Urinary Triclosan Concentrations during Pregnancy and Birth Outcomes

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Thesis

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Preface and Acknowledgements

This thesis is the start of my interest in environmental epidemiology and children's environmental health. I hope to continue a career that will allow me to better understand how the environment impacts our health, and how we can have healthier children.

I would like to thank Dr. Joseph M. Braun for the support as my advisor and for encouraging, and for sharing his enthusiasm for children's environmental health research.

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Introduction

Triclosan is an antimicrobial chemical that is widely used in some toothpastes, mouthwashes, soaps, cosmetics, lotions, textiles, toys, and kitchenware.[1] Exposure is ubiquitous among pregnant women in the United States [2-4]. The primary routes of exposure are oral and dermal, with the highest exposures hypothesized to be from dermally applied products.[5] Oral care products, including some toothpastes, are another major source of exposure, particularly among children.[5] The U.S. Food and Drug Administration banned over-the-counter consumer wash products that contain triclosan in September of 2016; however other potential sources of triclosan, such as some body lotions and toothpastes, remain in use.[6]

Triclosan may disrupt the actions within the hypothalamic-pituitary-thyroid axis.[7] Triclosan shares structural similarities with thyroid hormones, and triclosan exposure reduces thyroxine concentrations in pregnant, fetal, and juvenile rats.[8-10] Of particular concern is the potential for triclosan to disrupt thyroid hormone homeostasis during fetal development because thyroid hormones play a critical role in fetal growth and neurodevelopment; reduced thyroxine levels during gestation can have negative impacts on the developing fetus.[11-13]

Triclosan has been associated with adverse birth outcomes in epidemiological studies. Maternal prenatal urinary triclosan concentrations have been inversely associated with newborn's head circumference, but not with weight or length at birth.[14,15] Another study found that prenatal urinary triclosan concentrations were inversely associated with birth weight and length among newborn boys, but not with head circumference.[3] However, prior studies were limited by having only one measure of prenatal urinary triclosan during pregnancy and this may have resulted in exposure misclassification.

The objective of this study was to investigate the relationship of prenatal urinary triclosan concentrations at 16 and 26 weeks of pregnancy with birth weight, length, head circumference, and gestational duration in a prospective pregnancy and birth cohort. Based on previous animal and epidemiologic studies, we hypothesized that urinary triclosan concentrations would be inversely associated with neonatal anthropometry and gestational duration.

Materials and Methods

Study Participants

We used data collected from women and children enrolled in The Health Outcomes and Measures of the Environment (HOME) Study, an ongoing prospective pregnancy and birth cohort in the greater Cincinnati, Ohio metropolitan area. The HOME Study was designed to investigate the impact of exposure to common environmental contaminants on child health and development. We previously described details of the study.[16] Briefly, from March 2003 to January 2006 we recruited women living in the greater Cincinnati, Ohio metropolitan area who were: 18 years or older and 16±3 weeks of gestation, spoke English, lived in a home built before 1978, and had no history of HIV infection or other medical conditions such as diabetes, bipolar disorder, schizophrenia or cancer that resulted in radiation treatment or chemotherapy. Of the 1,263 eligible women we approached, 468 (37%) agreed to participate in our study. Of these, 67 dropped out before delivery leaving 401 women who delivered 389 singletons, 3 stillbirths, and 9 sets of twins.

The Cincinnati Children's Hospital Medical Center (CCHMC) and participating delivery hospitals' Institutional Review Boards (IRB) approved the HOME Study. After research assistants explained study protocols, all women provided written informed consent for themselves and their children. Brown University relinquished IRB authority to CCHMC through an Interagency Agreement. The Centers for Disease Control and Prevention also relied on CCHMC IRB and determined that their involvement did not constitute human subjects research.

Urinary Triclosan Biomarkers

Women provided two urine samples at an average of 16.0 (range: 10.4-22.6) and 26.5 (range: 19.1-34.6) weeks of gestation. We collected urine in polypropylene containers and stored it at -20^{o}C until we shipped it to the Centers for Disease Control and Prevention for analysis. We measured total (conjugated + free) urinary triclosan concentrations using online solid phase extraction coupled with high performance liquid chromatography-isotope dilution tandem mass spectrometry as previously described.[17] Concentrations below the limit of detection (LOD) of 2.3 ng/mL were given a value of LOD/√2 for the statistical analysis. Urinary triclosan concentrations were creatinine-standardized (µg/g creatinine) to control for individual variation in urine dilution. Because creatinine-standardized urinary triclosan concentrations were right skewed, we $\log_{10}$-transformed them to reduce the influence of extreme observations before taking the mean of the 16 and 26-week $\log_{10}$-transformed creatinine-standardized urinary triclosan concentrations. Ninety-four percent of women had triclosan measurements at 16 and 26 weeks; for those who had only one measure, we used that in place of the mean.

Birth Outcomes

We extracted birth weight, length, and head circumference, gestational duration, and the method for determining gestational age (last menstrual period, n=368; antenatal ultrasound, n=6; or Ballard Maturational Assessment, n=3) from newborn medical charts. We calculated sex and gestational age standardized birth weight z-scores using United States Natality datasets.[18]

Covariates

We considered adjusting for potential confounders that might be associated with both gestational triclosan concentrations and fetal growth or gestational duration using a directed acyclic graph (DAG) (Supplemental Figure S1). We collected information about sociodemographic factors including maternal race and ethnicity, age, education, marital status, and household income using a computer-assisted questionnaire administered by trained research staff during the second trimester of pregnancy. Depressive symptoms were measured with the Beck Depression Inventory (BDI-II) at 20 weeks of pregnancy.[19] We measured perinatal factors, including delivery method, parity, weight at 16 weeks gestation, and prenatal vitamin use using standardized interviews or medical chart reviews. We assessed tobacco smoke exposure using the average of serum cotinine concentrations taken at 16 and 26 weeks of pregnancy. [20]

S*tatistical Analysis*

Of the 389 women in the HOME Study who gave birth to singletons, we excluded women who had offspring with congenital or chromosomal abnormalities (n=2), were missing the method used to determine gestational age (n=1), or were missing covariate data (n=8). This left 378 mother-neonate pairs for analysis of birth weight z-score and gestational age. Eight children were missing the head circumference measurements and nine children were missing the birth length measurements, leaving 370 and 369 mother-neonate pairs for the analysis of head circumference and birth length, respectively.

We started our statistical analysis by calculating the geometric mean of the average prenatal triclosan concentrations and the mean birth weight z-score by covariates. Next, we calculated intraclass correlation coefficients (ICCs) between the 16 and 26 week $\log_{10}$-transformed creatinine-standardized urinary triclosan concentrations to estimate the reproducibility of the repeated urinary triclosan concentrations.[21]

We then determined whether there were non-linear relationships between urinary triclosan concentrations and birth outcomes using restricted cubic splines.[22] Because we observed a linear association (non-linearity p-values>0.3), we estimated the unadjusted and adjusted difference in birth weight z-score, gestational age, birth head circumference, and birth length associated with a 10-fold increase in gestational urinary triclosan concentration using multivariable linear regression. We examined whether child sex modified the association between triclosan and birth outcomes by creating a multivariable model including all variables in the final model plus child sex and a product interaction term between triclosan concentrations and child sex. To enhance the interpretability of the results, we also examined the adjusted mean value in birth outcomes across quartiles of prenatal urinary triclosan concentrations. Finally, we used modified Poisson regression with robust standard errors to calculate the relative risk of low-birth weight (<2500 grams), preterm birth (<37 weeks gestation), and small for gestational age (SGA, <10^{th} birth weight z-score percentile) for a 10-fold increase in prenatal triclosan concentrations.[23] We used SAS version 9.4 (SAS Institute, Inc. Cary, NC) and R version 3.2 for statistical analysis.

Sensitivity Analysis

We conducted several sensitivity analyses to evaluate the robustness of our results: 1) We adjusted for a reduced set of covariates that were shown to be the most influential confounders; these covariates included maternal age, race, education, marital status, income, and cotinine concentrations; 2) Since gestational diabetes and hypertensive disorders during pregnancy can influence fetal growth (Supplemental Figure S2), we excluded women who had either (n=10 for gestational diabetes and n=30 for hypertension disorders);[24] 3) Due to differences in methods of measuring gestational age, we restricted our analysis to women who had gestational age measured by LMP (n=368); 4) Because exposure to other phenols may impact birth outcomes (Supplemental Figure S2)[3,14], we adjusted for urinary 2,4-dichlorophenol, 2,5-dichlorophenol, bisphenol A, benzophenone-3, methylparaben, propylparaben, and butylparaben concentrations. 5) We excluded women who were missing a urine sample at either 16 or 26 weeks to ensure that these women were not unduly influencing our results; 6) To identify potential periods of heightened vulnerability during gestation, we examined the difference in birth outcomes associated with a 10-fold increase in prenatal urinary triclosan concentration at 16 and 26 weeks separately; 7) To determine if our methods to adjust for urine dilution were sensitive to various assumptions, we conducted additional analyses employing six other urine dilution adjustment methods described by O'Brien et al. (2016). We also excluded women who had extreme creatinine values < 0.3 g/L or > 3.0 g/L (n=7).

Results

Mothers in the cohort were predominately white (62%), college-educated (56%), multiparous (55%), married (65%), and between the ages of 25 and 34 (60%) (Table 1).

Triclosan was detected in 91 and 83% of the 16 and 26 week urine samples, respectively (Supplemental Table S1). Median urinary triclosan concentrations were slightly higher at 16 weeks (17 ng/mL, range: <LOD-1,985 ng/mL) than at 26 weeks (13 ng/mL, range: <LOD-1,657 ng/mL) (Supplemental Figure S3, Supplemental Table S1). The median of the average prenatal urinary triclosan concentration was 16 ng/mL (range: <LOD-1,501 ng/mL). Repeated un-standardized (ICC=0.42, 95% CI: 0.33, 0.50) and creatinine-standardized (ICC=0.53; 95% CI: 0.45, 0.60) urinary triclosan concentrations at 16 and 26 weeks had fair reproducibility. While not statistically significant, geometric mean prenatal urinary triclosan concentrations were slightly higher among women who were >25 to 35 years of age, white, had a bachelor's degree or higher, married, had household income more than $40,000 a year, had minimal depressive symptoms, took prenatal vitamins weekly or daily, and were non-smokers, but all differences were not statistically significant (Table 1).

The mean (±standard deviation [SD]) birth weight, weight z-score, length, head circumference, and gestation duration were 3,378 grams (±618 grams), 0.06 standard deviation units (±1.0), 51 cm (±3.0), 34 cm (±1.8), and 39 weeks (±1.8 weeks), respectively. Among live born singleton infants, 33 (8.7%) were low birth weight (<2,500 grams), 19 (5.0%) were preterm (<37 weeks of gestation), and 31 (8.2%) were SGA. Average birth weight z-scores were significantly lower among women who were <25 years of age at delivery, non-Hispanic black, less educated, had lower household income, unmarried, nulliparous, lower BMI, rarely or never took prenatal vitamins, exposed to secondhand tobacco smoke or smokers, and not diagnosed with gestational diabetes (Table 1).

In unadjusted models, we observed weak inverse or null associations between prenatal urinary triclosan concentrations and all four outcomes (Table 2). After adjusting for covariates, prenatal urinary triclosan concentrations were inversely associated with the four birth outcomes, although some of the 95% CI's included the null value (Table 2, Supplemental Figure S4). Each 10-fold increase in triclosan was associated with an estimated 125-gram decrease (95% CI: -216, -33) in birth weight, 0.15 standard deviation decrease (95% CI: -0.30, 0.00) in birth weight z-score, 0.4-cm decrease (95% CI: -0.8, 0.1) in birth length, 0.3-cm decrease (95% CI: -0.5, 0.0) in head circumference, and 0.3-week decrease (95% CI: -0.6, -0.1) in gestational age. There was no evidence of effect modification by child sex; all sex x triclosan effect modification p-values were $\geq$0.51 (Table 2).

We observed monotonic decreases in mean birth weight across increasing prenatal urinary triclosan concentration quartiles, whereas mean birth length, head circumference, and gestational duration generally decreased across the first three quartiles and then slightly increased from the third to fourth quartiles (Figure 1, Supplemental Table S2). For example, neonates born to women in the 4^{th} quartile of triclosan concentrations had 0.26 SD units lower birth weight z-score (95% CI: -0.55, -0.02) than neonates born to women in the 1^{st} quartile. Neonates born to women in the 2^{nd}, 3^{rd}, and 4^{th} quartile of triclosan concentrations had a gestational age that was 0.1 weeks (95% CI: -0.6, 0.4), 0.6 weeks (95% CI: -1.1, 0.1), and 0.5 weeks (95% CI: -1.0, 0.1) smaller, respectively, than neonates born to women in the 1^{st} quartile.

Higher prenatal urinary triclosan concentrations were associated with a slight and imprecise increase in risk for low birth weight, preterm birth, and SGA (Table 3). After adjusting for covariates, there was a 40% higher risk (RR=1.4, 95% CI: 0.8, 2.4) of low birth weight, a 40% higher risk (RR=1.4, 95% CI: 0.9, 2.3) of preterm birth, and a 30% higher risk (RR=1.3, 95% CI: 0.9, 2.1) of being SGA for every 10-fold increase in prenatal urinary triclosan concentrations.

Sensitivity Analyses

In trying to identify which individual covariates were responsible for the observed negative confounding, we found an inverse relation between prenatal urinary triclosan concentrations and birth outcomes when individually adjusting for maternal age, race, education, marital status, household income, and serum cotinine concentrations during pregnancy (Supplemental Figure S5). The magnitude and precision of our results from an adjusted model that only included these covariates was not appreciably different than the model that included our full set of covariates (Supplemental Table S3). Adjusting for gestational duration attenuated the association of prenatal urinary triclosan concentrations with birth length, head circumference, and birth weight (Supplemental Table S3). When adjusting for gestational duration, each 10-fold increase in prenatal urinary triclosan concentrations was associated with a 0.1 cm (95% CI: -0.4, 0.3) decrease in birth length, a 0.1 cm (95% CI: -0.4, 0.3) decrease in head circumference, and a 50 gram (95% CI: -122, 21) decrease in birth weight (Supplemental Table S3). Excluding women with gestational diabetes or hypertension disorders, restricting the analysis to women with only LMP gestational duration measurements, excluding women who only had one prenatal urine sample, and adjusting for other phenols did not appreciably change the association between prenatal urinary triclosan concentrations and birth outcomes (Supplemental Table S3).

The associations between prenatal triclosan concentrations taken during the second and third trimester and birth outcomes were slightly attenuated and had greater precision than the association between average prenatal triclosan concentration and birth outcomes (Supplemental Table S4). For example, each 10-fold increase in 16 and 26-week triclosan concentrations was associated with a 0.12 (95% CI: -0.27, 0.03) and 0.12 (95% CI: -0.26, 0.02) SD-unit decrease in birth weight z-score, respectively.

The associations between prenatal urinary triclosan concentrations and birth outcomes were similar when we used different methods to adjust for urine dilution (Supplemental Table S5). Excluding women with extreme creatinine values did not change our results.

Discussion

Among 378 mother-infant pairs in the HOME Study, we found that higher urinary triclosan concentrations measured during pregnancy were associated with modest reductions in birth weight, length, head circumference, and gestational duration. In addition, higher gestational urinary triclosan concentrations were associated with a modestly elevated risk of low birth weight, preterm birth, and SGA. We found an attenuation of birth weight, length, and head circumference estimates when adjusting for gestational duration. However, gestational age is a strong predictor of fetal growth and our gestational age-adjusted estimates would be attenuated because we were adjusting for a causal intermediate.

Some of our results are consistent with previous epidemiological studies observing inverse associations between prenatal urinary triclosan concentrations and birth outcomes. [3,14,15] Similar to Wolff et al. (2008), we found an inverse association between prenatal urinary triclosan concentrations and birth length and weight. However, Wolff et al. (2008) only found this association among boys. In contrast, Lassen et al. (2016) and Philippat et al. (2014) did not find any association between prenatal urinary triclosan concentrations and birth weight and length. Consistent with our findings, Philippat et al. (2014) and Lassen et al. (2016) observed an inverse association between prenatal triclosan concentrations and head circumference at birth, but this was not observed by Wolff et al. (2008). Wolff et al. (2008) did not observe an association between prenatal triclosan concentrations and gestational age; Lassen et al. (2016) and Philippat et al. (2014) did not examine gestational age as one of their outcomes.

Differences in the results of this and previous studies could be explained by the number and timing of urine sample collections. We had two measures of urinary triclosan concentrations during the 2nd and 3rd trimesters of pregnancy. In contrast, all three previous studies only had one measure taken during either the 2nd or 3rd trimester. Two measurements may enhance the accuracy of gestational triclosan exposure assessment. Others have concluded that a single measure of urinary triclosan concentrations could result in non-differential exposure misclassification and this could explain the lack of associations in some previous studies.[25]

It does not appear that discrepancies in study results are related to differences in the central tendency of triclosan concentrations. For instance, Lassen at al. (2016) observed an inverse association between prenatal triclosan concentrations and birth weight, despite having the lowest median urinary triclosan concentrations (1.0 ng/mL) compared to the HOME Study (median=15 ng/mL) and Philippat et al. (2014) study (median=30 ng/mL). Inconsistencies could also be due to differences in the attributes of the study participant characteristics. For instance, Philippat et al. (2014) studied a cohort of women who had male infants.

Previous research suggests that prenatal triclosan exposure could affect several biological mechanisms involved in fetal growth. Triclosan may disrupt the homeostasis of thyroid hormones by reducing the availability of thyroxine through increased hepatic metabolism.[7,8,26] Several studies have shown that triclosan exposure reduces thyroxine concentrations in pregnant and fetal rats.[7,10,26,27] A decrease in thyroxine availability during pregnancy could be detrimental for the growth and development of the fetus since the fetal thyroid does not become functional until the 12th week of gestation, making the fetus exclusively dependent on maternal thyroxine during the first trimester.[11] Because thyroid hormones play a critical role in brain development during fetal development, triclosan exposure may adversely affect brain development and growth.[12,13,28]

This study has some potential limitations that should be considered. First, triclosan exposure may be misclassified due to the moderate within-person variability of urinary triclosan concentrations. Triclosan has a half-life <24 hours [29] and exposures may be episodic in nature. Thus, accurate exposure assessment can be challenging. However, a strength of our study is that we had two triclosan measurements during the second and third trimesters of pregnancy and >94% of women had a measure in both trimesters. Assuming non-differential exposure misclassification of our urinary triclosan biomarker, it is likely that our reported results would be attenuated towards the null.[30]

Another limitation of the present study is that a majority of women had gestational age measured by LMP instead of an ultrasound, which is a more accurate method of pregnancy dating. However, previous research has shown that LMP reasonably approximates gestational age obtained from an ultrasound in the first trimester.[31] Generalizability is a third potential limitation of this study because our cohort is predominantly white and college educated (Table 1). However, the average triclosan concentration is consistent with the nationally representative National Health and Nutrition Examination Survey (NHANES) triclosan concentrations (17 ng/mL) and we have no evidence to suggest that women in the HOME Study are more susceptible to the effects of triclosan.[4,32] Fourth, different methods of adjusting for urine dilution could also result in exposure misclassification. Previous research based on simulations has shown that not adjusting for urine dilution introduces more bias compared to other statistical methods of urine dilution adjustment.[33] However, we used several different methods to adjust for urine dilution and our results did not vary by the method of adjustment we used.

Finally, while we adjusted for many potential confounders, there is the possibility of residual confounding from other factors associated with both triclosan exposure and fetal growth or gestational duration. We observed that the relationship between urinary triclosan concentrations and neonatal size or gestational duration was negatively confounded by sociodemographic factors.[34] Women who were white, older, more educated, married, and had a higher household income had higher urinary triclosan concentrations, and their infants had better birth outcomes compared to other women. This finding, in particular the association between household income and triclosan concentrations, is consistent with results from NHANES.[32] Given the observed negative confounding by sociodemographic factors in these data, the presence of any residual negative confounding from other unmeasured sociodemographic factors suggests that the results presented here would be biased towards the null. However, there is still a possibility of positive confounding from factors that are associated with poor birth outcomes and higher triclosan exposure. It is also important to consider other chemical exposures correlated with triclosan and birth outcomes as potential confounders. Reassuringly, our results did not change when we adjusted for several other urinary phenol biomarkers.

Conclusion

In this cohort, maternal urinary triclosan concentrations during pregnancy were associated with a decrease in birth weight, length, head circumference, and gestational duration. These findings suggest that triclosan exposure during pregnancy may be associated with adverse birth outcomes. While we did not find evidence that the association between triclosan and birth outcomes depended on the timing of exposure in the latter-two thirds of pregnancy, future research could attempt to identify windows of heightened vulnerability to triclosan using repeated assessments of exposure and statistically appropriate methods.[35] Finally, more research is needed to understand the relation between early life triclosan exposure and other infant and child health outcomes.

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Tables

Table 1. Urinary triclosan concentrations during pregnancy and birth weight z-scores by covariates among women and their infants in the HOME study (2003-2006).

	N (%)	Triclosan Geometric Mean, ng/mL (GSD)[a]	Mean Birth weight z-score (SD)
All	378	19 (4.1)	0.06 (1.03)
Maternal Age (years)			
18-25	90 (24)	16 (3.6)	-0.43 (0.86)
>25-35	227 (60)	20 (4.4)	0.23 (0.98)
>35	61 (16)	19 (3.5)	0.17 (1.22)
p-value		0.16	<0.01
Maternal Race			
White	235 (62)	20 (4.3)	0.28 (1.05)
Black	117 (31)	17 (3.8)	-0.32 (0.89)
Other	26 (7)	17 (3.9)	-0.20 (0.87)
p-value		0.56	<0.01
Maternal Education			
Bachelor's/Grad/Prof	191 (51)	21 (4.4)	0.27 (1.06)
Tech school/Some College	94 (25)	19 (4.2)	-0.02 (0.93)
High School	52 (14)	17 (3.6)	-0.19 (1.08)
<High School	41 (11)	14 (2.9)	-0.42 (0.79)
p-value		0.30	<0.01
Marital Status			
Married	245 (65)	21 (4.5)	0.25 (1.04)
Not married (Living Alone)	55 (15)	16 (3.7)	-0.21 (0.83)
Not married (Living w/ Someone)	78 (21)	15 (2.9)	-0.34 (0.96)
p-value		0.08	<0.01
Household Income			
>$80K	103 (27)	21 (3.9)	0.16 (0.97)
$40-80K	128 (34)	22 (4.9)	0.37 (1.09)
$20-40K	63 (17)	15 (3.9)	-0.07 (1.00)
<$20K	84 (22)	16 (3.1)	-0.44 (0.80)
p-value		0.17	<0.01
Parity			
Nulliparous	169 (45)	19 (4.2)	-0.11 (1.04)
1 to 2	117 (31)	18 (4.1)	0.19 (0.98)
3+	90 (24)	20 (3.9)	0.21 (1.04)
p-value		0.93	0.01
Delivery Method			
Vaginal	269 (71)	18 (3.9)	0.00 (0.99)
Cesarean	109 (29)	20 (4.4)	0.20 (1.12)
p-value		0.61	0.09
Depressive Symptoms			
Minimal	294 (78)	20 (4.4)	0.10 (1.03)
Mild	53 (14)	15 (3.2)	-0.09 (1.08)
Moderate/Severe	31 (8)	17 (2.9)	-0.04 (0.92)
p-value		0.30	0.39

Maternal BMI* (kg/m^2)			
<25	161 (43)	18 (3.9)	-0.12 (0.98)
25-30	124 (33)	21 (4.4)	0.14 (1.01)
>30	93 (25)	17 (4.0)	0.27 (1.08)
p-value		0.43	0.01
Prenatal Vitamin			
Rarely/Never	56 (15)	14 (3.3)	-0.23 (0.91)
Weekly/Daily	322 (85)	20 (4.2)	0.11 (1.04)
p-value		0.09	0.02
Child Sex			
Female	204 (54)	18 (4.0)	-0.02 (0.96)
Male	174 (46)	20 (4.2)	0.15 (1.10)
p-value		0.42	0.1
Tobacco Smoke Exposure[b]			
Unexposed	140 (37)	23 (4.5)	0.26 (1.08)
Secondhand Smoke	197 (52)	17 (4.0)	-0.03 (0.98)
Active	41 (11)	15 (3.0)	-0.17 (0.99)
p-value		0.12	0.01
Gestational Diabetes			
Yes	10 (3)	15 (3.2)	0.79 (1.55)
No	319 (97)	19 (4.2)	0.05 (1.03)
p-value		0.62	0.03
Hypertension Disorders			
Yes	30 (8)	21 (5.3)	-0.19 (1.06)
No	346 (92)	19 (4.0)	0.08 (1.03)
p-value		0.62	0.16

[a]Triclosan concentrations are from the mean of the 16-week and 26-week urinary concentrations. These values are not adjusted for urine dilution.

[b]Smoking status is based on prenatal serum cotinine concentrations; < 0.015ng/mL was classified as unexposed, 0.014 to ≤ 3.0 ng/mL was classified as secondhand smoke, and >3.0 ng/mL was considered active smoking exposure.

*-BMI: Body Mass Index

*p-values reflect the difference in the urinary triclosan geometric mean among the different categories.

Table 2. Difference in birth outcomes with 10-fold increase in prenatal urinary triclosan concentrations in the HOME Study (2003-2006). [a]

Birth Outcome	n	Unadjusted All Children (95% CI)	Adjusted All Children (95% CI)	n	Boys (95% CI)	n	Girls (95% CI)	Sex x Triclosan EMM p-value
Birth Weight (grams)	378	-28 (-120, 64)	-125 (-216, -33)	174	-82 (-216, 52)	204	-140 (-258, -22)	0.51
Birth Weight Z-Score (SD Units)	378	0.02 (-0.18, 0.13)	-0.15 (-0.3, 0.0)	174	-0.10 (-0.32, 0.12)	204	-0.18 (-0.38, 0.02)	0.60
Birth Length (cm)	369	0.1 (-0.4, 0.6)	-0.4 (-0.8, 0.1)	167	-0.4 (-1.1, 0.3)	202	-0.3 (-0.9, 0.3)	0.88
Head Circumference (cm)	370	0.0 (-0.3, 0.3)	-0.3 (-0.5, 0.0)	168	-0.3 (-0.7, 0.2)	202	-0.3 (-0.6, 0.1)	0.95
Gestational Age (weeks)	378	-0.1 (-0.4, 0.1)	-0.3 (-0.6, -0.1)	174	-0.3 (-0.7, 0.1)	204	-0.3 (-0.7, 0.0)	0.99

*Estimates are adjusted for maternal race, education, marital status, age at delivery, income, prenatal vitamin use, BDI score, and maternal BMI, and maternal serum cotinine concentrations during pregnancy. Head circumference is also adjusted for delivery method.

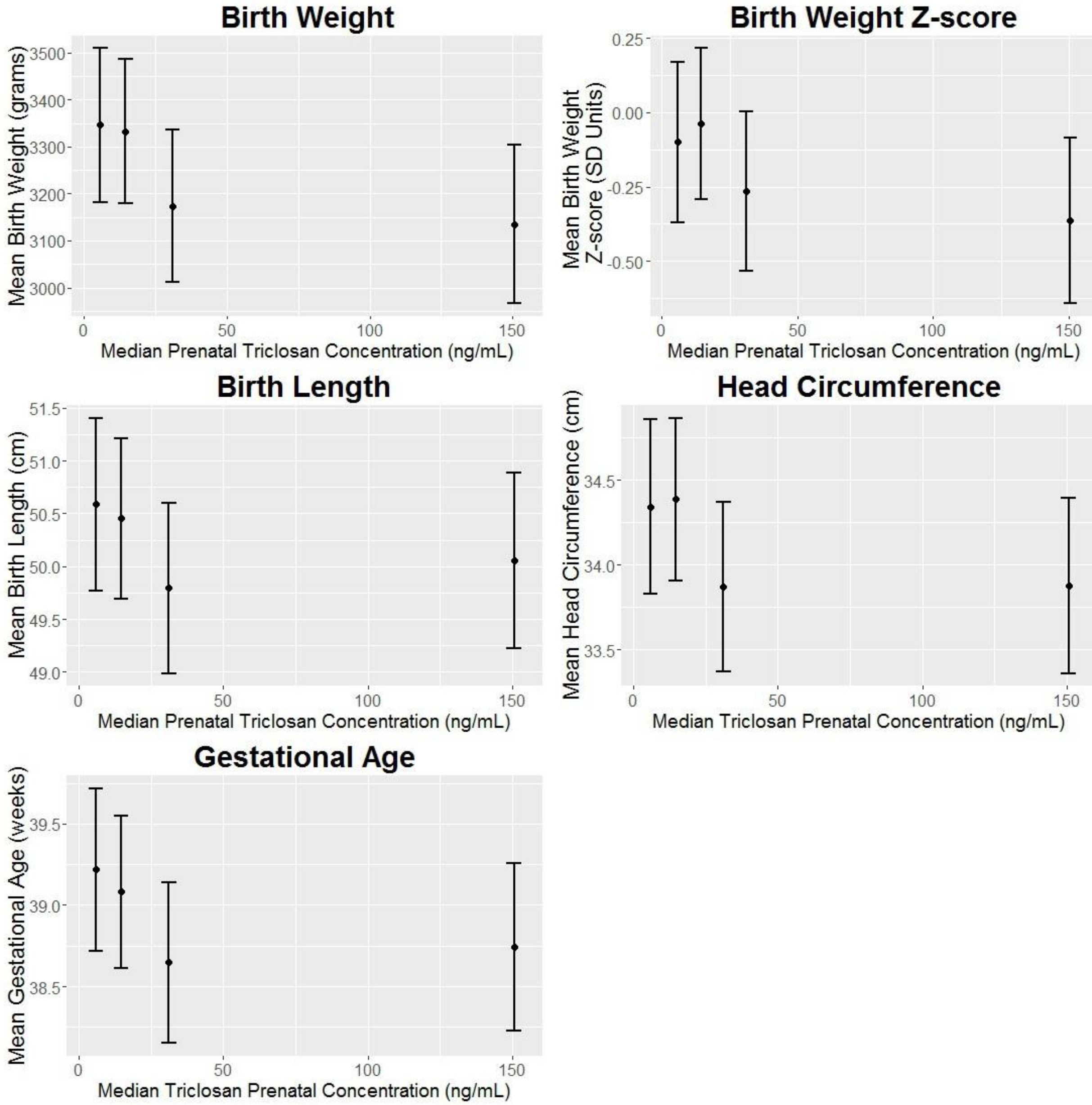
Table 3. Unadjusted and adjusted relative risk for low birth weight, preterm birth, and SGA per 10-fold increase in prenatal urinary triclosan concentrations among women in the HOME study (2003-2006).

Outcome	Number with outcome (%)	Crude RR (95% CI)	Adjusted RR (95% CI)[1]
Low birth weight (<2500 g)	33 (8.7)	1.1 (0.7, 1.9)	1.4 (0.8, 2.4)
Preterm Birth (<37 weeks)	19 (5.0)	1.2 (0.8, 1.9)	1.4 (0.9, 2.3)
SGA (<10th Percentile)	31 (8.2)	1.2 (0.8, 1.8)	1.3 (0.9, 2.1)

[1]Adjusted for mother's race, household income, age at delivery, education, marital status, BDI score, mother's BMI, prenatal vitamin use, and maternal serum cotinine concentrations during pregnancy.

Figures

Figure 1. Adjusted mean birth weight z-score, birth length, head circumference, and gestational age by maternal urinary triclosan quartiles in the HOME Study (2003-2006).



*Mean birth weight z-scores are adjusted for maternal race, education, marital status, age at delivery, income, prenatal vitamin use, delivery method, BDI score, and maternal serum cotinine concentrations during pregnancy. Head circumference is also adjusted for delivery method.
Mean birth outcomes are presented at the median triclosan concentration within each quartile

Supplemental Material

Table S1. Prenatal urinary triclosan concentrations by visit among women in the HOME Study (2003-2006).

Visit	# <LOD (%))[1]	Geometric Mean (ng/mL) (SD)	Minimum (ng/mL)	5th Percentile (ng/mL)	25th Percentile (ng/mL)	Median (ng/mL)	75th percentile (ng/mL)	95th Percentile (ng/mL)	Maximum (ng/mL)
16 Weeks	34 (9.0)	22	<LOD	<LOD	6.3	17	59	614	1,985
26 Weeks	65 (17.2)	16	<LOD	<LOD	4.9	13	52	369	1,657
Average	NA	25	<LOD	2.4	6.5	16	50	285	1,501

LOD = 2.3 ng/mL.
NA=Not Applicable

Table S2. Adjusted difference in birth outcomes across quartiles of prenatal urinary triclosan concentrations in the HOME Study (2003-2006).

Birth Outcome and Triclosan Quartile	**n**	**Difference (95% CI)**
Birth Weight (grams)		
1st Quartile (<LOD – 8.8 ng/mL)	94	Ref
2nd Quartile (8.794 – 22.3 ng/mL)	95	-13 (-179, 153)
3rd Quartile (22.294– 74.7 ng/mL)	95	-173 (-342, -4)
4th Quartile (76.921 – 1264 ng/mL)	94	-211 (-385, -37)
Birth Weight Z-Score (SD Unit)		
1st Quartile (<LOD – 8.8 ng/mL)	94	Ref
2nd Quartile (8.794 – 22.3 ng/mL)	95	0.06 (-0.21, 0.34)
3rd Quartile (22.294– 74.7 ng/mL)	95	-0.16 (-0.44, 0.11)
4th Quartile (76.921 – 1264 ng/mL)	94	-0.26 (-0.55, 0.02)
Birth Length (cm)		
1st Quartile (<LOD – 8.8 ng/mL)	92	Ref
2nd Quartile (8.794 – 22.3 ng/mL)	92	-0.1 (-1.0, 0.7)
3rd Quartile (22.294– 74.7 ng/mL)	94	-0.8 (-1.6, 0.0)
4th Quartile (76.921 – 1264 ng/mL)	91	-0.5 (-1.4, 0.3)
Head Circumference (cm)		
1st Quartile (<LOD – 8.8 ng/mL)	93	Ref
2nd Quartile (8.794 – 22.3 ng/mL)	92	0.0 (-0.5, 0.5)
3rd Quartile (22.294– 74.7 ng/mL)	94	-0.5 (-1.0, 0.0)
4th Quartile (76.921 – 1264 ng/mL)	91	-0.4 (-1.0, 0.1)
Gestational Age (weeks)		
1st Quartile (<LOD – 8.8 ng/mL)	94	Ref
2nd Quartile (8.794 – 22.3 ng/mL)	95	-0.1 (-0.6, 0.4)
3rd Quartile (22.294– 74.7 ng/mL)	95	-0.6 (-1.1, -0.1)
4th Quartile (76.921 – 1264 ng/mL)	94	-0.5 (-1.0 , 0.1)

*All estimates are adjusted for maternal race, education, marital status, age at delivery, income, prenatal vitamin use, BDI score, and maternal BMI, maternal serum cotinine concentrations during pregnancy. Head circumference is also adjusted for delivery method.

Table S3. Difference in birth outcomes with 10-fold increase in prenatal urinary triclosan concentrations among women in the HOME Study: Sensitivity analysis.

Adjustments/Exclusions	**n**	**Birth Weight, grams (95% CI)**	**n**	**Birth Weight Z-score (95% CI)**	**n**	**Birth Length, cm (95% CI)**	**n**	**Head Circumference, cm (95% CI)**	**n**	**Gestational Age, weeks (95% CI)**
Unadjusted	378	-28 (-120, 64)	378	0.02 (-0.18, 0.13)	369	0.1 (-0.4, 0.6)	370	0.0 (-0.3, 0.3)	378	-0.1 (-0.4, 0.1)
Adjusted Model-1[a]	378	-125 (-216, -33)	378	-0.15 (-0.30, 0.00)	369	-0.4 (-0.8, 0.1)	370	-0.3 (-0.5, 0.0)	378	-0.3 (-0.6, -0.1)
Adjusted Model-2[b]	378	-135 (-227, -44)	378	-0.19 (-0.34, -0.03)	369	-0.4 (-0.9, 0.0)	370	-0.3 (-0.6, 0.0)	378	-0.3 (-0.6, 0.0)
Adjust for GA[a]	378	-50 (-122, 21)	-	-	369	-0.1 (-0.4, 0.3)	370	-0.1 (-0.4, 0.1)	-	-
Adjust for other phenols[c]	378	-121 (-226, -16)	377	-0.16 (-0.34, 0.01)	369	-0.3 (-0.8, 0.2)	370	-0.3 (-0.7, 0.0)	378	-0.3 (-0.6, 0.0)
Exclude gestational diabetes[a]	368	-117 (-210, -26)	368	-0.14 (-0.29, 0.01)	359	-0.4 (-0.9, 0.1)	360	-0.2 (-0.5, 0.0)	368	-0.4 (-0.6, -0.1)
Exclude hypertension disorders[a]	348	-120 (-215, -24)	348	-0.12 (-0.28, 0.04)	339	-0.4 (-0.9, 0.1)	340	-0.2 (-0.5, 0.1)	348	-0.4 (-0.7, -0.1)
Women with LMP[a]	369	-129 (-223, -38)	369	-0.15 (-0.30, 0.01)	361	-0.4 (-0.9, 0.0)	362	-0.3 (-0.6, 0.0)	369	-0.4 (-0.7, -0.1)
Exclude women with missing 1 urine sample[a]	360	-107 (-200, -15)	360	-0.15 (-0.30, 0.01)	351	-0.3 (-0.7, 0.2)	353	-0.3 (-0.5, 0.0)	360	-0.2 (-0.5, 0.0)

[a]Adjusted for mother's race, education, marital status, age at delivery, household income, prenatal vitamin use, delivery method, BDI score, maternal BMI, and maternal serum cotinine concentrations during pregnancy. Head circumference is also adjusted for method of delivery.
[b]Adjusted for mother's race, education, marital status, age at delivery, household income, and maternal serum cotinine concentrations during pregnancy.
[c]Adjusted model plus adjusted for BP-3, BPA, PPB, MPB, BPB, 2,4-DCP, and 2,5-DCP.

Table S4. Difference in birth outcomes with 10-fold increase in 16 and 26 week prenatal urinary triclosan concentrations among women in the HOME Study.

Timing of Triclosan Measurement	**n**	**Birth Weight, g (95% CI)**	**n**	**Birth Weight Z-score (95% CI)**	**n**	**Birth Length, cm (95% CI)**	**n**	**Head Circumference, cm (95% CI)**	**n**	**Gestational Age, weeks (95% CI)**
2nd Trimester[a,b]	360	-103 (-187, -18)	360	-0.12 (-0.27, 0.03)	351	-0.2 (-0.7, 0.2)	353	-0.2 (-0.5, 0.1)	360	-0.2 (-0.4, 0.1)
3rd Trimester[a,b]	360	-79 (-167, 9)	360	-0.12 (-0.26, 0.02)	351	-0.3 (-0.7, 0.1)	353	-0.2 (-0.6, -0.1)	360	-0.3 (-0.5, 0.0)
Average[a,b]	360	-110 (-202, -18)	360	-0.15 (-0.31, 0.00)	351	-0.3 (-0.7, 0.2)	353	-0.3 (-0.6, 0.0)	360	-0.3 (-0.5, 0.0)

[a] Adjusted mother's race, education, marital status, age at delivery, household income, prenatal vitamin use, BDI score, maternal BMI, and maternal serum cotinine concentrations during pregnancy. Head circumference is also adjusted for delivery method.

[b] Restricted to women with both a 2nd and 3rd trimester sample available.

Table S5. Difference in birth outcomes with 10-fold increase in prenatal urinary triclosan concentrations among women in the HOME study using different methods to account for urine dilution.[a]

Creatinine Adjustments	n	Birth Weight (grams)	n	Birth Weight Z-score (SD Units)	n	Birth Length (cm)	n	Head Circumference (cm)	n	Gestational Age (weeks)
No Urine Dilution Adjustment	378	-120 (-208, -33)	378	-0.15 (-0.29, 0.00)	369	-0.4 (-0.8, 0.0)	370	-0.2 (-0.5, 0.0)	378	-0.4 (-0.6, -0.1)
Creatinine Standardized	378	-125 (-216, -33)	378	-0.15 (-0.30, 0.00)	369	-0.4 (-0.8, 0.1)	370	-0.3 (-0.5, 0.0)	378	-0.3 (-0.6, -0.1)
CAS	376	-128 (-218, -39)	376	-0.15 (-0.30, 0.00)	367	-0.4 (-0.8, 0.1)	368	-0.3 (-0.6, 0.0)	376	-0.4 (-0.7, -0.1)
Covariate Adjustment	378	-131 (-222, -41)	378	-0.16 (-0.31, -0.01)	369	-0.4 (-0.8, 0.1)	370	-0.3 (-0.6, 0.0)	378	-0.4 (-0.7, -0.1)
Standardization plus Covariate Adjustment	378	-125 (-217, -33)	378	-0.15 (-0.31, 0.00)	369	-0.4 (-0.8 0.1)	369	-0.3 (-0.5, 0.0)	378	-0.4 (-0.6, -0.1)
CAS plus Covariate Adjustment*	376	-129 (-219, -39)	376	-0.15 (0.30, 0.00)	367	-0.4 (-0.8, 0.1)	367	-0.3 (-0.5, 0.0)	376	-0.4 (-0.7, -0.1)
Exclude women with extreme creatinine values[a]	371	-124 (-217, -31)	371	-0.15 (-0.31, 0.00)	362	-0.4 (-0.8, 0.1)	363	-0.3 (-0.6, 0.0)	371	-0.3 (-0.6, -0.1)

[a] Adjusted mother's race, education, marital status, age at delivery, household income, prenatal vitamin use, BDI score, maternal BMI, and maternal serum cotinine concentrations during pregnancy. Head circumference is also adjusted for delivery method.

CAS: Covariate Adjusted Standardization.

*For the covariate adjusted standardization we adjusted the model for mother's race, household income, age at delivery, education, marital status, BDI score, mother's BMI, prenatal vitamin use, maternal serum cotinine concentrations during pregnancy, and parity.

Figure S1. Directed Acyclic Graph used to select covariates in the association between prenatal urinary triclosan concentrations and birth outcomes.

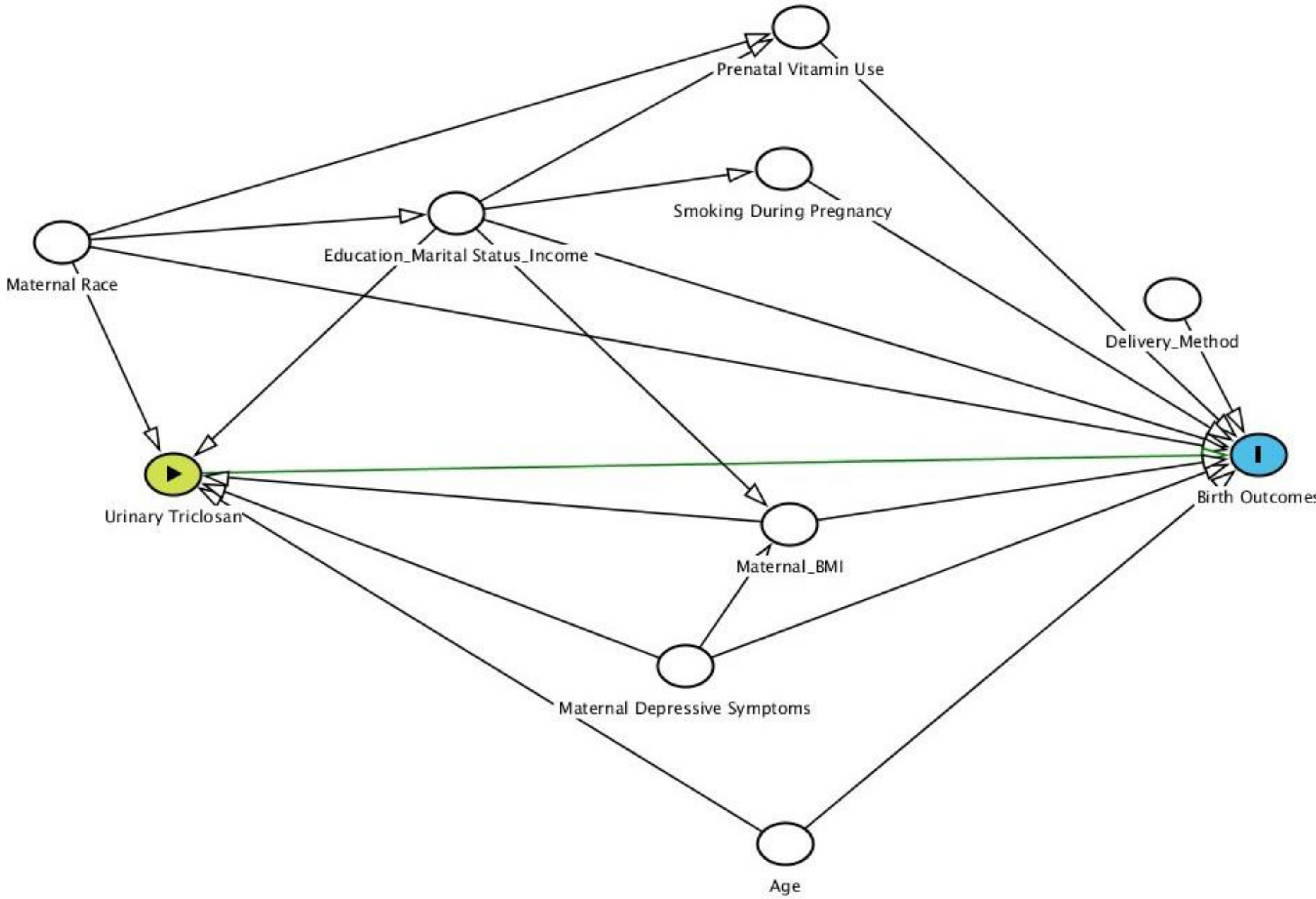


Figure S2. Directed Acyclic Graph used to select covariates in the primary model and potential confounders adjusted for in sensitivity analyses examining association between prenatal urinary triclosan concentrations and birth outcomes.

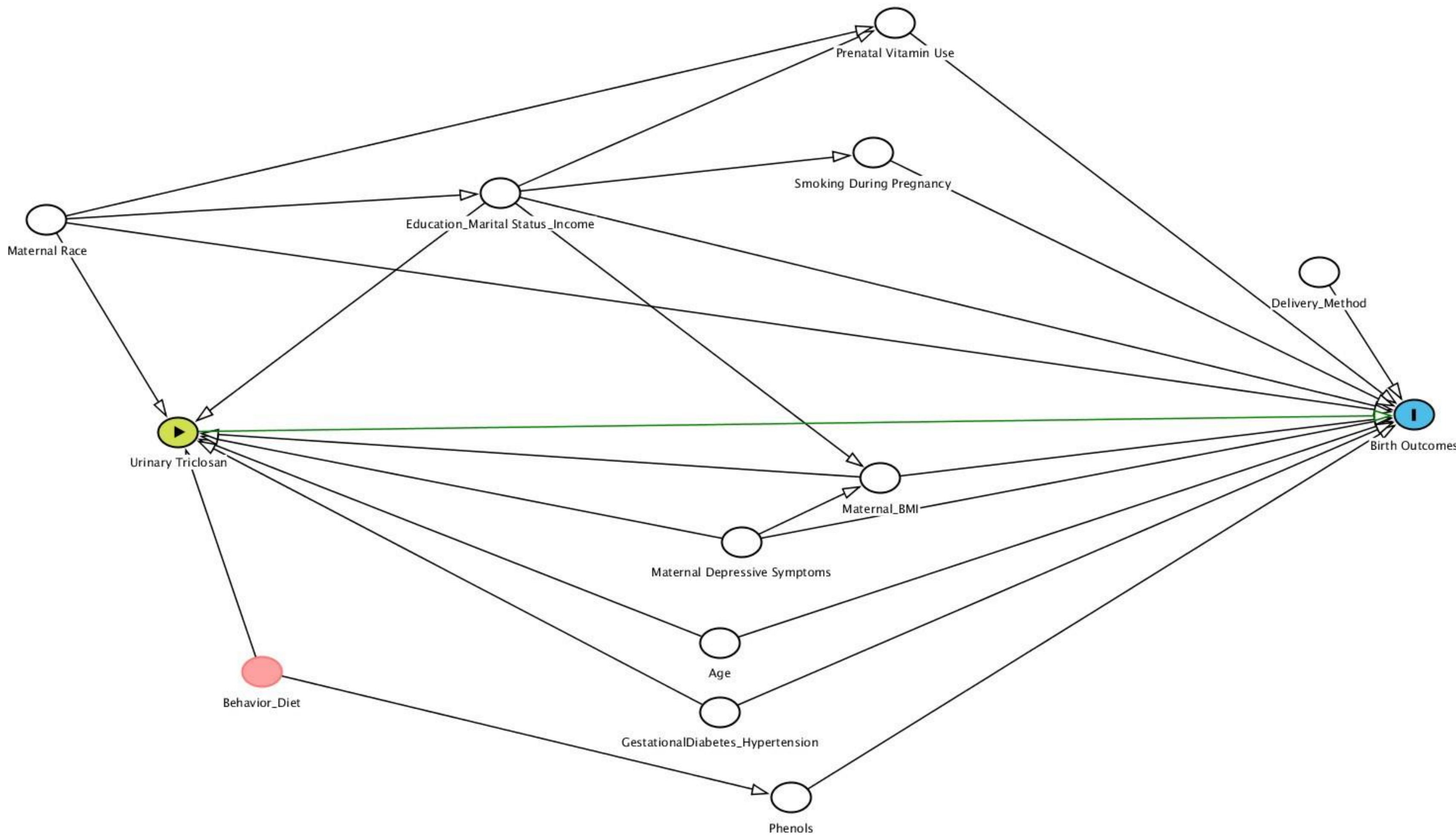
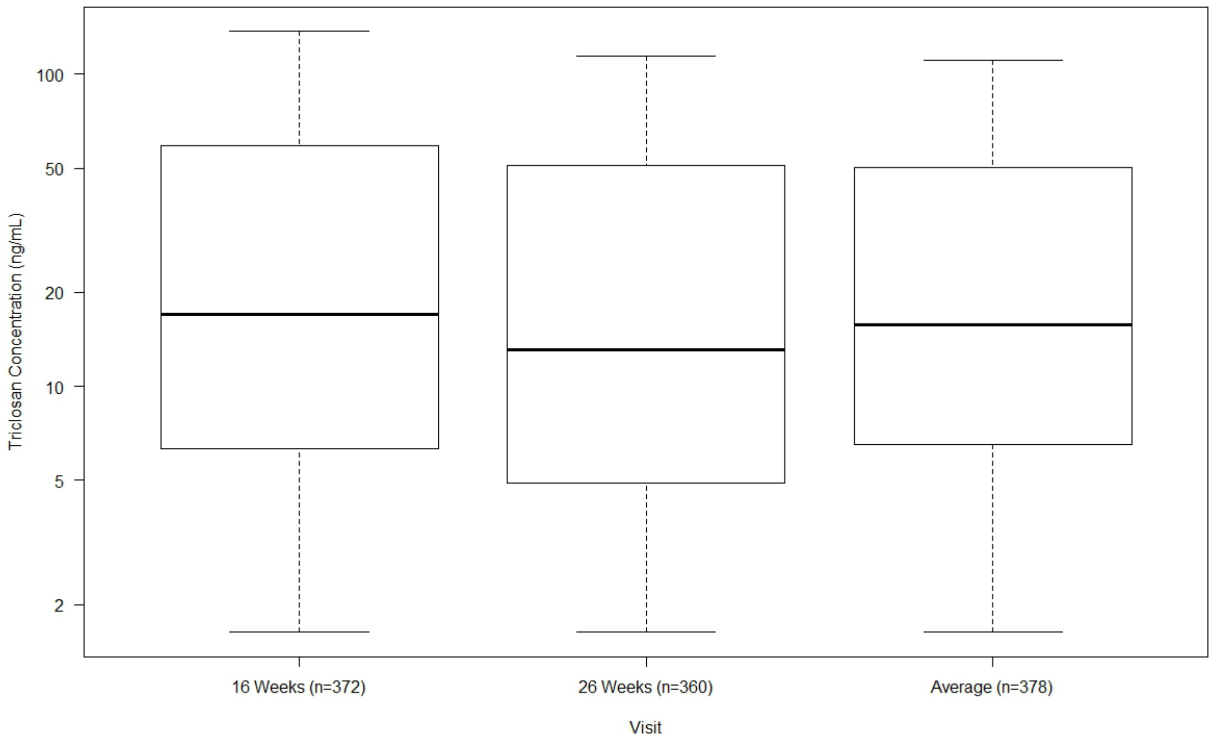
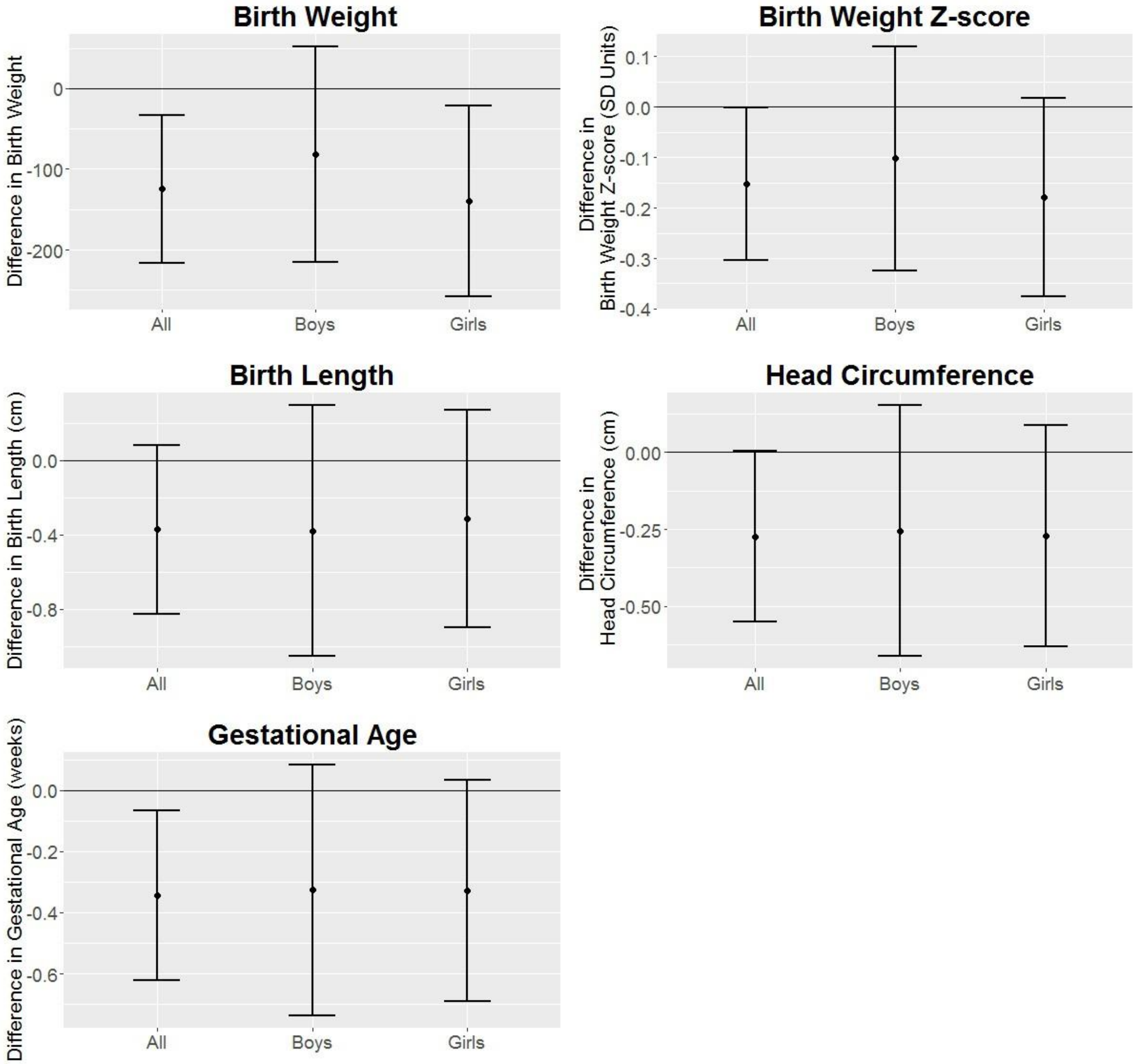


Figure S3. Prenatal urinary triclosan concentrations by visit among women in the HOME Study (2003-2006).



*The average prenatal urinary triclosan concentrations were obtained by taking the mean of the log_{10}-transformed 16 and 26 week triclosan concentrations. If there was only a 16 or 26 week triclosan concentration for a woman, then that that was used as the average prenatal urinary triclosan concentration.

Figure S4. Adjusted differences in birth outcomes per 10-fold increase in prenatal urinary triclosan concentrations in the HOME study (2003-2006).

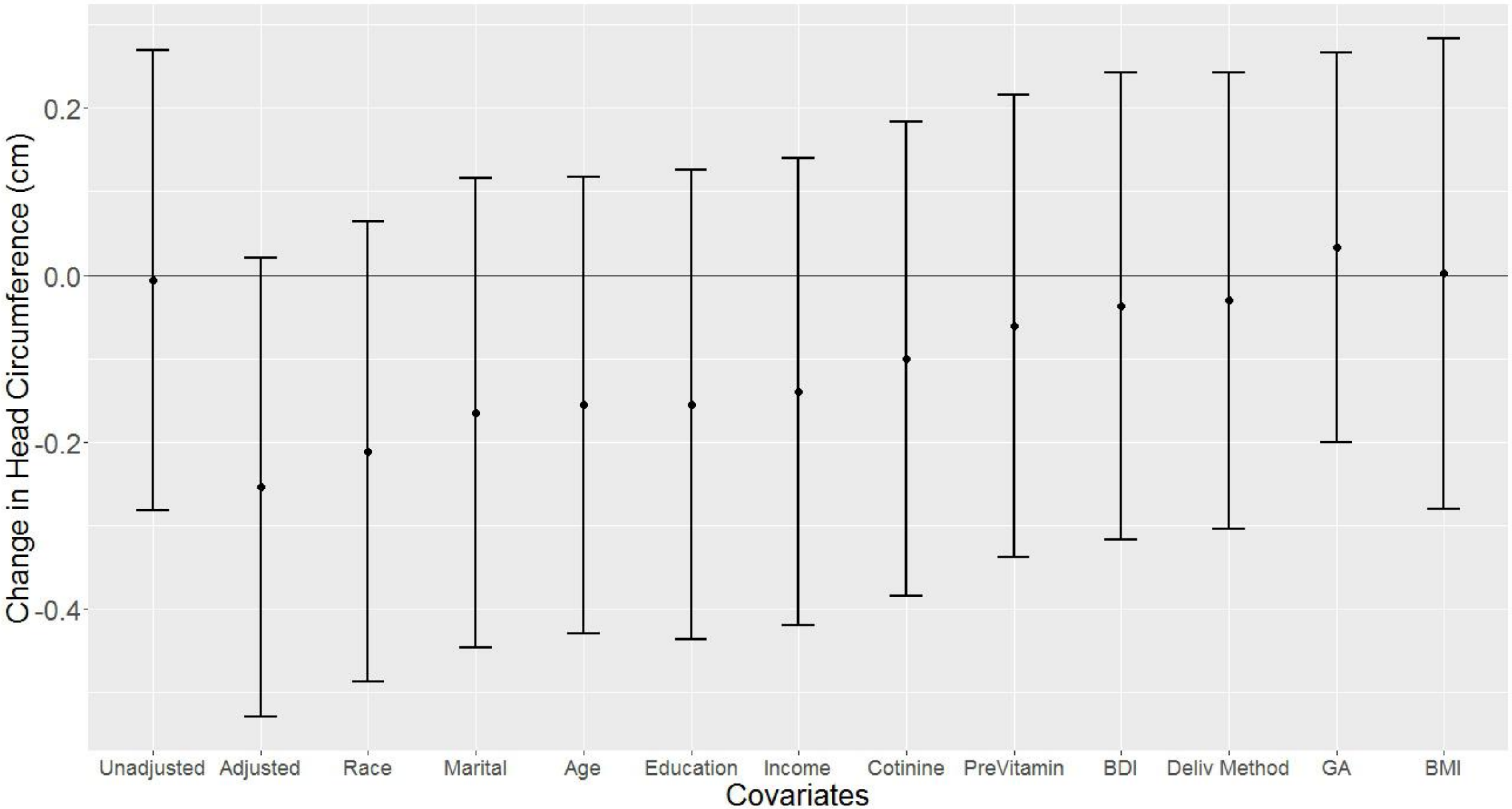


[1]The p-values for the effect modification between urinary triclosan concentrations and child sex were 0.56, 0.85, 0.92, and 0.81 for birth weight z-score, birth length, head circumference, and gestational age, respectively. Separate boy and girl effect estimates were derived from a model including triclosan, child sex, their product interaction term, and covariates.

[2]All effect estimates are adjusted for maternal race, education, marital status, age at delivery, income, prenatal vitamin use, maternal BMI, BDI score, and maternal serum cotinine concentrations during pregnancy. Head circumference is also adjusted for delivery method.

[3]There are 377 mother-child pairs for birth weight z-score and gestational age, 368 mother-child pairs for birth length, and 369 mother-child pairs for head circumference.

Figure S5. Change in head circumference per 10-fold increase in prenatal urinary triclosan concentrations in the HOME Study with unadjusted, adjusted, and single-covariate adjusted models.



* There was a null association between increased prenatal triclosan concentrations and birth weight z-score in the unadjusted model. There was an inverse relationship between prenatal triclosan concentrations and birth weight z-score in the adjusted model. This was because maternal age, education, marital status, race, and household income were inversely associated with prenatal triclosan concentrations and positively associated with birth weight z-score.