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Second Trimester Amniotic Fluid Bisphenol A Concentration is Associated with Decreased Birth Weight in Term Infants

Sara E. Pinney^{*,1,2}, Clementina A. Mesaros^{3,4}, Nathaniel W. Snyder^{3,5}, Christine M. Busch^{3,4}, Rui Xiao⁶, Sara Aijaz¹, Naila Ijaz¹, Ian A. Blair^{3,4}, and Jeanne M. Manson⁷

¹Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia

²Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, United States

³Penn SRP Center and Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania Philadelphia, PA 19104, United States

⁴Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania Philadelphia, PA 19104, United States

⁵AJ Drexel Autism Institute, Drexel University, Philadelphia, PA 19104, United States

⁶Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States

⁷Canadensis, PA 18325, United States

Abstract

Bisphenol A (BPA) is an endocrine disrupting chemical with ubiquitous environmental exposure. Animal studies have demonstrated that *in utero* BPA exposure leads to increased adult body weight. Our aim was to characterize human fetal BPA exposure by measuring BPA concentration in second trimester amniotic fluid (AF) samples and to study its relationship with birth weight (BW) in full term infants. To achieve these goals, we developed a total BPA assay utilizing derivatization with pentafluorobenzyl followed by analysis with LC-ECAPCI-MS/MS with a limit of detection of 0.08 ng/mL and limit of quantification (LOQ) of 0.25 ng/mL. The mean BW of infants with AF BPA 0.40-2.0 ng/mL was 241.8 grams less than infants with AF BPA less than the LOQ after controlling for covariates ($p=0.049$). No effect was seen outside this range indicating a non-monotonic effect. Our data suggest that low level BPA exposure *in utero* decreases BW and needs further study.

*Corresponding author: Sara E. Pinney, Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, Philadelphia, PA 19104. pinneys@email.chop.edu; Phone: 215-590-3174; Fax: 215-590-3053.

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Keywords

Bisphenol A; In utero exposure; Developmental Origins of Health and Disease

1. Introduction

Bisphenol A (BPA) is an organic synthetic compound used in the production of plastics for beverage bottles, epoxy resins to line beverage/food cans and thermal receipt paper, and is a major component of dental sealants [1-6]. BPA is an endocrine disrupting chemical (EDC) that exerts estrogenic activity, affects cellular antioxidant mechanisms, inhibits cell cycle regulation and disrupts cell differentiation [7, 8]. Animal studies have demonstrated that *in utero* exposure to BPA results in increased body weight later in life [9-12], while studies looking at postnatal BPA exposure have yielded inconsistent effects on adult weight [13, 14]. In addition, prenatal and perinatal exposures to BPA have been linked to increased adiposity and abnormalities in glucose regulation in the adult offspring [7, 12, 15]. Prior epidemiological studies have shown an association between BPA exposure *in utero* and decreased BW in the offspring, but this finding has not been consistent, likely due in part to technical and bioanalytical challenges in accurately assessing fetal exposure to BPA [16-18].

Animal studies have shown that *in utero* exposure to BPA during pregnancy and lactation can result in decreased birth weight (BW) of the offspring [12] and later development of glucose intolerance later in life [9-11, 13, 14, 19]. Epidemiologic and animal studies support the concept that there is a critical window of fetal development in which exposure to environmentally or maternally derived agents *in utero* can make an individual more susceptible to diseases later in life such as obesity and diabetes [20]. In addition, *in utero* exposures to conditions such as maternal smoking, gestational diabetes and maternal obesity, and maternal hypertension can lead to placental insufficiency and decreased BW of the infant. We hypothesize that *in utero* exposure to BPA leads to compromised placental function, which can manifest with decreased infant BW. This hypothesis is supported by a recent study demonstrating a significant negative correlation between placenta BPA concentration and BW [21]. However, no relationship between amniotic fluid (AF) BPA concentration and BW has been described. Thus, our primary aim was to investigate the relationship between second trimester AF BPA concentration and BW in full term human infants.

Challenges in measuring fetal exposure to BPA include accessibility of the fetal space, as well as the relevance of sampling surrogate tissues (such as maternal blood and urine) to fetal exposure due to differential intrauterine distribution and metabolism [22]. Maternal urine levels reflect the quantity of excreted BPA, and do not necessarily represent the internal exposure to the fetus. Absolute demonstration of fetal exposure comes only from biosamples derived from within the fetal compartment, including AF from amniocentesis or delivery, meconium, cord blood and neonatal blood and urine [23, 24] but repeated measures of these samples are difficult or impossible to obtain.

These challenges are compounded by bioanalytical difficulties in measurement of BPA including achieving adequate sensitivity and ubiquitous laboratory contamination [25, 26].

Only recently have methods consistently reported limits of detection (LODs) below 0.1 ng/mL allowing pharmacokinetic studies in humans at doses approximating estimated dietary exposures [27]. The issue of appropriate and accurate quantitation of BPA exposure is critical to the process of evaluating the relationship between *in utero* BPA exposure and human fetal development [28]. Our secondary aim was to characterize *in utero* BPA exposure in the human developing human fetus by measuring BPA concentration in second trimester AF samples with a novel highly sensitive and specific assay for total BPA.

To achieve these analytical goals, we developed an assay utilizing derivatization with the electron-capturing pentafluorobenzyl (PFB) followed by analysis with normal phase liquid chromatography-electron capture atmospheric pressure chemical ionization-tandem mass spectrometry (LC-ECAPCI-MS/MS). This method was then validated in control human AF samples and subsequently used to measure BPA in AF samples obtained via amniocentesis from pregnant women during the second trimester of gestation.

2. Methods

2.1 Study Population and Sample Collection

Women who contributed AF samples for this study had healthy singleton pregnancies with infants born at term (defined as 37-42 weeks gestation) and without any reported maternal health conditions, pregnancy complications, fetal anomalies or exposures to maternal smoking or illicit drugs. AF specimens were collected from women undergoing clinically indicated amniocentesis from 2004-2006 using a 22 gauge needle and 20-30 mL polypropylene syringe, without use of any plastic tubing. The samples were stored in polypropylene cryogenic vials at -80°C adhering to a strict research protocol for sample collection and management that was consistent during the study period. Data abstracted from reproductive genetics charts and post-birth outcome surveys included maternal age, race and ethnicity, gestational age (GA) at amniocentesis, indication for amniocentesis, cytogenetic testing results, sex of offspring and pregnancy outcome data including BW, GA at birth, and maternal health history including complications encountered throughout the duration of the pregnancy. Written informed consent was obtained from subjects prior to participating in the study. The study was approved by The Children's Hospital of Philadelphia and University of Pennsylvania Institutional Review Boards (IRB-13-010626).

2.2 BPA Analysis

Total AF BPA concentration was measured via a novel method that converts BPA into PFB-BPA followed by quantification via LC-ECAPCI-MS/MS. One hundred thirty (130) AF samples were analyzed for BPA content in 2 batches on 2 separate days. The full description of the method, as well as the method validation is included in Supporting Information. Briefly, a 500 μL aliquot of AF was measured with a glass syringe and transferred to a 10 mL glass test tube containing 0.3 mL of 0.1 sodium acetate buffer (pH of 5, containing 2 ng of $^{13}\text{C}_{12}$ -BPA). *Helix pomatia* β -glucuronidase/arylsulfatase enzyme (30uL) was added followed by overnight incubation at 37°C . Formic acid (20 μL) was added to acidify each sample and halt enzymatic hydrolysis. To create the blank controls, the same procedure was performed with 500 μL of water. After equilibration at room temperature, samples were

extracted with 4 mL of methyl tert-butyl ether. The organic layer was transferred to a new glass test tube and then evaporated to dryness under nitrogen steam. The dried residue was derivatized with PFB using NaOH as a catalyst. The samples were then evaporated to dryness, resuspended, and analyzed by LC-ECAPCI-MS/MS as described in the supplemental methods. All data analysis for the BPA-PFB measurements was performed using Xcalibur software, version 2.0 SR2 (Thermo Electron Corporation).

We defined the LOD as 3 times the standard deviation of multiple blank determinations containing only internal standard [29], resulting in a value of 0.08 ng/mL for this assay. The limit of quantification (LOQ) was estimated at 0.25 ng/mL and defined as the lowest calibration level, which could be fitted to the calibration curve with a residual of less than 10% and peak area ratio 10 times higher than the background. No appreciable batch effects were observed with AF BPA measurements, as the calibration constructed in pooled AF had identical slopes in 3 different days and the co-efficient of variation of all QC samples were within 15%.

2.3 Data analysis

Two sample t-tests or Mann-Whitney tests were used to compare means between two groups for continuous variables depending on the distribution, linear regressions for more than two groups, and chi-squared test or Fisher exact test for categorical variables as appropriate. Geometric means of BPA were calculated and compared for groups defined by gender of offspring, GA at the time of amniocentesis (< 19 weeks vs. ≥ 19 weeks), GA at birth (37-39 weeks vs. 40-42 weeks), race/ethnicity (white non-Hispanic vs. others) and parity (= 0 vs. > 0) using the least square means obtained from generalized linear models. Linear regression analyses were performed to evaluate associations between BW and AF BPA concentration initially as a categorical variable and subsequently as a continuous variable, adjusted for other covariates of interest including sex of offspring, GA at amniocentesis, parity, and gravidity (= 0 vs. >0). Subgroup analysis was conducted on samples with AF BPA concentrations between 0.25 –2.0 ng/mL (n=46) to capture a potential linear relationship between BW and AF BPA concentration. Linear regression analyses were also performed to evaluate associations between BW and GA at birth and other independent variables of interest including sex of offspring, GA at amniocentesis, parity, and gravidity (= 0 vs. >0). Dunnett's method was applied to correct for multiple comparisons among the BPA groups using the unexposed group as the reference. All the statistical analyses were performed with SAS 9.4 or Graph Pad Prism.

3. Results

3.1 BPA quantification by PFB Derivatization and LC-ECAPCI-MS/MS

The PFB derivative of BPA was successfully analyzed by negative ion mode LC-ECAPCI-MS/MS. Both phenolic groups were derivatized as PFB ethers under strong basic conditions (NaOH) resulting in a single product as a bis-derivative. One of the PFB groups was lost during in-source fragmentation resulting in the product ion $[M-PFB]^-$. Mass spectra obtained by MS/MS analysis of $[M-PFB]^-$ contained specific fragments for the loss of the second PFB group and loss of the PFB group plus a methyl (Figure 1A). We analyzed a

middle concentration quality control (MQC) sample (8 ng/mL) both with and without derivatization (Figure 1B). The PFB derivative eluted earlier in the gradient (7.5 min) than the underivatized sample (10.2 min), corresponding to expected polarities. The sensitivity of the samples after derivatization was at least 2 orders of magnitude higher than without PFB derivatization (Figure 1B). As shown in Figure 1B and 1C, derivatization minimizes low level background BPA detection while increasing signal intensity from the same MQC sample. Using the derivatization method for total BPA measurement the LOD was estimated at 0.08 ng/mL and the LOQ at 0.25 ng/mL. We adopted this conservative LOD and LOQ to avoid erroneous exposure estimates, but notably, it is in line with the most recent methods for measuring BPA from other biofluids with LODs in the sub-ng/mL range [27].

3.2 BPA concentration in AF

The majority of the AF samples analyzed for BPA came from white women (78.5%) who were non-Hispanic (94.6%) (Table 1). AF samples were collected from women with ages ranging from 16–45 years and GA ranging from 16–22 weeks, with a mean GA of 17.2 weeks. The most common indication for amniocentesis was advanced maternal age (>35 years) (71.5%). Other indications for amniocentesis included abnormal results from the multiple marker screening and AFT test (i.e. triple screen test, a maternal blood screening test to screen for fetal genetic disorders) (23.1%), concern for fetal anomaly on ultrasound (6.2%), family history of a single gene disorder (3.1%) and history of a previous child with a birth defect (2.3%) and other (3.1%), with some women reporting more than one indication for amniocentesis.

Among the 130 samples with AF BPA concentration measured, 52 (40%) had BPA concentration greater than the LOQ (Table 1). We did not impute BPA values for samples with BPA concentrations less than the LOQ due to the large proportion (60%) of our samples in this category [30]. There were no significant differences in maternal race, maternal ethnicity, maternal age, GA at amniocentesis, BW, gravidity, parity and distribution of offspring sex between the samples with AF BPA concentration less than the LOQ and samples with AF BPA concentration greater than the LOQ. In the AF samples with BPA greater than the LOQ (n=52), the range of BPA concentrations was 0.25–23.74 ng/mL (median = 0.36 ng/mL) (Table 1). For additional analysis, samples were divided into 4 categories based on the distribution of AF BPA measurements (Figure 2A). Group 1 consisted of samples with BPA $\geq$ LOQ ($\geq$ 0.25 ng/mL; n=78). Group 2 contained samples with BPA concentrations from 0.25–0.40 ng/mL (n=29) and Group 3 consisted of samples with BPA concentration from 0.41–2.0 ng/mL (n=17). Due to the extremely skewed distribution of AF BPA concentrations, six AF samples with BPA concentrations > 2.0 ng/mL were considered statistical outliers (>2SD greater than the mean BPA concentration) and were placed in Group 4 (Figures 2B and 2C). None of the 3 groups significantly differed from Group 1 in maternal race, maternal ethnicity, maternal age, GA at amniocentesis, GA at birth, BW, gravidity, parity, and distribution of offspring sex (Table 2).

The geometric mean of AF BPA concentration for samples with BPA concentrations between 0.25–2.0 ng/mL (Groups 2 and 3) was calculated for groups defined by gender of offspring, GA at the time of amniocentesis (< 19 weeks vs. $\geq$ 19 weeks), GA at birth (37–39

weeks vs. 40-42 weeks), race/ethnicity (white non-Hispanic vs. others) and parity (= 0 vs. > 0) (Table 3). Variables such as sex of the offspring, gestational age at amniocentesis, maternal race/ethnicity and parity had no effect on the geometric mean of AF BPA. However, the geometric mean of AF BPA from offspring born at 40-42 weeks GA (0.68 ng/mL) was greater than the geometric mean for infants born at 37-39 weeks GA (0.46 ng/mL) but this did not reach statistical significance ($p=0.089$). In addition, the geometric mean of AF BPA concentration was calculated for samples with BPA > LOQ (Groups 2,3 and 4), but was heavily influenced by the AF BPA concentrations measured in Group 4 (Supplementary Table 1).

3.3 BPA and Birth weight

Multi-variable linear regression analysis was performed to examine the association between BW and independent variables including AF BPA concentration categorized as 4 groups, sex of offspring, GA at amniocentesis, and parity (Table 4). The regression analysis indicates that the mean BW in Group 3 (0.40 ng/mL < AF BPA ≤ 2.0 ng/mL) is 241.8 grams lower than that of group 1 (BPA ≤ LOQ (≤ 0.25 ng/mL) when holding other covariates constant ($p=0.049$). This comparison was not significant after correcting for the multiple comparisons using Dunnett's method ($p=0.11$). The BW was not significantly different for infants with AF BPA concentrations greater than 2.0 ng/mL or less than 0.40 ng/mL compared to infants in Group 1, suggesting that *in utero* BPA exposure may have a non-monotonic dose effect on BW. Sex of offspring was marginally significantly associated with BW in this model, with male offspring weighing on average, 152.3 grams more than female offspring ($p = 0.064$). We explored the interaction of sex of offspring with BPA concentration in the regression model with awareness of underpower due to small sample size, and no significant association was observed (data not shown).

Since the linear regression analysis with BPA as categories provided evidence that AF BPA concentration has a non-monotonic dose effect on BW, we performed a subgroup analysis on samples with AF BPA concentrations between 0.25 – 2.0 ng/mL (Groups 2 and 3, $n=46$) as a continuous variable, to better define the relationship (Table 5). In this subgroup analysis, each 1 ng/mL increase in AF BPA concentration was associated with a decrease in the BW by 252.0 grams when adjusted for GA at amniocentesis, gravidity, parity, and sex of the offspring, but this relationship did not reach statistical significance ($p=0.12$). The BWs of male offspring were on average 321.0 heavier than female offspring in this model ($p=0.038$).

In the regression analyses described in Table 4, we did not incorporate GA at birth into our model due to the fact that GA at birth is highly correlated with BW [31, 32]. However, we wanted to explore whether the significant relationship detected between second trimester AF BPA concentration and BW was in fact due to a significant relationship between second trimester BPA concentration and GA at birth. Therefore, we performed multi-variable regression analysis between GA at birth and the independent variable AF BPA (based on categories), adjusting for covariates and found no significant relationship (Table 6). These results indicate that there is a relationship between second trimester AF BPA concentration

and BW that is independent of GA. In addition, this regression analysis revealed a significant relationship between parity (1 vs. 0) and GA at birth ($p=0.02$).

4. Discussion

Our data represent, to our knowledge, the largest cohort of human amniotic fluid samples in which BPA concentrations have been measured and correlated with BW outcome data. Developing a highly sensitive and specific assay to measure BPA concentrations in biological fluids has been particularly challenging given the ubiquitous nature of environmental background contamination with BPA which can effectively increase the LOD and LOQ for the assay. There are only a few investigators that have reported BPA measurements in AF using a liquid chromatography coupled with mass spectrometry (LC-MS) method with electrospray ionization (ESI) or ELISA [17, 33-36].

Although *in utero* exposure to EDCs has been hypothesized to affect the developing fetus and could play a significant role in programming the rapidly developing fetal cells, modeling methods for assessing fetal exposures throughout the gestational period have proven difficult. Many studies estimate fetal BPA exposure through maternal serum or urine measurements at one time or with repeated measures during gestation [16, 37-41] but these studies have failed to demonstrate consistent relationships between maternal serum or urinary BPA concentration and the outcome of BW. Animal and human studies have shown that BPA is transported across the placenta into amniotic fluid [42-44] but the data are not clear as to whether BPA is concentrated in AF and if the levels are consistent throughout gestation [33-36, 39, 45]. Previous studies have measured AF BPA in an attempt to assess *in utero* exposure and have demonstrated measurable BPA concentrations in AF at both second and third trimesters and at delivery but employed less sensitive assays for BPA measurement and had smaller sample sizes [33-36].

Within our population of term pregnancies, there is an indication that the second trimester AF BPA concentration may be higher in infants who delivered at 40-42 weeks gestation compared to those who delivered at 37-39 weeks gestational age, but the difference did not reach statistical significance. Other studies have examined the relationship between *in utero* BPA exposure and gestational length with inconsistent results but these studies all assessed fetal exposure by maternal serum or urine measurements during pregnancy and used less sensitive methods for BPA measurement [17, 46-48].

The major finding in our study was that second trimester AF samples with BPA concentrations between 0.40-2.0 ng/mL are associated with a significantly lower BW. Animal studies examining the effects of *in utero* BPA exposure indicate that the programming effects on the offspring are dependent on the dose during prenatal exposure and the effects may be representative of a non-monotonic dose relationship [7, 12, 14, 49, 50]. A non-monotonic dose response curve is defined as one where the curve contains a point of inflection where the slope switches sign from positive to negative or vice versa [51]. Although prenatal exposure to BPA has been associated with significant effects on body weight and beta cell insulin secretory capacity, these effects are more pronounced with lower dose BPA exposure *in utero* than they are with the higher dose exposures [12, 14]. The

mechanisms responsible for the non-monotonic effects of prenatal exposure to BPA are not definitively known but in general non-monotonic responses can be attributable to factors such as cell cytotoxicity at high dose exposure, cell and tissue specific receptor and cofactor populations, receptor factors including selectivity, down-regulation and desensitization and competition and negative feedback signaling pathways [51].

While animal studies highlight the link between BPA exposure in the intrauterine environment and the development of obesity and abnormalities in glucose homeostasis later in life, the molecular mechanisms underlying this phenomenon are still being elucidated. Many animal studies linking *in utero* exposure to BPA with increased body weight in adulthood did not report BW [9, 10, 14, 15]. However, Susiarjo *et al* reported that male mice exposed to low dose BPA *in utero* (10ug/kg/day) had significantly lower BWs than controls but this effect was not seen in mice exposed to high dose BPA *in utero* (10mg/kg/day)[12]. Mice exposed to both low dose and high dose BPA *in utero* developed obesity and impaired glucose tolerance in adulthood but the low dose exposure to BPA *in utero* was associated with impaired insulin secretion in isolated islets while high dose BPA exposure *in utero* was associated with increased basal insulin levels, a marker of insulin resistance [12].

In interpreting the significance of our results, the mean decrease in BW of infants with AF BPA concentrations between 0.40-2.0 ng/mL of 241.8 grams could represent a population shift in the BW of infants exposed to low level BPA *in utero*. Given the rapid rise of the prevalence of Type 2 diabetes and obesity over the past several decades, there is interest in identifying additional factors contributing to the epidemic other than poor nutrition and lack of exercise, including environmental factors and developmental exposures. Our finding that low level BPA exposure *in utero* is associated with decreased BW is concerning when considering the effect this could have on the population as a whole, as there have been a number of meta-analyses demonstrating significant inverse linear relationships between BW and development of Type 2 diabetes [52, 53] indicating a need for further investigation into the mechanisms involved.

Our findings are limited by the fact that there was a single measurement of AF BPA concentration used to approximate BPA exposure throughout gestation. However, there is data to support that a single biofluid measurement of BPA concentration provides a reasonable degree of temporal reliability for assessing BPA exposure over a 6 month time period and is appropriate to use as a biomarker [54]. The biospecimen repository utilized for this study did not contain subsequent measurements throughout gestation or correlating measurements in maternal samples, and in the future such correlative studies will greatly contribute to our ability to understand gestational BPA exposure. We acknowledge that it is possible that our data may include exposure misclassification since sample collection materials were not tested for BPA prior to collection, but the materials and processes were consistent throughout the collection period and the sample collection and storage protocol were developed to minimize contamination.

Although free BPA measurements quantify the active form of BPA, due to limited sample volume (less than 1 mL), we could only measure total BPA concentration. Edlow *et al*, measured total BPA concentration in second trimester AF samples and reported values for

total BPA concentration (Conjugated + Free) that were similar to the values measured in this study (Edlow: mean total BPA, 2nd Trimester AF = 0.45 ng/mL; Pinney mean total BPA, 2nd Trimester AF = 0.38, $p=0.11$) [33]. Edlow *et al*'s study of second trimester AF samples that included free BPA concentration measurements, free BPA accounted for 83% of the total concentration and thus did not represent a substantial difference from the total values [33]. Furthermore, due to the high activity β -glucuronidase in human placenta tissue and decreased capacity of the fetal liver to perform BPA glucuronidation (due to the fact that UDP-glucuronosyltransferase activity is low or absent at this gestational age), it is possible that increased levels of free BPA concentration in AF reported by Edlow *et al* are physiologic [21, 55, 56]. Since it has been demonstrated that a high percentage of BPA measured in second trimester AF is free BPA, the absence of free BPA data in our cohort is not major limitation of this study.

The population of women contributing AF samples for this study are not necessarily representative of the general population of pregnant woman therefore caution should be used when extrapolating these results. In addition, we were unable to investigate whether there were relationships between AF BPA concentration and socio-economic status, or family income level. Finally, even though our data represent the largest cohort of human amniotic fluid samples in which BPA concentrations have been correlated with BW outcome data, some of the exposure groups contain relatively few samples. Therefore, it is possible that the lack of association between BPA concentration and BW in the group with the highest BPA concentrations may only reflect a lack of statistical power to detect such an association.

Our data indicate that the developing fetus is exposed to BPA as it passes through the placenta and into the AF during gestation. In our population of AF samples from healthy second trimester term singleton pregnancies, BPA concentrations in the range of 0.40-2.0 ng/mL were associated with lower BW. The development of a novel assay for total BPA measurement in body fluids through the derivatization of PFB-BPA permitted a sensitive and specific method for the detection of BPA exposure to the fetus. Given that animal studies have shown that low level BPA exposure *in utero* is associated increased adult body weight, adiposity and beta cell defects in the offspring, our study suggests that the low level BPA exposure *in utero* needs to be studied more carefully in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BPA	bisphenol A
AF	amniotic fluid
EDC	endocrine disrupting chemical
LOD	limit of detection
LOQ	limit of quantification
PFB	pentafluorobenzyl
LC-ECAPCI-MS/MS	liquid chromatography-electron capture atmospheric pressure chemical ionization-tandem mass spectrometry
GA	gestational age
MQC	middle concentration quality control
SRM	selected reaction monitoring
NL	normalized intensity

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Figure 1B

8 ng/mL BPA **without**
derivatization

Figure 1C

8 ng/mL BPA **after** PFB
derivatization

Figure 1.

A. Tandem mass spectrometry spectrum of BPA-PFB. Analytical specificity comes from the use of collision induced dissociation of the parent ion $[\text{M-PFB}]^-$ (407.05) resulting in the formation of a specific product ion (211.03) from an alpha cleavage adjacent to the hydroxyl group. Selected reaction monitoring (SRM) can be performed on diagnostic parent and product ions, which provide high specificity and increased sensitivity. **B and C:** LC-SRM/MS chromatograms. Top panels are chromatograms for BPA and lower panels are chromatograms for the internal standard $[^{13}\text{C}_{12}]$ -BPA) that was spiked in the beginning of the extraction for quantification in each sample; m/z : mass to charge ratio, NL; normalized intensities. **B.** Standard sample at 8 ng/mL concentration without derivatization. **C.** Standard sample at 8 ng/mL concentration after PFB derivatization. NLs in panel C (after derivatization) are 2 orders of magnitude higher than in panel B.

Figure 2A:

Figure 2B:

Figure 2C:

Figure 2.

A. Distribution of total AF BPA concentrations for samples with BPA >0.25 ng/mL (n=52).

B. Box plot of amniotic fluid BPA concentration for samples measured above the LOQ

(n=52) Black bar indicates mean AF BPA concentration with black line indicating 2 standard deviations greater than the mean. Six AF samples had BPA concentrations measured > than 2SD from the mean measurement and were thus considered statistical outliers.

C. Distribution of offspring BWs and AF BPA concentration measured at amniocentesis, limited to samples with AF BPA concentration between 0.251 –2.0 ng/mL (n=46). Male offspring indicated by black circles, female offspring indicated by gray circles.

Table 1

Total BPA Measurements in Amniotic Fluid

	All Samples (n=130)	BPA 0.25 ng/mL ^a (n=78)	BPA > 0.25 ng/mL ^b (n=52)	P value
Race, n (%)				0.23
White	102 (78.5)	62 (79.5)	40 (76.9)	
Black	13 (10)	5 (6.4)	8 (15.4)	
Asian/Pacific Islander	12 (9.3)	9 (9.0)	3 (5.8)	
Ethnicity, n (%)				0.46
Non-hispanic	123 (94.6)	73 (93.6)	50 (96.2)	
Hispanic	6 (4.6)	5 (6.4)	1 (1.9)	
Maternal Age, yrs	35.0 ± 4.6	34.6 ± 4.6	35.5 ± 4.6	0.31
Range	16-45	16-44	20-45	
Advanced Maternal Age, n (%)	93 (71.5)	53 (67.9)	40 (76.9)	0.36
Gestational Age at Amniocentesis, wks	17.2 ± 1.6	17.0 ± 1.4	17.5 ± 1.8	0.11
Range	16-22	16-22	16-22	
Birth Weight, grams	3428±456	3447 ± 461	3399 ± 451	0.65
Range	2211-4763	2523-4763	2211-4366	
BPA, ng/mL				
Mean			1.61 ± 3.93	
Median		LOQ = 0.25	0.36	
Range			0.251-23.74	
Gravidity ^c	2.5±1.6	2.4 ± 1.4	2.4 ± 1.5	0.88
Parity ^c	0.9±1.1	0.85 ± 0.91	0.77 ± 0.94	0.50
Sex of offspring, male, n (%)	70 (53.8)	30 (57.7)	40 (51.3)	0.59

^abelow the limit of quantification for BPA^babove the limit of quantification for BPA^cat time of amniocentesis

Table 2

BPA Measurement in Amniotic Fluid Grouped by BPA Category

	Group 1: BPA = 0.25 ng/mL (n=78)	Group 2: 0.25 < BPA = 0.40 ng/mL (n=29)	Group 3: 0.41 < BPA = 2.0 ng/mL (n=17)	Group 4: BPA > 2.0 ng/mL (n=6)
Race, n (%)				
White	62 (79.5)	22 (76)	13 (76)	5 (83)
Black	5 (6.4)	4 (14)	3 (18)	1 (17)
Asian/Pacific Islander	9 (9.0)	3 (10)	0 (0)	0 (0)
Ethnicity, n (%)				
Non-Hispanic	73 (93.6)	27 (93)	17 (100)	6 (100)
Hispanic	5 (6.4)	1 (3)	0 (0)	0 (0)
Maternal Age, yrs (Mean ± SD)	34.6 ± 4.6	35.3 ± 3.8	35.6 ± 4.6	35.7 ± 8.3
Range	16-44	24-40	29-45	20-45
Advanced Maternal Age, n (%)	53 (68)	23 (78)	12 (71)	5 (83)
Gestational Age at Amniocentesis, wks (Mean ± SD)	17.0 ± 1.4	17.4 ± 1.7	17.8 ± 2.1	17.5 ± 2.1
Range	16-22	16-21	16-22	16-21
Birth Weight, grams (Mean ± SD)	3447 ± 461	3455 ± 465	3228.8 ± 428	3608 ± 327.9
Range	2523-4763	2381-4366	2211-4026	3317-4253
Gestational Age at Birth, wks (Mean ± SD)	39.5 ± 1.21	39.3 ± 1.26	39.7 ± 1.26	41.2 ± 1.17
BPA, ng/mL (Mean ± SD)	LOQ = 0.25	0.30 ± 0.03 ^b	1.05 ± 0.43 ^b	9.65 ± 8.28 ^b
Median		0.3	1.02	6.64
Range		0.251-0.38	0.43-1.90	2.52-23.74
Gravidity ^c (mean ± SD)	2.4 ± 1.4	2.55 ± 1.86	2.12 ± 1.05	2.5 ± 0.84
Parity ^c (mean ± SD)	0.86 ± 0.91	0.72 ± 1.07	0.88 ± 0.86	0.67 ± 0.52
Sex of offspring, male, n (%)	40 (51)	17 (59)	11 (65)	2 (33)

^a below the limit of quantification for BPA^b p<0.05

Table 3

Geometric mean (SD) of total BPA (ng/mL) for values between 0.25 to 2.0 ng/mL simultaneously adjusted for sex, GA at amniocentesis, GA at birth, race/ethnicity, and parity.

	Sex		GA at amniocentesis (weeks)		GA at birth (weeks)		Race/Ethnicity		Parity	
	Male n=28	Female n=18	< 19 n=35	19 n=11	37-39 n=26	40-42 n=20	White Non-Hispanic n=34	Others n=132	=0 n=24	>0 n=22
BPA, ng/ml	0.62 (0.10)	0.52 (0.11)	0.56 (0.09)	0.59 (0.13)	0.46 (0.10)	0.68 (0.11)	0.59 (0.09)	0.55 (0.13)	0.47 (0.10)	0.67 (0.10)
P-value	0.43		0.84		0.089		0.76		0.14	

Table 4

Multi-variable regression analysis: Relationship between AF total BPA level (categorical) and birth weight, adjusted for covariates (n=129)^a.

		Estimate (SD)	P value
BPA Category^b	Group 2 vs. 1	−4.6 (98.7)	0.96
	Group 3 vs. 1	−241.8 (121.8)	0.049
	Group 4 vs. 1	235.4 (192.8)	0.22
Gestational age at amniocentesis		4.48 (25.2)	0.86
Parity	1 vs. 0	−77.3 (92.3)	0.40
	> 1 vs. 0	70.5 (104.6)	0.50
Sex (male vs. female)		152.3 (81.4)	0.064

^aParity data available for 129 patients

^bGroup 1: AF total BPA ≤ 0.25 ng/mL, Group 2: 0.25 ng/mL < AF total BPA ≤ 0.40 ng/mL, Group 3: 0.40 ng/mL < AF total BPA ≤ 2.0 ng/mL, Group 4: AF total BPA > 2.0 ng/mL

Table 5

Multi-variable regression analysis: Subgroup analysis for association between birth weight and total BPA level as continuous, adjusted for covariates. Subjects with AF total BPA level greater than 0.25 ng/mL and less than 2.0 ng/mL were included (N=46).

	Estimate (SD)	P value
BPA, ng/mL	-252.0 (159.1)	0.12
GA, amniocentesis	18.5 (37.4)	0.62
Parity=1 vs. Parity=0	-181.9 (166.2)	0.28
Parity>1 vs. Parity=0	45.8 (179.3)	0.80
Sex (male vs. female)	321.0 (149.9)	0.038

Table 6

Multi-variable regression analysis (n=129)^a: Relationship between AF total BPA level (categorical) and GA at birth, adjusted for covariates.

		Estimate (SD)	P value
BPA Category^b	Group 2 vs. 1	−0.20 (0.27)	0.45
	Group 3 vs. 1	0.29 (0.33)	0.38
	Group 4 vs. 1	0.80 (0.53)	0.13
GA, amniocentesis		−0.026 (0.069)	0.71
Parity	1 vs. 0	−0.59 (0.25)	0.020
	> 1 vs. 0	−0.29 (0.29)	0.31
Sex (male vs. female)		−0.26 (0.22)	0.25

^aParity data available for 129 patients

^bGroup 1: AF total BPA ≤ 0.25 ng/mL, Group 2: 0.25 < AF total BPA ≤ 0.40 ng/mL, Group 3: 0.41 < AF total BPA ≤ 2.0 ng/mL, Group 4: AF total BPA > 2.0 ng/mL