)
light[c] cranberry juice cocktail	17.8 ± 1.2 20.1 ± 0.5	21.5 (0.5)
concord[b] grape flavored water	4.0 ± 0.1 3.8 ± 0.1	3.6 (0.7)

[a] Error calculated according to EPA Method 8261A. [b] Calorie free. [c] Low calorie.

thermal equilibration time, illustrates that the amount of benzene found did not change with longer equilibration times.

A progressive HS analysis was conducted to determine whether benzene-d_6 was at equilibrium in 15 min at 60 °C. A carbonated fruity beverage fortified with benzene-d_6 only and previously found to contain 10.7 ng/g benzene was analyzed in a 60 °C HS oven in 15 min increments from 15 to 120 min. The response for benzene-d_6 showed that equilibration was reached in 60 min. The average amount of benzene found over the range of the progressive analysis was 9.7 ng/g, and the RSD was 2.5%. This result suggests that increased sample throughput could be achieved with a shorter equilibration without sacrificing accuracy. Increasing the oven temperature would in all likelihood increase sensitivity and shorten the equilibration time but could also increase the chance for benzene formation.

To further evaluate the method, five split samples were analyzed by using the modified HS GC/MS method and EPA Method 8261 using VD GC/MS. The VD GC/MS method does not heat test portions during the analysis and would not be expected to generate benzene in beverages containing benzoate and ascorbic acid. The EPA's Office of Research and Development Laboratory in Las Vegas, NV, conducted the VD GC/MS analyses. **Table 3** summarizes the amount of benzene found using the two methods and shows that comparable results were obtained.

Survey Results. The bar graph in **Figure 3** shows the distribution of benzene found in 199 beverage samples and shows that only 9% of the samples representing nine products were found to contain greater than 5 ng/g benzene. Some of these samples represent multiple lots of the same product.

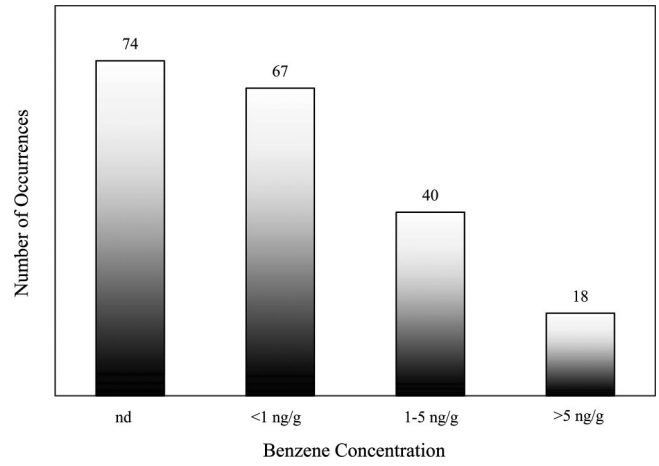

Figure 3. Distribution of benzene found in 199 beverage samples. nd = not detected.

Table 4. Minimum and Maximum Amount of Benzene Found in 199 Samples and Number of Samples with 5 ng/g Benzene or More, Labeled To Contain Added Benzoate, AA/EA, and EDTA/SHMP

beverage type	n	min	max	$n \geq 5$, ng/g
colas, regular				
no benzoate, AA/EA, or EDTA	1	nd[c]	nd	
benzoate	2	nd	0.5	
benzoate, AA/EA, and EDTA	1	0.1	0.1	
colas, low or calorie free				
benzoate	14	nd	1.3	
benzoate, AA/EA, and EDTA	4	nd	2.3	
other carbonated beverages, regular				
benzoate[a]	15	nd	0.9	
benzoate and AA/EA	18	nd	11.2	2
benzoate, AA/EA, and EDTA	5	nd	0.4	
other carbonated beverages, low or calorie free				
benzoate[a]	21	nd	3.5	
benzoate and AA/EA	12	0.2	79.2	5
AA/EA	3	nd	0.3	
benzoate, AA/EA, and EDTA	9	nd	1.4	
noncarbonated beverages, regular				
benzoate	3	nd	0.4	
benzoate and AA/EA	16	nd	88.9	2
AA/EA	3	nd	0.1	
cranberry juice[b]	8	nd	2.3	
benzoate, AA/EA, and EDTA/SHMP	17	nd	6.6	3
noncarbonated beverages, low or calorie free				
benzoate	5	nd	nd	
benzoate and AA/EA	7	nd	23.4	2
AA/EA	7	nd	2.5	
cranberry juice[b]	14	nd	9.9	2
benzoate, AA/EA, and EDTA/SHMP	14	nd	82.2	2

[a] May contain EDTA. [b] May contain naturally occurring benzoic acid. AA was added to all products except two products that were reformulated by removing AA. [c] Not detected.

Seventy-one percent of the samples analyzed contained less than 1 ng/g benzene. The amount of benzene found in multiple lots of the same product varied, probably due to variations in handling, storage, and shelf-life conditions. **Table 4** reports the minimum and maximum amounts of benzene found in the samples and the number of samples with 5 ng/g benzene or more. The data are reported according to beverage type and whether the beverages contained benzoate, ascorbic or erythorbic acids (AA/EA), and ethylenediaminetetraacetic acid or sodium hexametaphosphate (EDTA/SHMP). This information was obtained from the product label and, in most cases, was not verified with the manufacturer. Cranberry juice also was represented as a separate category. None of the cranberry juices

Table 5. Maximum Benzene Concentrations Found in Old and New Product Formulations

reformulated products	benzene, ng/g old	benzene, ng/g new	product reformulation
diet[a] orange soda, product 1	79.2	nd[c]	removed AA
diet[a] orange soda, product 2	0.2	1.1	added EDTA
pineapple soda, product 1	9.2	nd	removed AA
pineapple soda, product 2	11.2	nd	removed AA
light[b] cranberry juice	9.9	0.5	removed AA
black cherry beverage	88.9		manufacturer discontinued product
strawberry-flavored water[a]	23.4	nd	removed AA
orange beverage	6.6	nd	reduced benzoate and used benzoate/sorbate blend
cherry beverage	5.2	nd	removed benzoate
light orange beverage[b]	82.2	nd	removed calcium

[a] Calorie free. [b] Low calorie. [c] Not detected.

analyzed contained added benzoate but may have contained naturally occurring benzoic acid.

None of the cola products were found to contain benzene above 5 ng/g. Three of the carbonated products (not colas) were found to contain greater than 5 ng/g benzene, one of which was a diet beverage. All of the products contained added benzoate and AA. The highest benzene level found in the regular calorie beverages was 11.2 ng/g. In comparison, the highest benzene level found in the diet beverages was 79.2 ng/g. The lack of nutritive sweeteners in the diet beverage may have contributed to higher levels of benzene formation in this particular product.

Six of the noncarbonated products were found to contain greater than 5 ng/g benzene, including a cranberry juice, two products with added benzoate and AA, and three products with benzoate, AA, and EDTA/SHMP. Two of the 22 cranberry juice samples analyzed were found to contain 5.4 and 9.9 ng/g benzene. The two samples represented two lots of a light cranberry juice cocktail. This product contained added ascorbic acid and probably naturally occurring benzoic acid.

Two noncarbonated products with added benzoate and AA were found to contain greater than 5 ng/g benzene. Strawberry-flavored water was found to contain 10 and 23 ng/g benzene. A black cherry beverage was found to contain 40 and 89 ng/g benzene. The elevated benzene levels found likely were the result of the products' handling in the retail market. The two black cherry samples analyzed were purchased from a retail store several months beyond the manufacturer's sell-by date.

Three noncarbonated products with added benzoate, AA, and EDTA/SHMP were found to contain greater than 5 ng/g benzene. The highest levels found were 6.6 ng/g benzene in an orange beverage and 80 ng/g in the light orange beverage. The high benzene levels in the light orange beverage probably were due to the low level of nutritive sweetener in the product and the addition of calcium, which forms a complex with EDTA. Uncomplexed metal ions that remain in solution would be free to initiate the formation of hydroxyl radicals that potentially react with benzoate to form benzene.

Beverages found to contain more than 5 ng/g benzene were reformulated by the manufacturer. One of the products was discontinued. The remaining products were reformulated to eliminate or minimize benzene formation. Samples of the reformulated products were provided by the manufacturers or collected from retail stores. **Table 5** reports the maximum concentration of benzene found in the old and new formulations and how the products were reformulated. Five products were reformulated by removing added AA. One product was reformulated by adding EDTA. The three remaining products were reformulated by using a blend of benzoate and sorbate and reducing the benzoate concentration, removing benzoate, or removing calcium. All of the reformulated products were found to contain 1.1 ng/g benzene or less. Only 0.2 ng/g benzene was found in one of the diet orange sodas manufactured according to the old formulation. The manufacturer reformulated this product on the basis of elevated benzene levels found by a private laboratory (data not available). Sample handling in the retail market was likely the cause for the difference in the amount of benzene found by the FDA and the private laboratory.

In summary, the modified HS GC/MS method was found to be reliable at 1 ng/g benzene. HS temperature and time studies showed that benzene was not generated as a result of a 15 min thermal equilibration time in a 60 °C HS oven or HS oven temperatures that ranged from 35 to 105 °C for 15 min. A relatively small number of the samples surveyed were found to contain benzene above 5 ng/g. All of these samples contained benzoate and AA and represented multiple lots of nine products. Product formulation and shelf-life conditions were factors contributing to benzene formation. The affected products were reformulated. The FDA survey results are comparable to results reported by other food safety organizations (*11, 13–15*). On the basis of these results and actions taken by the beverage industry, the FDA concluded that the benzene found in the beverage samples did not pose a safety concern for consumers.

ACKNOWLEDGMENT

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NOTE ADDED AFTER ASAP PUBLICATION

Several minor text changes have been made after the original ASAP posting of December 12, 2007. Footnotes have been added to Tables 2, 4, and 5, and the appearance of Table 4 has been altered in the posting of December 19, 2007.

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