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# **Exposure to Free and Conjugated Forms of Bisphenol A and Triclosan among Pregnant Women in the MIREC Cohort**

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**Running title:** Bisphenol A and triclosan in maternal urine

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## **Abstract**

**Background:** Bisphenol A (BPA) and triclosan (TCS) are two non-persistent chemicals that have been frequently measured in spot urine samples from the general population but less so in pregnant women; however, data are limited on the free (bioactive) and conjugated forms of these phenols.

**Objectives:** The Maternal-Infant Research on Environmental Chemicals (MIREC) Study addressed these data gaps by utilizing stored maternal urine samples from a large multi-center cohort study of Canadian pregnant women.

**Methods:** Concentrations of free and conjugated forms of BPA and TCS were measured in about 1890 first trimester urine samples by UPLC-MS/MS using isotope dilution.

**Results:** The glucuronides of BPA and TCS were the predominant forms of these chemicals measured (detected in 95% and 99% of samples, respectively), while the free forms were detected in 43% and 80% of samples, respectively. The geometric mean urinary concentrations for glucuronides of BPA and TCS were 0.80 µg/L (95% CI: 0.75, 0.85) and 12.30 µg/L (95% CI: 11.08, 13.65), respectively. Significant predictors of BPA included maternal age <25 vs. ≥ 35 years, current smoking, low vs. high household income, and low vs. high education. For TCS, urinary concentrations were significantly higher in women ≥ 25 years of age, never vs. current smokers, and women with high household income and high education.

**Conclusions:** The results from this study represent the largest national-level data on urinary concentrations of free and conjugated forms of BPA and TCS in pregnant women and suggest maternal characteristics predicting elevated urinary concentrations of these phenols largely act in opposite directions.

## Introduction

Leaching of bisphenol A (BPA) has been reported from food cans and polycarbonate bottles, paper receipts, as well as dental sealants and fillings (Hoyle and Budway 1997; Joskow et al. 2006; Lu et al. 2013). BPA does not bioaccumulate and has a very short half-life in humans with elimination of the conjugated BPA in about 6 hours in the urine (Völkel et al. 2002). Orally administered BPA is rapidly and efficiently absorbed from the gastrointestinal tract and undergoes first-pass metabolism in the gut wall and liver, biotransforming BPA to its conjugated forms: BPA glucuronide, which is devoid of estrogenic activity (Matthews et al. 2001) and BPA mono- and disulfate.

Exposure to BPA is widespread with over 90% of the populations of the US and Canada having detectable urinary concentrations (Calafat et al. 2008a; Health Canada 2013). Evidence regarding effects of prenatal BPA exposure on fetal growth and birth weight is conflicting. For example in a Dutch cohort study, BPA exposure was associated with lower fetal growth rates and lower birth weight (Snijder et al. 2013); however, BPA was not associated with birth weight in a Chinese cohort (Tang et al. 2013). Similarly, some studies have estimated significant associations between maternal urinary concentrations of BPA and child behaviour (Harley et al. 2013; Perera et al. 2012; Braun et al. 2011), while others have reported no associations (Miodovnik et al. 2011; Yolton et al. 2011). These ambiguous findings may reflect differences in study populations or methodological issues related to exposure assessment.

Triclosan (TCS) is an antibacterial compound used in some cosmetic products, toothpaste, treated textiles, and food contact materials, such as cutting boards and countertops (Health Canada and Environment Canada 2012). TCS may be an endocrine disruptor, with some

evidence in laboratory animals of effects on thyroid hormone homeostasis and possibly the reproductive axis (Dann and Hontela 2011). Only two epidemiologic studies have explored potential health effects of prenatal exposure to TCS on birth size, and both reported no significant association (Wolff et al. 2008; Philippat et al. 2012).

Triclosan is highly lipid soluble, rapidly absorbed from the gut, and has a urinary elimination half-life of eleven hours, with an estimated 0.5% present in the unconjugated form within 24 h of exposure and the majority of the compound as the glucuronide (Sandborgh-Englund et al. 2006). TCS was detected in approximately three-quarters of the urines collected as part of NHANES 2003-2004 survey of the US population (Calafat et al. 2008b) and the 2009-2011 Canadian Health Measures Survey (Health Canada 2013).

Given the unique vulnerability of pregnant women and their fetuses and the possibility that these chemicals may be endocrine disruptors, it is important to examine the extent of exposure in this population and especially during the critical exposure window of the first trimester. As the free form may be more toxicologically active than the conjugated forms of these phenols, measurement of urinary concentrations of free BPA and TCS may provide a superior metric of the biologically effective dose.

The objectives of the current study were to: (1) measure the extent of exposure to free and conjugated forms of BPA and TCS during pregnancy among a population of Canadian women; and (2) identify predictors of elevated body burdens of these chemicals. This research offered a unique opportunity to efficiently examine these issues in a large diverse population using stored biological specimens and data collected in this prospective pregnancy cohort study.

## **Methods**

### **Participants**

The Maternal-Infant Research on Environmental Chemicals (MIREC) Study is a national-level pregnancy cohort of approximately 2,000 women recruited in the first trimester of pregnancy from 10 cities across Canada between 2008 and 2011 (Arbuckle et al. 2013). The protocol was approved by ethics committees at Health Canada and at Sainte-Justine University Hospital Center and study subjects gave written informed consent.

### **Data collection**

We collected detailed information on demographic and lifestyle factors from questionnaires administered at recruitment in the first trimester. The date and time of the urine collection, as well as the time since last urine void were also noted.

### **Urine collection and field blanks**

A spot urine sample was collected during the 1<sup>st</sup> trimester clinic visit in polypropylene cups, aliquoted into 30-mL Nalgene® tubes, frozen at -20°C and shipped on dry ice to the MIREC Biobank. Of the 2,001 participants, 43 did not consent to the Biobank, 18 women subsequently withdrew from the study, 47 urine samples were not collected, and 3 samples were insufficient, leaving a total of 1,890 urine samples for analysis.

We included field blanks to assess the potential contamination from the material used for collection and storage of urine samples as well as from the environment of collection sites. Water (Steril.O reagent grade deionized distilled water) was used as a surrogate matrix for urine during the process. Water was poured in the polypropylene cup and transferred in polypropylene storage

tubes using the same material as for urine samples. Water samples were analysed following pentafluorobenzyl bromide derivatization by GC-MS/MS using the method previously developed in our laboratory and described in Provencher et al. (2014). Results showed that all field blanks were free of BPA and TCS contamination. All materials in contact with urine samples had been pre-screened and found not to be a source of contamination.

### **Analytical methods**

The LC-MS/MS methods for the analysis of free and conjugated forms of BPA and TCS in urine were previously described (Provencher et al. 2014). Briefly, free BPA and TCS and their isotope-labelled standards,  $^{13}\text{C}_{12}$ -BPA and  $^{13}\text{C}_{12}$ -TCS, were derivatized with dansyl chloride directly in 1 mL of urine. A liquid-liquid extraction with hexane was subsequently performed and the organic phase evaporated prior to reconstitution in a solution of acetonitrile:H<sub>2</sub>O (50:50, v:v). The LC-MS/MS (UPLC Acquity and Xevo TQ-S; Waters, Milford, MA) was operated in electrospray positive and multiple reaction monitoring (MRM) mode. Chromatographic separation was achieved on an Acquity UPLC HSS T3, 1.8  $\mu\text{m}$ , 50 x 2.1 mm analytical column (Waters) using a mobile phase gradient with 0.1 % aqueous formic acid solution and acetonitrile.

Conjugated metabolites, BPAS, BPADS, BPAG, TCSS, TCSG and their isotope-labeled standards, BPAS-*d*<sub>6</sub>, BPADS-*d*<sub>6</sub>, BPAG-*d*<sub>6</sub>, TCSS-*d*<sub>3</sub> and TCSG-*d*<sub>3</sub>, were extracted from 1.5 mL of urine by solid phase extraction using a weak anion exchange phase (Strata X-AW; Phenomenex, Torrance, CA). Analytes were eluted from the cartridge using a 1 % NH<sub>4</sub>OH in methanol solution. The extracts were evaporated to dryness and reconstituted in a solution of 25% methanol in water. The same LC-MS/MS instrument and analytical column were used as for the free species, but the MS/MS was operated in the electrospray negative and MRM mode.

A mobile phase gradient from aqueous ammonium hydroxyde (2%) to an ammonium hydroxyde methanolic solution (0.1%) was used to obtain proper chromatographic resolution of conjugated compounds.

### **Laboratory quality control (QC)**

Several QC samples, reagents blanks and urine blanks were incorporated in each batch of samples. Low- and high-level in-house reference materials were prepared by spiking human urine to yield concentrations of 0.18 µg BPA/L and 0.9 µg TCS/L, and 1.5 µg BPA/L and 7.5 µg TCS/L, respectively. For conjugated species, human urine was spiked to obtained reference materials at three different concentration levels: low (0.2 µg/L), medium (2 µg/L for sulfate metabolites; 3 µg/L for glucuronide metabolites) and high (15 µg/L for sulfate metabolites; 60 µg/L for glucuronide metabolites). The intraday precision varied from 2.5 % to 7.7 % and the interday precision from 4.3 % to 13 % depending on the analyte. The accuracy was -3.7% for free BPA and -1.0 % for free TCS. The accuracy for conjugated forms ranged from -2.1 to 13.3 % depending on the analyte. Detailed QA/QC procedures are described in Provencher et al. (2014).

### **Statistical analysis**

Two different approaches were used to calculate summary statistics for the biomonitoring results that were below the limits of detection (LOD). The first approach used values generated by the laboratory instrument and observations that were reported as zero were replaced by half the next smallest value (other than zero) for that contaminant. In the second approach, censoring methods were used by applying survival analysis techniques to left-censored data which have been demonstrated by other authors (Helsel 2012, Nysen et. al. 2012) to improve estimation and

reduce bias. To account for non-detects, the geometric mean (GM) from a lognormal random variable with censoring was calculated using the maximum likelihood method (MLE) and compared to the empirical median from the Kaplan-Meier (K-M) approach. The Greenwood estimate of variance was used for K-M confidence intervals. We report summary statistics for both the unadjusted and specific gravity (SG)-adjusted contaminants.

In order to compare concentrations of free and conjugated forms of BPA or TCS, we expressed the concentrations of glucuronides and sulfates as BPA (or TCS) equivalents. Total BPA or TCS was calculated by summing the free and conjugated forms and the most conservative LOD of the components was assigned to the total to determine the percentage below the LOD.

We calculated geometric mean (GM) urinary concentrations for each level of potential predictive variables for all analytes having at least 50% of the data above the LOD in all groups (as justified in Helsel 2012). For analysis of the associations between potential predictors and the urinary metabolite, specific gravity was included as a covariate in the regression model using analysis of covariance (ANCOVA) (Kutner et. al., 2005). ANCOVA adjusts the mean values compared in each level of the potential predictor such that the levels are compared at the same value of the covariate (in this case SG). The assumptions of ANCOVA are similar to those of ANOVA (normality, and constant variance of residuals) with an additional assumption, the slopes of the relationship between the covariate (SG) and the urinary metabolite must be similar in each level of the potential predictor. The assumptions of normality and equal variance of residuals were tested using the Anderson Darling test and Levene's test, respectively. The assumption of equal slopes between levels of the potential predictor and the covariate is crucial for ANCOVA to be valid. This reduces to testing the interaction between the potential predictor and SG. When the

assumption of equal slopes is not validated ( $p < 0.05$ ), separate treatment regression lines need to be estimated and then compared (Kutner et.al, 2005). This implies fitting the ANCOVA model with the interaction between the potential predictor and SG and then comparing the means of the urinary metabolite in each of the groups of the potential predictor at the 25th, 50th, and 75th percentile of the covariate SG. When the assumptions of normality and constant variance (for the ANCOVA model) were not satisfied then non-parametric methods were applied. Essentially this involves running the models on the ranks of the data. When the overall F-test for group differences of the potential predictor from ANCOVA models was significant ( $p < 0.05$ ), then pairwise comparisons were carried out using Scheffé correction for multiple comparisons to determine significant group differences. This ensures that the overall false positive rate from multiple comparisons is less than 0.05.

Statistical analysis was performed using SAS (Statistical Analysis System) Enterprise Guide 4.2 and R (R Core Development Team). For the censoring methods, we used functions from the R libraries NADA and SURVIVAL. Unless otherwise indicated, a 5% significance level ( $\alpha = 0.05$ ) was implemented throughout.

## **Results**

### **Participant characteristics**

Our participants tended to be well-educated, over 30 years of age, born in Canada, had never smoked, had an underweight or normal body mass index (BMI) prior to pregnancy and had a parity of 0 or 1 (see Supplemental Material, Table S1).

## **BPA**

Detectable urinary concentrations of free BPA, BPA glucuronide (BPA-G), and BPA mono-sulfate (BPA-MS) were found in 43.24%, 94.75%, and 23.43%, respectively of the samples (Table 1). We did not detect BPA disulfate in any samples. The GM urinary concentration for BPA-G using the machine readings was 0.80  $\mu\text{g/L}$  (95% CI: 0.75, 0.85), similar to the result using the censoring methods (Supplemental Material, Table S2). The GMs of the ratio of free BPA to total BPA was 1.01%, and the ratio of BPA-G to total BPA was 90.27% (data not shown).

The log of BPA-G was positively correlated with the log of free BPA ( $r=0.38$ ,  $p<0.0001$ ), and log of total BPA ( $r=0.98$ ,  $p<0.0001$ ) (data not shown). The log of free BPA was positively correlated with the log of total BPA ( $r=0.42$ ,  $p<0.0001$ ) (Supplemental Material, Figure S1a). As log total urinary BPA increased, free BPA also increased and the ratio of free to total BPA decreased ( $r= -0.22$ ,  $p<0.0001$ ) (Supplemental Material, Figure S1b).

In the analysis of characteristics associated with urinary concentrations, BPA-G (Table 2) and total BPA concentrations (Supplemental Material, Table S3) were significantly higher in the urine of women  $< 25$  years of age compared with women  $\geq 35$ , in smokers compared with former or never smokers, and in women with lower household income ( $< \$50,000$  vs.  $> \$100,000$ ) and lower education ( $\leq$  high school vs.  $\geq$  university degree). A significant interaction was observed between SG and time of urine collection; for example, at the 25<sup>th</sup> and 50<sup>th</sup> percentile of SG, urine voids after 3 p.m. had significantly higher BPA-G and total BPA than earlier in the day. However, at the 75<sup>th</sup> percentile of SG, adjusted geometric mean concentrations of BPA-G and

total BPA were similar with respect to time of urine collection (Table 3 and Supplemental Material, Table S4).

### **Triclosan**

Most of the women had detectable concentrations of total TCS, TCS glucuronide (TCS-G) and free TCS (Table 1). The geometric mean urinary concentration for total and TCS-G were 12.64 (95% CI: 11.38, 14.03) and 12.30 (95% CI: 11.08, 13.65)  $\mu\text{g/L}$ , respectively. The GMs of the ratio of free TCS to total TCS was 0.50 %, and the ratio of glucuronide to total TCS was 97.82% (data not shown).

All the log-transformed triclosan metabolites were highly correlated with each other (data not shown). TCS-G was correlated with free TCS ( $r=0.81$ ,  $p<0.0001$ ), and total TCS ( $r=1.00$ ,  $p<0.0001$ ). Similarly, free TCS was correlated with total TCS ( $r=0.82$ ,  $p<0.0001$ ).

Women had significantly lower urinary concentrations of total TCS (Supplemental Material, Table S5) and TCS-G (Table 4) if they were smokers vs. never smokers, <25 vyears of age, did not have a university degree, and  $\leq \$100,000$  household income. Only free TCS varied by time of urine collection, with urine collected between 9 a.m. and noon significantly higher than between 3 and 6 p.m. A significant interaction between specific gravity and parity was observed. Specifically at the 25<sup>th</sup> and 50<sup>th</sup> percentiles of SG, TCS levels were statistically similar between the different parity groups; however, at the 75<sup>th</sup> percentile of SG, adjusted geometric mean TCS concentrations were higher in parity 0 than parity 1 (Table 5 and Supplemental Table S6).

Comparing the two methods for dealing with values < LOD, the median from Kaplan-Meier was similar to the median based on machine readings, and the GM from censored MLE compared

well with the GM based on machine readings (Table 1 and Supplemental Material, Table S2). Overall, p-values for the censoring methods were similar to machine readings, but generally more conservative (data not shown).

## **Discussion**

Given potential health concerns of prenatal exposure to BPA and TCS on the developing infant and the physiological and behavioral changes during pregnancy that can affect exposure to and disposition of chemicals in the body (Moya et al. 2014), it is critical to have biomonitoring data on a large diverse population of pregnant women for the critical window of development. Furthermore, data on the free and conjugated compounds will contribute to understanding the toxicokinetics and potential health risks of the biologically active compounds.

Total BPA urinary concentrations (unadjusted for urine dilution) have been measured in several cohorts of pregnant women in the US with medians ranging from 1.0 - 2.0  $\mu\text{g/L}$  (Braun et al. 2011; Harley et al. 2013; Hoepner et al. 2013; Mortensen et al. 2014; Wolff et al. 2008), somewhat higher than has been reported in MIREC (0.89  $\mu\text{g/L}$ ). Urinary concentrations of BPA in US women  $\geq 20$  years of age (NHANES 2009-2010) (CDC 2013) were higher at the 50<sup>th</sup> (1.8 vs. 0.9  $\mu\text{g/L}$ ) and 95<sup>th</sup> (9.6 vs. 6.2  $\mu\text{g/L}$ ) percentiles than in MIREC (2008-2011). A previous comparison of US and Canadian urinary concentrations of BPA in the general population was not able to identify any methodological differences to explain the statistically significant lower levels in Canada (Lakind et al. 2012), which might suggest that there are population differences (e.g., consumer product formulations or avoidance of BPA-containing products) in Canada.

Only one study has reported urinary concentrations of free BPA in pregnant women; however, contamination of the samples from exogenous sources during collection likely occurred (Vandentorren et al. 2011). Free BPA has been measured in the urine of non-pregnant adult populations with widely varying detection rates of  $\leq 10\%$  (Ye et al. 2005; Völkel et al. 2008; Fox et al. 2011) to  $> 70\%$  (Schöringhumer and Cichna-Markl 2007; Liao and Kannan 2012). The comparable figure in our study was 43%.

A few studies have measured the conjugated forms of BPA in adult populations (e.g., Kim et al. 2003; Ye et al. 2005; Liao and Kannan 2012). In a study of 163 subjects in France, the geometric mean urinary concentration of BPA-G was 4.64  $\mu\text{g/L}$  (corresponding to 2.62  $\mu\text{g/L}$  BPA) which represented 79% of urinary total BPA (Harthé et al. 2012). The geometric mean concentration of BPA-G in our study was considerably lower at 0.80  $\mu\text{g/L}$  with a ratio of BPA-G to total BPA of 90%,

There are a number of possible explanations for differences between studies in levels or proportions of free and total BPA, including disparities in the laboratory methods and their sensitivity, differences in the study populations, hydrolysis of the conjugates and contamination of the samples. While free BPA has been measured in several studies (reviewed in Vandenberg et al. 2010), others have argued that the detection of free BPA was due to contamination of the sample or deconjugation of the BPA-G during storage or sample preparation (Dekant and Völkel, 2008; Teeguarden et al. 2011). In our study, because derivatization with dansyl chloride is the first step of our analytical procedure for free BPA and TCS determination, contamination along the subsequent steps of sample preparation by the free forms of these ubiquitous phenols is prevented (Provencher et al. 2014). Furthermore, our in-house-reference materials did not show

any decrease in conjugate concentrations after several months of storage at  $-20^{\circ}\text{C}$  and our field blanks and pre-screening of collection materials did not provide any evidence of contamination with BPA. Therefore we believe we have minimized as much as possible potential sources of external contamination that could artifactually inflate urinary concentrations of free BPA in our participants. It should be noted however, that on average only about 1% of the total BPA was present in the unconjugated form and that the median free BPA was at the method's limit of detection.

There are some inconsistencies among studies identifying major predictors of urinary BPA concentrations in pregnant women. For example, while our study and one in Spain (Casas et al. 2013) found higher BPA concentrations in younger women, studies in Puerto Rico (Meeker et al. 2013), and the US (Braun et al. 2011; Quirós-Alcalá et al. 2013; Robledo et al. 2013) reported no significant associations with maternal age. Smoking and low education ( $< 12$  years) were significant predictors of higher BPA concentrations in MIREC and in studies in Cincinnati ( $n = 388$ ) (Braun et al. 2011) and Spain ( $n = 479$ ) (Casas et al. 2013), but not in studies in California ( $n = 470$ ) (Quirós-Alcalá et al. 2013), New York City ( $n = 568$ ) (Hoepner et al. 2013), Puerto Rico ( $n = 105$ ) (Meeker et al. 2013) or Korea ( $n = 757$ ) (Lee et al. 2014).

Similarly to BPA, the predominant TCS metabolite measured in MIREC was the glucuronide form; however, free TCS was detected in about 80% of the urine samples. The median ratio of free to total TCS (0.57%) was comparable to that reported following oral ingestion of TCS in 10 volunteers, where 0.9% of the TCS excreted was reportedly in the free form 24-48 h after exposure (Sandborgh-Englund et al. 2006).

A handful of studies have measured total triclosan in the urine of pregnant women (see Supplemental Material, Table S7). Median urinary concentrations were considerably higher in studies conducted in Puerto Rico (Meeker et al. 2013) and France (Philippat et al. 2012) than in California (California 2013), New York City (Wolff et al. 2008; Philippat et al. 2013), Spain (Casas et al. 2011), or MIREC. Regional and population differences may be related to the use and/or availability of consumer products containing TCS. There was no significant difference between median urinary TCS concentrations in US (NHANES 2009-2010) (CDC 2013) or Canadian women (CHMS 2009-2011) (Health Canada 2013) (11.1 and 16  $\mu\text{g/L}$ , respectively) and MIREC (8.7  $\mu\text{g/L}$ ).

Data on major predictors of TCS exposure are limited. In MIREC, urinary TCS concentrations were significantly elevated in women with a university degree vs. not, > \$100,000 vs.  $\leq$  \$100,000 household income,  $\geq 25$  vs. < 25 years of age and who had never smoked vs. former or current smokers. In the US general adult population, TCS concentrations were significantly higher in households with  $\geq$  \$20,000 and appeared to peak in the third decade of life and then decline slowly thereafter (Calafat et al. 2008b). In Puerto Rico, maternal urinary TCS was higher in older pregnant women (>30 years) but was not associated with maternal education or income (Meeker et al. 2013).

A major concern in conducting biomonitoring studies is potential contamination of the biospecimen by materials and processes (Longnecker et al. 2013; Ye et al. 2013; Salgueiro-González et al. 2012) resulting in higher measured concentrations than were actually present. Alternatively, concentrations may be artifactually reduced if for example, free TCS adheres to the collection containers or other materials (Provencher et al. 2014). In MIREC, the ratio of free

to total TCS varied from a minimum of less than 1% to 82.5%, indicating that this was likely not a consistent problem. The use of field and laboratory blanks and pre-screening of collection materials can assist with identifying potential sources of contamination, as was done in this research and found not to be a concern.

Consideration of the ability of a single spot urine sample to predict an individual's exposure over a period of time is especially important for short-lived chemicals such as BPA and TCS. Previous studies have indicated that while the intra-class correlation coefficient (ICC) for BPA is low ( $< 0.25$ ) across pregnancy (Braun et al. 2011; Meeker et al. 2013; Philippat et al. 2013; Fisher et al. 2014), the ICC for TCS is better ( $> 0.47$ ) (Meeker et al. 2013; Philippat et al. 2013; Bertelsen et al. 2014), so the results presented here should be interpreted with this in mind.

In conclusion, this study is one of only a few to measure the extent of exposure to free and conjugated forms of BPA and TCS and especially during the critical period of pregnancy. Our median concentrations were lower than the estimated biomonitoring equivalents (24-h average concentrations that are consistent with an existing health-based exposure guideline such as a reference dose or tolerable daily intake) for total BPA (1 – 2 mg/L) (Krishnan et al. 2010a) and total triclosan (2.6 – 6.4 mg/L) (Krishnan et al. 2010b) in urine.

Maternal age, household income, education and smoking status were significant predictors for both total BPA and TCS exposure; however in opposite directions. The consumer products used by individuals (and thus their exposure to various chemicals) can vary by age and education (Barrett et al. 2014; Biesterbos et al. 2013; Wu et al. 2010) and possibly by culture or ethnicity. These data will be important in assessing potential risks of these chemicals and developing profiles of exposure, particularly in identifying women with elevated exposures.

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**Table 1.** Summary of first trimester urinary concentrations of bisphenol A (BPA) and triclosan (TCS), volumetric and specific gravity (SG) adjusted ( $\mu\text{g}$  equivalents/L). (n = 3 missing SG).

Contaminant	N	LOD	%<LOD	Min	Machine Readings <sup>b</sup>						
					GM <sup>a</sup>	95% Confidence Interval		5th Percentile	Median	95th Percentile	Max
						Lower	Upper				
BPA disulfate	1890	0.47	100	ND	0.01	0.01	0.01	0.01	0.01	0.01	0.36
SG-adjusted	1887			ND	0.01	0.01	0.01	0.00	0.01	0.04	0.61
BPA glucuronide	1889	0.11	5.2	ND	0.80	0.75	0.85	0.11	0.84	6.07	136.15
SG-adjusted	1886			ND	0.91	0.86	0.95	0.24	0.87	4.65	252.86
BPA free	1885	0.012	56.8	ND	0.01	0.01	0.01	0.00	0.01	0.15	2.82
SG-adjusted	1882			ND	0.01	0.01	0.01	0.00	0.01	0.15	2.52
BPA mono-sulfate	1885	0.03	76.6	ND	0.00	0.00	0.00	0.00	0.01	0.12	1.79
SG-adjusted	1882			ND	0.00	0.00	0.00	0.00	0.01	0.11	2.25
Total BPA	1879	0.47	30.8	ND	0.88	0.83	0.93	0.13	0.89	6.19	137.82
SG-adjusted	1876			ND	1.00	0.96	1.04	0.29	0.92	4.78	255.95
Total TCS	1861	0.12	0.6	ND	12.64	11.38	14.03	0.43	8.74	697.58	6784.3
SG-adjusted	1858			ND	14.36	13.01	15.85	0.72	9.18	571.10	4199.8
TCS glucuronide	1868	0.12	0.6	ND	12.30	11.08	13.65	0.43	8.42	676.47	6760.29
SG-adjusted	1865			ND	13.94	12.63	15.40	0.70	9.03	559.97	4184.94
TCS free	1882	0.008	20.0	ND	0.06	0.05	0.07	0.00	0.06	7.70	784.47
SG-adjusted	1879			ND	0.07	0.06	0.08	0.00	0.06	5.74	329.34
TCS sulfate	1890	0.02	85.3	ND	0.00	0.00	0.00	0.00	0.00	0.08	4.26
SG-adjusted	1887			ND	0.00	0.00	0.00	0.00	0.00	0.06	3.69
% free/total BPA	1879		56.8	0.0006	1.01	0.93	1.10	0.03	1.18	12.25	63.47
% free/total TCS	1861		20.0	0.0006	0.50	0.46	0.54	0.01	0.58	6.17	82.5

ND – Estimate below the laboratory’s limit of detection.

<sup>a</sup>Geometric Mean. <sup>b</sup>Some statistics are below the laboratory method’s limit of detection and should be interpreted with caution.

**Table 2.** Predictors of maternal urinary concentrations of free and conjugated bisphenol A (BPA) (reported as  $\mu\text{g}$  BPA equivalents/L) including specific gravity as a covariate.

Characteristic	N	BPA Glucuronide				BPA Free			
		% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>	% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>
Maternal Age									
<25	125	0.80	0.0013	A	1.06 (0.88, 1.28)	39.52	<0.0001	A	0.02 (ND, 0.02)
25-29	441	4.76		AB	0.83 (0.75, 0.91)	52.50		AB	ND (ND, ND)
30-34	682	5.43		AB	0.83 (0.76, 0.90)	57.27		B	ND (ND, ND)
>=35	641	6.24		B	0.72 (0.66, 0.78)	62.50		C	ND (ND, ND)
Parity									
0	834	4.68	0.0816		0.85 (0.79, 0.92)	55.10	0.0774 <sup>d</sup>		ND (ND, ND)
1	765	6.27			0.78 (0.72, 0.84)	58.19			ND (ND, ND)
2+	288	4.17			0.73 (0.65, 0.83)	57.49			ND (ND, ND)
Maternal Smoking Status									
Current/quit during pregnancy	225	3.11	<0.0001 <sup>d</sup>	A	1.01 (0.88, 1.16)	45.33	0.0056	A	0.01 (ND, 0.02)
Former	521	4.03		B	0.82 (0.75, 0.90)	59.92		B	ND (ND, ND)
Never	1141	6.22		B	0.76 (0.71, 0.81)	57.59		B	ND (ND, ND)
Maternal Education									
High school or less	168	4.17	0.0068 <sup>d</sup>	A	0.90 (0.76, 1.06)	42.86	<0.0001 <sup>d</sup>	A	0.01 (ND, 0.02)
College courses or diploma	540	4.63		AB	0.83 (0.75, 0.90)	51.49		A	ND (ND, ND)
University degree	1179	5.68		B	0.78 (0.73, 0.83)	61.09		B	ND (ND, ND)
Place of Birth									
Elsewhere	353	6.80	0.0029 <sup>d</sup>	A	0.70 (0.63, 0.79)	60.23	0.5268		ND (ND, ND)
Canada	1536	4.88		B	0.83 (0.78, 0.87)	55.97			ND (ND, ND)
Pre-pregnancy BMI (kg/m <sup>2</sup> )									
< 25 (underweight-normal)	1108	5.60	0.2206 <sup>d</sup>		0.78 (0.73, 0.83)	62.90	<0.0001	A	ND (ND, ND)
25 – 29 (overweight)	384	6.25			0.81 (0.73, 0.90)	50.91		B	ND (ND, ND)
≥ 30 (obese)	261	3.83			0.84 (0.73, 0.96)	42.53		B	0.01 (ND, 0.02)
Household Income									
≤ \$50,000	326	3.68	0.0045 <sup>d</sup>	A	0.89 (0.79, 1.01)	45.23	<0.0001	A	0.01 (ND, 0.02)
\$50,001 – 100,000	754	5.17		AB	0.82 (0.76, 0.88)	55.38		B	ND (ND, ND)
> \$100,000	726	5.79		B	0.76 (0.70, 0.82)	63.90		C	ND (ND, ND)

Characteristic	N	BPA Glucuronide				BPA Free			
		% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>	% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>
Season Urine Collected									
Fall	547	4.94	0.0720 <sup>d</sup>		0.80 (0.73, 0.87)	60.81	0.0002	A	ND (ND, ND)
Winter	456	4.17			0.84 (0.76, 0.93)	61.95		A	ND (ND, ND)
Spring	442	7.01			0.73 (0.66, 0.81)	53.17		B	ND (ND, ND)
Summer	444	4.95			0.85 (0.76, 0.93)	50.11		B	ND (ND, ND)
Fasting Status									
No	1827	5.36	0.7425		0.80 (0.76, 0.84)	57.05	0.0037	A	ND (ND, ND)
Yes	37	0.00			0.85 (0.60, 1.20)	45.95		B	0.02 (ND, 0.04)
Time Since Last Urination (min.)									
≤ 75	488	7.58	0.0020	A	0.76 (0.69, 0.84)	65.23	<0.0001 <sup>d</sup>	A	ND (ND, ND)
76 - 120	596	6.21		A	0.74 (0.67, 0.80)	59.26		AB	ND (ND, ND)
121 - 170	266	2.26		B	0.98 (0.86, 1.11)	51.50		BC	ND (ND, ND)
> 170	446	2.69		AB	0.85 (0.77, 0.94)	45.29		C	0.01 (0.01, 0.02)

ND: less than the limit of detection (LOD). Although values generated by the laboratory instrument were available and were subsequently used in the analysis, when the point estimate fell below the limit of detection it was reported as ND in the table.

<sup>a</sup>p-value for overall group effect based on machine readings. <sup>b</sup>Pairwise comparisons were only made when the overall group effect was significant (p<0.05). Group levels with the same letter indicate no significant difference using Scheffé multiple comparisons. Groups with different letters indicate significant differences in contaminant levels. <sup>c</sup>GM: geometric mean; 95% CI: the 95% confidence intervals for the GM were corrected for multiple comparisons using Scheffé correction. <sup>d</sup>ANCOVA model based on the ranks of the data (non-parametric ANCOVA). Each group is assumed to have the same slope with respect to the covariate [i.e., the interaction between the potential predictor (characteristic) and specific gravity is not significant (p>0.05)].

**Table 3.** Comparisons of maternal urinary concentrations of free and conjugated bisphenol A (BPA) (reported as  $\mu\text{g}$  BPA equivalents/L) at the various times of urine collection, with respect to different levels of specific gravity.

Chemical	Characteristic	Grouping of characteristic	P25 of SG <sup>a</sup>		P50 of SG <sup>a</sup>		P75 of SG <sup>a</sup>	
			Differences	Adjusted Geometric Mean (95% CI)	Differences	Adjusted Geometric Mean (95% CI)	Differences	Adjusted Geometric Mean (95% CI)
BPA Glucuronide <sup>b</sup>	Time of Urine Collection	6:00 - 9:00	AB	0.27 (0.15, 0.48)	AB	0.62 (0.41, 0.94)		1.65 (1.03, 2.62)
		9:00 - 12:00	A	0.34 (0.31, 0.37)	A	0.66 (0.62, 0.71)		1.44 (1.30, 1.60)
		12:00 - 15:00	A	0.34 (0.30, 0.38)	A	0.69 (0.63, 0.75)		1.56 (1.41, 1.73)
		15:00 - 18:00	B	0.57 (0.48, 0.67)	B	0.94 (0.84, 1.06)		1.72 (1.52, 1.94)
		18:00 - 24:00	AB	0.73 (0.41, 1.29)	AB	1.21 (0.81, 1.80)		2.18 (1.51, 3.15)
BPA Free <sup>b</sup>	Time of Urine Collection	6:00 - 9:00		0.01 (0.00, 0.01)	AB	0.01 (0.00, 0.01)	ABC	0.01 (0.00, 0.02)
		9:00 - 12:00		0.01 (0.01, 0.01)	A	0.01 (0.01, 0.01)	A	0.02 (0.01, 0.02)
		12:00 - 15:00		0.01 (0.00, 0.01)	B	0.01 (0.01, 0.01)	AB	0.01 (0.01, 0.02)
		15:00 - 18:00		0.01 (0.00, 0.01)	B	0.01 (0.01, 0.01)	C	0.01 (0.01, 0.01)
		18:00 - 24:00		0.01 (0.00, 0.02)	AB	0.01 (0.00, 0.01)	BC	0.01 (0.00, 0.01)

<sup>a</sup>P25 of SG is the 25th percentile of specific gravity = 1.007; P50 of SG is the 50th percentile of specific gravity = 1.013; P75 of SG is the 75th percentile of specific gravity = 1.020. <sup>b</sup>ANCOVA model, assuming separate slopes for each of the groups of the characteristic, since the interaction between time of urine collection and SG was significant  $p < 0.05$ . Pairwise comparisons are made at each level of SG only when the overall difference between the characteristic was significant at that level of SG. Therefore, where no pairwise comparisons were made, there was no significant difference between the groups of the characteristic.

**Table 4.** Predictors of maternal urinary concentrations of free and conjugated triclosan (TCS) (reported as µg TCS equivalents/L) with specific gravity as a covariate.

Characteristic	N	TCS Glucuronide				Free TCS			
		% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>	% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>
Maternal Age									
<25	122	0.81	<0.0001 <sup>d</sup>	A	5.11 (3.48, 7.51)	17.89	0.1173		0.04 (0.02, 0.06)
25-29	437	0.23		B	13.28 (10.83, 16.29)	17.50		0.07 (0.06, 0.09)	
30-34	672	0.89		B	13.08 (11.09, 15.42)	20.67		0.07 (0.05, 0.08)	
>=35	630	0.47		B	12.93 (10.91, 15.33)	21.51		0.06 (0.05, 0.07)	
Maternal Smoking Status									
Current/quit during pregnancy	220	0.45	0.0030	A	8.55 (6.41, 11.42)	20.09	0.0236 <sup>d</sup>	AB	0.05 (0.03, 0.07)
Former	515	0.97		AB	10.82 (8.96, 13.06)	22.59		A	0.05 (0.04, 0.06)
Never	1124	0.44		B	13.97 (12.30, 15.87)	18.89		B	0.07 (0.06, 0.09)
Maternal Education									
High school or less	163	0.60	0.0001	A	7.34 (5.25, 10.28)	13.94	0.1232 <sup>d</sup>		0.05 (0.04, 0.08)
College courses or diploma	530	0.38		A	10.31 (8.57, 12.41)	22.72		0.06 (0.04, 0.07)	
University degree	1166	0.69		B	14.33 (12.64, 16.24)	19.61		0.07 (0.06, 0.08)	
Place of Birth									
Elsewhere	347	0.86	0.3025		11.01 (8.75, 13.86)	21.02	0.8749 <sup>d</sup>		0.06 (0.05, 0.08)
Canada	1514	0.53			12.60 (11.28, 14.06)	19.80		0.06 (0.05, 0.07)	
Pre-pregnancy BMI (kg/m <sup>2</sup> )									
< 25 (underweight-normal)	1089	0.55	0.7914 <sup>d</sup>		11.80 (10.37, 13.43)	22.76	0.0002 <sup>d</sup>	A	0.05 (0.04, 0.06)
25 – 29 (overweight)	383	0.26			12.29 (9.88, 15.29)	19.79		AB	0.07 (0.05, 0.09)
≥ 30 (obese)	258	0.39			12.44 (9.52, 16.26)	10.38		B	0.11 (0.08, 0.15)
Household Income									
≤ \$50,000	318	0.31	<0.0001	A	9.27 (7.31, 11.76)	16.51	0.1950		0.06 (0.05, 0.09)
\$50,001 – 100,000	743	0.40		A	10.69 (9.15, 12.50)	20.61		0.05 (0.05, 0.07)	
> \$100,000	717	0.84		B	16.07 (13.70, 18.85)	21.10		0.07 (0.06, 0.09)	
Season Urine Collected									
Fall	540	0.19	0.3043	A	14.16 (11.77, 17.04)	20.66	0.6682 <sup>d</sup>		0.06 (0.05, 0.08)
Winter	448	0.44		A	12.24 (10.00, 14.98)	21.41		0.05 (0.04, 0.07)	
Spring	436	0.91		B	11.21 (9.14, 13.76)	20.09		0.07 (0.05, 0.09)	
Summer	437	0.92		B	11.34 (9.23, 13.92)	17.79		0.07 (0.05, 0.09)	
Fasting Status									
No	1799	0.61	0.2239		12.27 (11.09, 13.57)	20.38	0.6824 <sup>d</sup>		0.06 (0.05, 0.07)

Characteristic	N	TCS Glucuronide				Free TCS			
		% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>	% <LOD	p-value <sup>a</sup>	Pairwise <sup>b</sup>	GM (95% CI) <sup>c</sup>
Yes	37	0.00			7.88 (3.89, 15.97)	8.11			0.08 (0.03, 0.19)
Time of Urine Collection									
6:00 - 9:00	28	3.57	0.4257		13.71 (6.09, 30.85)	14.29	0.0002 <sup>d</sup>	AB	0.09 (0.03, 0.24)
9:00 - 12:00	797	0.63			13.66 (11.73, 15.92)	20.25		A	0.09 (0.07, 0.10)
12:00 - 15:00	635	0.63			11.68 (9.85, 13.85)	20.28		AB	0.06 (0.05, 0.07)
15:00 - 18:00	364	0.27			10.83 (8.64, 13.57)	20.22		B	0.04 (0.03, 0.05)
18:00 - 24:00	35	0.00			9.62 (4.70, 19.70)	11.43		AB	0.04 (0.01, 0.09)
Time Since Last Urination (min.)									
≤ 75	480	1.04	0.1182		10.20 (8.38, 12.40)	27.72	<0.0001	A	0.04 (0.03, 0.05)
76 - 120	593	0.67			13.34 (11.19, 15.90)	21.48		B	0.07 (0.05, 0.08)
121 - 170	262	0.38			14.18 (10.88, 18.47)	16.23		B	0.07 (0.05, 0.10)
> 170	438	0.00			13.28 (10.82, 16.31)	10.61		B	0.10 (0.07, 0.12)

<sup>a</sup>p-value for overall group effect using machine readings. <sup>b</sup>Pairwise comparisons were only made when the overall group effect was significant ( $p < 0.05$ ). Group levels with the same letter indicate no significant difference using Scheffé multiple comparisons. Groups with different letters indicate significant differences in contaminant levels. <sup>c</sup>GM: geometric mean; 95% CI: the 95% confidence intervals for the GM was corrected for multiple comparisons using Scheffé correction. <sup>d</sup>ANCOVA model based on the ranks of the data (non-parametric ANCOVA). Each group is assumed to have the same slope with respect to the covariate [i.e., the interaction between the potential predictor (characteristic) and specific gravity is not significant ( $p > 0.05$ )].

**Table 5.** Comparisons of maternal urinary concentrations of free and conjugated triclosan (TCS) (reported as  $\mu\text{g}$  TCS equivalents/L) at the various parity groups, with respect to different levels of specific gravity.

Chemical	Characteristic	Grouping of Characteristic	P25 of SG <sup>a</sup>		P50 of SG <sup>a</sup>		P75 of SG <sup>a</sup>	
			Differences	Adjusted Geometric Mean (95% CI)	Differences	Adjusted Geometric Mean (95% CI)	Differences	Adjusted Geometric Mean (95% CI)
Triclosan Glucuronide <sup>b</sup>	Parity	0		6.25 (5.13, 7.60)		12.30 (10.60, 14.28)	A	27.13 (22.20, 33.14)
		1		6.98 (5.64, 8.63)		10.93 (9.33, 12.80)	B	18.43 (15.22, 22.32)
		2+		5.15 (3.61, 7.35)		9.53 (7.35, 12.37)	AB	19.53 (14.30, 26.67)
Free Triclosan <sup>c</sup>	Parity	0		0.02 (0.01, 0.02)		0.06 (0.05, 0.07)	A	0.26 (0.20, 0.33)
		1		0.02 (0.02, 0.03)		0.05 (0.04, 0.06)	B	0.15 (0.12, 0.19)
		2+		0.02 (0.01, 0.02)		0.04 (0.03, 0.06)	B	0.16 (0.11, 0.23)

<sup>a</sup>P25 of SG is the 25th percentile of specific gravity = 1.007; P50 of SG is the 50th percentile of specific gravity = 1.013; P75 of SG is the 75th percentile of specific gravity = 1.020. <sup>b</sup>ANCOVA model, assuming separate slopes for each of the groups of the characteristic, since the interaction between parity and SG was significant  $p < 0.05$ . Pairwise comparisons were made at each level of SG only when the overall difference between the groups of the characteristic was significant at that level of SG. Therefore where no pairwise comparisons are made there was no significant difference between the groups of the characteristic. <sup>c</sup>ANCOVA model based on the ranks of the data (non-parametric) model, assuming separate slopes for each of the groups of the characteristic, since the interaction between parity and SG was significant  $p < 0.05$ . Pairwise comparisons were made at each level of SG only when the overall difference between the groups of the characteristic was significant at that level of SG. Therefore, where no pairwise comparisons are made, there was no significant difference between the groups of the characteristic.