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# Determination of free Bisphenol A (BPA) concentrations in breast milk of U.S. women using a sensitive LC/MS/MS method

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## HIGHLIGHTS

- A sensitive method was developed to detect free BPA in human breast milk.
- Free BPA was detected in 62% of the milk samples (range $\leqslant$ 0.22–10.8 ng mL$^{-1}$).
- Caucasian women had significantly higher levels of free BPA in their breast milk.
- This is the first study to find that BPA levels in breast milk may vary with race.

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## ABSTRACT

Bisphenol A (BPA) is a synthetic, endocrine-disrupting compound. Free BPA has been detected in human samples indicating that humans are internally exposed to estrogenically active BPA. The purpose of this study was to develop a sensitive method to detect free BPA in human breast milk. BPA was isolated from the milk of 21 nursing mothers in the U.S. by solid-phase extraction. It was then derivatized to improve sensitivity and subsequently analyzed by ultra high performance liquid chromatography–tandem mass spectrometry. Free BPA was detected in 62% of the milk samples ($\leqslant$0.22–10.8 ng mL$^{-1}$, median 0.68 ng mL$^{-1}$, mean 3.13 ng mL$^{-1}$). No statistical difference in BPA concentrations was observed between women with a low or high Body Mass Index (BMI) (<30 ($n$ = 11) and > 30 ($n$ = 10), respectively). However, there was a significant association between BPA concentration and race. Caucasian women had significantly higher levels of free BPA in their breast milk than non-Caucasian women (mean = 4.44 ($n$ = 14) and 0.52 ($n$ = 7), respectively; $p < 0.05$). The difference between races could be attributed to variations in exposure, lifestyle or metabolism and suggests that certain populations should take extra precautions to limit BPA exposure, particularly during pregnancy and lactation.



## 1. Introduction

Bisphenol A (BPA) is a synthetic compound with structural similarities to the hormone 17β-estradiol. BPA is a major component of plastic and epoxy resins, which are used in the production of plastic food and beverage containers, metal can linings, dental sealants, thermal receipt paper and household paper products (Chapin et al., 2008; Liao and Kannan, 2011). Concern about BPA arises because incomplete polymerization as well as exposure to high temperatures and acidic or basic conditions causes BPA monomers to leach from food-related commercial products into their surrounding environment (Vandenberg et al., 2010). Therefore, the main exposure route of BPA is thought to be oral (Chapin et al., 2008). However, secondary exposure routes include inhalation and dermal absorption (Geens et al., 2012). Because BPA is such a widely used chemical, many people are exposed to BPA on a daily basis. Quantifiable amounts of BPA have been measured in a variety of biological samples including human urine, blood, saliva, amniotic

*Abbreviations:* BPA, Bisphenol A; BMI, Body Mass Index; EPA, Environmental Protection Agency; RfD, reference dose; SPE, solid-phase extraction; MTBE, methyl-tert-butyl-ether; BPA-PS, BPA-pyridine sulfonyl; ESI+, electrospray ionization positive; MRM, multiple reaction monitoring; LOD, limit of detection; IUPAC, International Union of Pure and Applied Chemistry; PS, pyridine-3-sulfonyl; RRF, relative response factor; NHANES, National Health and Nutrition Examination Study; BPA-G, BPA-glucuronide; UGT, UDP-glucuronosyltransferase.

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fluid, placental tissue, colostrum and breast milk (Kuruto-Niwa et al., 2007; Vandenberg et al., 2010; Cao et al., 2012).

BPA binds to the estrogen receptor and has been reported to disrupt normal estrogenic endocrine function (Nagel et al., 1997; Gould et al., 1998). However, as reviewed in Vandenberg et al. (2009), controversy exists over whether environmental exposures to BPA are harmful. The U.S. Environmental Protection Agency (EPA) has established a BPA reference dose (RfD) of 50 μg $kg^{-1}$ $d^{-1}$ based on the lowest-observable-adverse-effect level (Vandenberg et al., 2009). Despite the established RfD, animal studies have indicated that adverse effects, including changes in brain structure and function, behavior, male and female reproductive tracts, metabolism, hormone signaling, and the immune system occur in response to BPA levels below the RfD (reviewed in (Richter et al., 2007)).

Initially, BPA was thought to be metabolized *via* glucuronidation or sulfonation, forming conjugates that are rapidly excreted from the body (Völkel et al., 2002). However, as recently reviewed, unconjugated BPA (free BPA) has been detected in a variety of human samples indicating that humans are exposed internally to estrogenically active BPA (Vandenberg et al., 2010). This is not surprising considering that deconjugation of BPA can occur by β-glucuronidase (reviewed in (Ginsberg and Rice, 2009)), an enzyme present in human liver, spleen, kidney, intestine, lung, muscle and serum (Sperker et al., 1997). The length of time that free BPA remains in the body and the extent to which it accumulates in tissues, such as the breast, is unknown.

Developmental exposure to BPA is particularly important because of the increased susceptibility of the brain and other organs to estrogens during this time (Vandenberg et al., 2009). Furthermore, it has been suggested that infants may be exposed to elevated levels of BPA due to their lack of metabolic enzymes capable of conjugating BPA (Mykkänen et al., 1997; Taylor et al., 2008; Vandenberg et al., 2010). Breastfeeding mothers who have been exposed to BPA may be unknowingly exposing their children to harmful levels of BPA. Therefore, it is imperative to monitor the amount of BPA in breast milk. In this study, free BPA was extracted from breast milk samples from 21 nursing women in the U.S. by a solid-phase extraction (SPE) method, derivatized and subsequently analyzed by ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). The influence of the demographic parameters, Body Mass Index (BMI) and ethnicity, on free BPA concentrations was also investigated.

## 2. Materials and methods

### 2.1. Sample collection

Twenty-one breast milk samples were selected from an archive of a larger national study investigating breast cancer risk for which University of Massachusetts Institutional Review Board Approval included secondary analysis of environmental contaminants. Methods of milk collection and storage for the larger study have been published (Browne et al., 2011). Briefly, milk was collected in acid-washed glass bottles and shipped on ice along with a detailed questionnaire to the laboratory *via* overnight express carrier. One- to five-mL aliquots of whole milk were stored at −20 °C for future analyses. The BMI values, calculated from height and weight provided by the participants at the time they donated the milk, were used to select ten milk samples from women with a BMI < 30 (Low BMI), and eleven milk samples from women with a BMI > 30 (High BMI). The women who donated the 21 samples lived in 14 different states (CA, CO, FL, GA, IA, IL, MA, MD, MN, NJ, NY, OH, WA, WI). Fourteen of the women identified themselves as Caucasian and seven identified themselves as non-Caucasian. Of the non-Caucasian, five identified themselves as African American and two identified themselves as Hispanic. One 10 mg $mL^{-1}$ solution of infant formula (NIST SRM 1846 Infant Formula, Gaithersburg, MD, USA) was analyzed along with the 21 milk samples.

### 2.2. Control of background BPA contamination

All necessary precautions were taken to avoid contamination with BPA during sample preparation (Ye et al., 2013). Glass SPE cartridges as well as glass pipettes were used. All glassware used in the extraction procedure was washed and baked for 8 h at 500 °C. Water was filtered through a Millipore purification system in which new cartridges and UV lamp were installed prior to sample analysis. The concentration of BPA in the organic solvents examined was found to be below the limit of detection. To evaluate the potential for BPA contamination during the analysis, a large number of procedural water blanks were used in the study relative to the number of samples analyzed (8 blanks and 21 samples).

### 2.3. Chemicals

Native and $^{13}C_{12}$-ring-labeled BPA standards (100 ng $\mu L^{-1}$ dissolved in acetonitrile) were purchased from Cambridge Isotopes (Andover, MA). Acetonitrile (LC/MS Grade), methanol (Optima Grade and LC/MS Grade), n-hexane (Optima Grade), methyl-tert-butyl ether (HPLC Grade), dichloromethane (Optima Grade), acetic acid (Glacial HPLC) and sodium bicarbonate were purchased from Fisher Scientific (Pittsburgh, PA). Ammonium hydroxide was purchased from Acros Organics (Belgium). Pyridine-3-sulfonyl chloride, 97% was purchased from Matrix Scientific (Columbia, SC).

### 2.4. Processing of sample batches

Over the course of three consecutive weeks, samples were processed in batches consisting of three milk samples and one water blank. Samples were processed in groups that included either one High BMI milk sample and two Low BMI milk samples or two High and one Low. Milk samples were thawed and vortexed for 1 min prior to sample processing as described below.

### 2.5. Hexane/acetonitrile partitioning

A partitioning step (adapted from (Yi et al., 2010)) was utilized prior to SPE. Either 1 mL of water or milk was transferred to a 15-mL glass centrifuge tube and spiked with 6 ng of $^{13}C_{12}$-BPA (1 ng $\mu L^{-1}$ in methanol) then vortexed for 30 s. Three milliliters of acetonitrile were then added to each sample and the sample was vortexed for 30 s. Then 6 mL of hexane were added and the samples were inverted by hand for 5 min, vortexed for 30 s and centrifuged at 5500 rpm, 5 °C for 10 min. The hexane layer was discarded and the process was repeated with an additional 6 mL of hexane. After discarding the second hexane layer, as much of the aqueous layer as possible was transferred to a new centrifuge tube and evaporated down to approximately 1 mL under $N_2$, 37 °C water bath.

### 2.6. Solid-phase extraction

The SPE procedure from (Markham et al., 2010), initially developed for use with urine samples, was followed with a few adjustments. Glass Oasis™ HLB (5 mL/200 mg) cartridges were purchased from Waters (Milford, MA) and conditioned with six, 4-mL washes with methyl-tert-butyl-ether (MTBE), 3 mL of methanol, and 5 mL of water. Samples were then diluted with 9 mL of

1:8 methanol:water and vortexed for 30 s. The samples were then loaded onto the column without vacuum and the sample vials were rinsed with 5 mL of water, which was also loaded without vacuum. The SPE column was then rinsed with 3 mL of 50:50 methanol:water followed by 3 mL of 10:2:88 methanol:ammonium hydroxide:water and dried for 5 s under $N_2$ with medium vacuum. A gas flow of approximately 8 L $min^{-1}$ was used during the drying steps. Medium and high vacuum indicate a manifold reading of −25 kPa and −50 kPa, respectively. The column was washed with 5 mL of water and dried for 5 s under $N_2$ with medium vacuum, 3 mL of 50:50 methanol:water and dried again for 5 s under $N_2$ with medium vacuum. The final wash consisted of 3 mL of dichloromethane followed by 1 min drying time under $N_2$ with high vacuum. BPA was eluted from the column with 4 mL of MTBE without vacuum and collected in a 5-mL reacti-vial. The sample was then derivatized (see procedure below) prior to analysis.

### 2.7. Synthesis of pyridine sulfonyl derivative

Upon completion of sample extraction, a BPA-Pyridine sulfonyl (BPA-PS) derivative was synthesized for improved MS sensitivity and detection in electrospray ionization positive (ESI+) mode. The procedure was adapted from Xu (2007). The 4-mL eluent from SPE was evaporated to dryness under $N_2$, 37 °C water bath. Then 95 μL of 1.13 mg $mL^{-1}$ pyridine-3-sulfonyl chloride in acetone followed by 100 μL of 0.1 M sodium bicarbonate were added to the reacti-vial and vortexed for 30 s. The vial was then placed in a 60 °C heating block and allowed to react for 7 min and immediately cooled on ice for 8 min. The solution was allowed to reach room temperature and two liquid–liquid extractions were performed each with 1 mL of hexane. The organic portion was saved and transferred to a new vial, evaporated to dryness under $N_2$, 37 °C water bath and reconstituted with 1 mL of 50:50 water:acetonitrile. The sample was vortexed for 30 s and 0.5 mL was analyzed immediately (Round I analysis). The remaining 0.5 mL was transferred to an autosampler vial and stored at 4 °C, until it was analyzed upon completion of the last sample batch (Round II analysis).

### 2.8. Instrumental analysis

Identification and quantification of BPA were performed, each with a Micromass Quattro PremierXE electrospray triple quadrupole mass spectrometer (ESI + MS/MS, Waters, Milford, MA) coupled with an Aquity UHPLC system (2996 PDA Detector) (Waters, Milford, MA). Chromatographic separation of the 10 μL sample injection was achieved with a BEH $C_{18}$ chromatographic column (21 × 100 mm, 1.7 μm; Waters, Milford, MA). A flow rate of 0.433 mL $min^{-1}$ was used with the mobile phase initially consisting of 30% acetonitrile and 70% 0.1% acetic acid held for 0.5 min, then increased linearly to 61% acetonitrile from 0.5 min to 6.5 min, held there for 2.5 min and finally reversed to 30% acetonitrile over 1 min, with a three-min hold between injections. BPA-PS derivative was detected with the MS operating in the ESI+ multiple reaction monitoring (MRM) mode. The parameters used for detection are described in Supplementary Table S1. The transitions of native- and $^{13}C_{12}$-BPA-PS derivative monitored were 511–354 and 523–366 *m/z*, respectively. Argon was used as the collision gas and nitrogen was used as the nebulizer, desolvation and cone gas. Using a flow injection system, the MS/MS parameters were optimized by infusing the native and labeled compounds. Triplicate injections were made for all sample extracts and standard solutions.

### 2.9. Data analysis

Demographic data were organized and analyzed using the statistical software analysis program, STATA 10: Data Analysis & Statistical Software (Statacorp, LP, College Station, TX). The figures and corresponding statistical analyses were conducted in GraphPad (GraphPad Software Inc., La Jolla, CA).

The limit of detection (LOD) was determined by the procedure of the International Union of Pure and Applied Chemistry (IUPAC) (see Section 3.2). For statistical analyses BPA concentrations below the LOD were assigned values equal to the LOD divided by the square root of two (Hornung and Reed, 1990).

## 3. Results and discussion

### 3.1. Method development

Due to the complex matrix of breast milk, several established methods for the extraction of BPA from other biological samples were tested and modified for efficiency prior to sample analysis. As described by Kang and Kondo, a hexane partitioning step was utilized to remove non-polar lipids from a mixture of acetonitrile and milk (Kang and Kondo, 2003). Additionally, the multiple steps in the Oasis HLB SPE procedure developed by Markham et al. (2010) provided the best sample cleanup compared to the other SPE methods that were tested. Upon using negative ion UHPLC/MS/MS, we discovered that the ionization efficiency was too low for analysis of BPA in our breast milk samples. Therefore, we utilized a derivatization reaction, which added basic pyridine-3-sulfonyl (PS) groups to BPA allowing our samples to be analyzed using ESI+ conditions with high efficiency. The related 5-(dimethylamino) naphthalene sulfonyl (dansyl) derivative has been used to measure multiple classes of phenolic compounds such as BPA and estrogens in wildlife blood plasma samples (Chang et al., 2010) and to analyze BPA in human urine samples (Fox et al., 2011). In this study, a PS derivative was prepared instead of a dansyl derivative to ensure a complete reaction with both hydroxyl groups of BPA (Xu, 2007). The BPA-PS derivative also ensures specificity, as a BPA-specific ion is formed as the major product ion in the collision-induced dissociation of BPA-PS whereas dansyl moieties are the major product ions formed from dansyl-BPA (Xu and Spink, 2008). Monitoring an MS/MS transition involving an analyte-specific product ion rather than a reagent-specific product ion is beneficial in terms of both qualitative and quantitative analysis. The PS derivatization procedure was adapted from work on detecting BPA in aquatic samples (Xu, 2007) and on the analysis of estrogen in charcoal-stripped fetal-bovine serum (Xu and Spink, 2008). The reaction conditions used were similar to those used to analyze free estrogens in the presence of conjugated estrogens (Xu et al., 2007). Additionally, a study using a similar SPE method during the cleanup of BPA from urine samples found no breakdown of conjugated BPA over the course of their experiment (Markham et al., 2010).

### 3.2. Quality assurance and quality control

Calibration standards were prepared from mixtures of equal concentrations of the native and $^{13}C_{12}$-BPA standards. The overall native/$^{13}C_{12}$ relative response factor (RRF) was 0.97. This was determined by calculating the ratio of the slope of the native calibration curve to the slope of the $^{13}C_{12}$ calibration curve (graphed in Excel). The range of daily RRF values from Round I analysis was 0.89–1.04. In general when a labeled internal standard is used in mass spectrometric analysis, calibration is carried out by using a range of native standards to encompass the range of concentrations expected in the sample set. Each native standard would also

contain a fixed concentration of labeled standard equivalent to the fixed concentration added to each sample prior to analysis. In our case, based on method development experiments, we expected that we would have variable recoveries of labeled internal standard throughout the analytical process. For this reason we considered it prudent to determine the RRF value by comparing the slopes of the curves for a series of native and labeled standards. The curves had a slightly negative y-intercept indicating that there was no background BPA contamination in the native or the labeled internal standard solutions. Forcing the curves through zero had no effect on the calculated RRF.

A procedural blank was processed with each batch of three samples. The free BPA concentration in the eight blanks analyzed during the complete study was determined to be 0.10 ± 0.04 ng $mL^{-1}$ (mean ± SD). Prior to replacing the UV lamp and the cartridges in the Milli-Q water purification system, the average BPA concentration in laboratory blanks was approximately 0.4 ng $mL^{-1}$. Therefore the purity of the laboratory water supply was an essential element in maintaining a low background BPA concentration. Based on the blank results, the LOD was determined to be $0.1 + (3 \times 0.04) = 0.22$ ng $mL^{-1}$. Using this IUPAC criterion there is <1% chance of having a false positive result (Long, 1983). Precision was determined by analyzing three aliquots of the same milk sample, S1–S3. Mean BPA concentration in this sample was determined to be 2.10 ± 0.07 ng $mL^{-1}$, which is in close agreement with the seven previous method development replicate measurements of the same sample (1.96 ± 0.24 ng $mL^{-1}$).

To evaluate instrument sensitivity and stability over the time course of this study, each sample was divided into two fractions upon completion of sample processing as described in Section 2. The first fraction was analyzed as each batch was processed (Round I analysis) and the second was analyzed after the completion of all sample processing (Round II analysis). The concentrations of BPA determined from Round I and II analyses were nearly identical ($R^2 = 0.9897$ for procedural blanks, $R^2 = 0.9996$ for milk samples). These correlations demonstrate that consistent results were obtained over the three-week period of sample processing and analysis.

After the samples were subjected to the Round I and Round II analyses, the samples were spiked with 6 ng native BPA-PS derivative in order to determine the recovery of $^{13}C_{12}$-BPA. The native BPA-PS signals from the original extract analyses were subtracted from the native BPA-PS signals of the spiked samples and the resulting data were used to determine the $^{13}C_{12}$ recoveries. Recoveries from Round I and Round II analyses ranged from 20% to 58% (49 ± 9%) and 17% to 54% (40 ± 8%), respectively. Due to the complexity of the sample preparation process variable BPA recoveries among samples would be expected. As a consequence of this variability, the recovery data from Rounds I and II were not as strongly correlated ($R^2 = 0.6435$ for procedural blanks, $R^2 = 0.6837$ for milk samples) as the concentration data discussed above. However, the recoveries were used only as a measure of the efficiency of sample preparation and have no effect on the isotope dilution calculations of BPA concentrations.

### 3.3. BPA concentrations in breast milk

One infant formula and 21 breast milk samples collected from women across the U.S. were analyzed by the extraction and analysis method developed in the present study. Background information on the study participants is presented in Section 2and their demographics are summarized in Table 1. The mother's age, baby's age and number of children were similar for women with Low (<30) and High (>30) BMI and for Caucasians and non-Caucasians. The summary statistics for free BPA found in our study and comparative data from other studies are shown in Tables 2 and 3, respectively (Otaka et al., 2003; Sun et al., 2004; Ye et al., 2006b, 2008; Kuruto-Niwa et al., 2007; Yi et al., 2010; Mendonca et al., 2014). The median concentrations found in two Japanese studies and two studies conducted in the U.S. ranged from 0.40 to 0.65 ng $mL^{-1}$, values that are comparable to the median concentration of 0.68 ng $mL^{-1}$ found in our study (Otaka et al., 2003; Sun et al., 2004; Ye et al., 2006b, 2008). However, a median free BPA concentration of ⩽LOD was found in a recent U.S. study (Mendonca et al., 2014). While the median free BPA concentration was 6.6 ng $mL^{-1}$ in a Korean study (Yi et al., 2010). There are also noticeable differences in the range of free BPA concentrations found in the different studies. In a small Japanese study (3 samples) the range of free BPA concentrations was ⩽LOD – 0.7 ng $mL^{-1}$ (Otaka et al., 2003) whereas in a larger Korean study the range was 0.65–29.9 ng $mL^{-1}$ (Yi et al., 2010). In our study the range was ⩽LOD – 10.8 ng $mL^{-1}$. The detection frequency in our study was 62%, which can be compared to a detection frequency of 20–100% in the other reported studies.

The present study focused on the determination of free BPA, which is estrogenically active whereas the conjugated form is considered inactive (Matthews et al., 2001). Although other routes of BPA exposure exist, the main route of human exposure to BPA is considered to be oral (reviewed in (Völkel et al., 2002; Rubin, 2011)). An earlier study (Völkel et al., 2002) investigating BPA metabolism concluded that BPA is rapidly metabolized to conjugates and excreted after ingestion. However, there is mounting evidence that humans are internally exposed to unconjugated BPA. As reviewed by Vandenberg et al. (2010), free BPA has been detected in urine, blood/serum, amniotic fluid, placental tissue, and breast milk (Vandenberg et al., 2010). Additionally, data collected from the National Health and Nutrition Examination Study (NHANES) suggest that urinary BPA levels do not decrease with increasing lengths of fasting (Stahlhut et al., 2009); a decrease would have been expected if rapid BPA metabolism and excretion occurred. The authors concluded that (1) BPA has a longer half-life than expected, (2) additional routes of exposure (comparable to oral) exist, or (3) conjugation/deconjugation cycling occurs. If BPA-glucuronide (BPA-G) is not removed quickly from the body and becomes deconjugated by β-glucuronidase prior to excretion, then high levels of free BPA may be present in breast milk and other tissues of women with high exposure. In fact by treating samples with β-glucuronidase concentrations of total BPA (free + conjugated) were determined in four of the seven studies referenced in Table 3. For three of these studies median free BPA concentrations as a percentage of the total median BPA concentrations were 36%, 72% and 63%, respectively (Ye et al., 2006a, 2008; Yi et al., 2010). The median free BPA value in the fourth study (Mendonca et al., 2014) was ⩽LOD but if geometric means of free/total BPA are compared, the ratio value for this study is 50%.

### 3.4. BPA concentration and BMI

Based on the literature indicating a possible link between BPA exposure and BMI, we predicted that women with a high BMI would have high BPA levels in their breast milk. Indeed, a study investigating urinary BPA concentration and BMI from the NHANES 2003–2008 data found high BPA concentrations to be associated with high BMI (Shankar et al., 2012). Elevated urinary levels of BPA in children and adolescents were also found to be associated with obesity (Trasande et al., 2012). However, we found no association between BMI and BPA concentration in breast milk ($p > 0.05$; see Table 4). BMI may be dependent on a number of other variables that could affect BPA exposure such as ethnicity, genetics and lifestyle tendencies with respect to exercise, employment or medical conditions.

**Table 1**
Calculated BPA concentrations and potentially relevant demographic factors for each of the 21 participants.

| Sample | Calculated BPA Conc. (ng mL$^{-1}$)[a] | Age | BMI | Race[b] | Baby's Age (days) | No. of children | Breast pump[c] |
|---|---|---|---|---|---|---|---|
| 20 | ⩽LOD | 30 | 20.5 | Non-Caucasian (AA) | 356 | 2 | n/a |
| 18 | ⩽LOD | 35 | 18.0 | Non-Caucasian (AA) | 240 | 1 | Medela |
| 6 | ⩽LOD | 26 | 31 | Non-Caucasian (AA) | 150 | 2 | n/a |
| 21 | ⩽LOD | 31 | 31.9 | Non-Caucasian (AA) | 270 | 1 | Avent |
| 16 | ⩽LOD | 31 | 18.2 | Caucasian | 420 | 1 | Medela |
| 14 | ⩽LOD | 37 | 30 | Non-Caucasian (H) | 30 | 1 | Medela |
| 13 | ⩽LOD | 28 | 18.9 | Caucasian | 240 | 2 | Medela |
| 7 | <LOD | 37 | 18.6 | Caucasian | 255 | 2 | Medela |
| 9 | 0.37 | 36 | 33.1 | Caucasian | 14 | 3 | Medela |
| 3 | 0.40 | 33 | 18.9 | Caucasian | 180 | 1 | Medela |
| 19 | 0.68 | 31 | 32.6 | Non-Caucasian (AA) | 210 | 2 | Ameda |
| 15 | 2.12 | 31 | 33.3 | Caucasian | 375 | 2 | Medela |
| 8 | 2.18 | 34 | 27.5 | Non-Caucasian (H) | 30 | 1 | Ameda Elite |
| 11 | 3.37 | 32 | 19.9 | Caucasian | 300 | 2 | Limerick |
| 5 | 5.51 | 36 | 34.9 | Caucasian | 70 | 1 | Medela |
| 1 | 6.04 | 41 | 38.2 | Caucasian | 780 | 3 | Ameda Purely Yours |
| 17 | 6.11 | 36 | 18 | Caucasian | 21 | 2 | Medela |
| 10 | 6.42 | 31 | 31.1 | Caucasian | 90 | 2 | Medela |
| 12 | 9.81 | 31 | 19.4 | Caucasian | 180 | 4 | Lansinoh Manual |
| 2 | 10.7 | 36 | 18.4 | Caucasian | 540 | 1 | Evenflo |
| 4 | 10.8 | 32 | 33.5 | Caucasian | 224 | 3 | Medela |

[a] LOD = Limit of detection (0.22 ng mL$^{-1}$).
[b] AA = African American, H = Hispanic.
[c] n/a = not available.

**Table 2**
Percentiles and summary statistics of Bisphenol A concentrations (ng mL$^{-1}$) in breast milk ($n$ = 21).

| Detection frequency (%) | Percentiles | | | | Mean | Geometric mean |
|---|---|---|---|---|---|---|
| | 25th | 50th | 75th | 95th | | |
| 62 | <LOD | 0.68 | 6.04 | 10.7 | 3.13 | 1.00 |

**Table 3**
Reported studies of Bisphenol A (BPA) in breast milk.

| Study population | Sample size | Detection Frequency (%) | BPA (ng mL$^{-1}$) | | | References |
|---|---|---|---|---|---|---|
| | | | Mean | Median | Range | |
| Japanese | 3 | 67 | | 0.65 | <LOD – 0.7 | Otaka et al. (2003) |
| Japanese | 23 | 100 | 0.61 | 0.61 | 0.28–0.97 | Sun et al. (2004) |
| U.S. | 20 | 60 | 1.3 | 0.40 | <LOD – 6.3 | Ye et al. (2006) |
| Japanese (colostrum)[a] | 101 | 100 | 3.4 | | 1–7 | Kuruto-Niwa et al. (2007) |
| U.S. | 4 | 100 | 0.80 | 0.62 | 0.41–1.5 | Ye et al. (2008) |
| Korean | 100 | 100 | | 6.6 | 0.65–29.9 | Yi et al. (2010) |
| U.S. | 23 | 20 | 1.7 | <LOD | <LOD – 23.6 | Mendonca et al. (2014) |
| U.S. | 21 | 62 | 3.13 | 0.68 | <LOD – 10.8 | The current study |

[a] The samples were collected within three days of delivery and analyzed by ELISA for both free and conjugated BPA. Result from the other reported studies are for free BPA and were obtained by LC/MS/MS, GC/MS or HPLC/Fluorescence.

**Table 4**
Relationship between demographics and levels of Bisphenol A (BPA) in breast milk.

| Characteristic | BPA ng mL$^{-1}$ mean (SD) | $n$ | $p$ |
|---|---|---|---|
| BMI < 30 | 3.03 (4.04) | 11 | |
| BMI ⩾ 30 | 3.24 (3.37) | 10 | >0.05 |
| Non-Caucasian | 0.52 (0.76) | 7 | |
| Caucasian | 4.44 (4.05) | 14 | <0.05 |

### 3.5. BPA concentration and race

Although we found no correlation between BMI and BPA levels, we found a significant difference in BPA levels by race. Caucasian women had significantly higher levels of free BPA in their breast milk (mean 4.44 ng mL$^{-1}$, $n$ = 14) than non-Caucasian women (mean 0.52 ng mL$^{-1}$, $n$ = 7) ($p$ < 0.05; see Table 4). Non-Caucasian women included two Hispanic and five African American women. Three samples in the Caucasian data set and five in the non-Caucasian data set had BPA levels equal to or below the MDL. A recent study found that total (free and conjugated) urinary concentrations of BPA were significantly higher in Caucasians than Asians (Liao and Kannan, 2012). In contrast, Unal et al. (2012) found 10-fold higher total BPA concentrations in maternal serum in African American women compared to Caucasian women (median BPA concentration 30.13 compared to 3.14 ng mL$^{-1}$, respectively). BPA concentrations in maternal serum from the Unal et al. study ranged from 0 to 153.5 ng mL$^{-1}$. Their sample size was comparable to our study, 27 women (8 African–American, 8 Caucasian and 11 Hispanic) (Unal et al., 2012). However, these concentrations are much greater than what others have measured in blood or serum. As summarized in a recent review, the majority of studies have found the range of BPA concentrations in blood or serum to be less than 2 ng mL$^{-1}$

(Vandenberg et al., 2010). The highest concentration measured by Unal et al. (2012) in maternal serum (153.5 ng $mL^{-1}$) is over 10 times greater than the highest value measured in the present study in breast milk (10.81 ng $mL^{-1}$). The apparent discrepancy between the results of the present study and that conducted by Unal et al. (2012) may be due to sampling error or the fact that we measured free BPA and their study measured total BPA (free and conjugated). The difference in BPA concentrations could also reflect differences in lifestyle and exposure.

The results described in the present study could be partially explained by an overall greater amount of internally accumulated free BPA in Caucasians. While the significant association between race and BPA levels observed in the present study could be an artifact of the limited sample size, it may indicate differences in metabolism or lifestyle factors between racial groups that lead to increased exposure. BPA is metabolized primarily in the liver where it is conjugated to BPA-G by UDP-glucuronosyltransferase (UGT), which has several different protein isoforms (Hanioka et al., 2008; Vandenberg et al., 2009). The main isoform responsible for BPA glucuronidation in humans is UGT2B15 (Hanioka et al., 2008). UGT2B15 has several allelic variants and those variants that possess the D85Y substitution (UGT2B15.2 and UGT2B15.5) have decreased enzymatic function compared to wild type (Hanioka et al., 2011). The D85Y polymorphism has been found to be more common in Caucasians than Asians (Lampe et al., 2000). However, it is unclear from the literature how prevalent this allele is in other populations. The presence of the D85Y polymorphism may vary with race, resulting in a difference in free BPA and BPA-G concentrations in biological samples. In the future, it will be important to investigate the relationship between different UGT2B15 genotypes and BPA levels in breast milk. It would also be of value to examine BPA levels in breast milk in relation to levels in urine as it would be expected that women who do not metabolize BPA effectively would have high levels of BPA in breast milk and low urinary levels of BPA-G.

## 4. Conclusions

In summary, using a sensitive LC/MS method, our study found a wide range of BPA levels in the breast milk of women from the U.S., ranging from below the detection limit (0. 22 ng $mL^{-1}$) to approximately 11 ng $mL^{-1}$. Using BMI as an approximation of BPA exposure, we found no significant relationship between BMI and BPA levels. However, we did find a significant association between BPA levels and race. Caucasian women ($n = 14$) had higher levels of free BPA in their breast milk than non-Caucasian ($n = 7$) (mean 4.44 ng $mL^{-1}$ compared to 0.52 ng $mL^{-1}$, respectively). Although these results are preliminary and the sample size was small, it will be important in the future to investigate the manner in which race may affect BPA exposure. This could be determined, for example, by a more detailed questionnaire that assesses dietary or other exposures to BPA that may vary with race. Studies investigating racial disparities in enzyme metabolism function are also necessary. High levels of BPA in breast milk may be due to a combination of differences in exposure and lifestyle as well as inefficient metabolism. Decreasing exposure could offset potential differences in metabolism. Therefore, nursing women could make changes to their diet to reduce both their exposure as well as their child's exposure. However, further studies are necessary to understand how free BPA levels in breast milk are related to exposure and metabolism. Monitoring other fluids, including maternal serum or urine, for BPA levels will also prove important as there may be increased sensitivity of the fetus to BPA during development.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2013.12.085.

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