Female exposure to phenols and phthalates and time to pregnancy: the Maternal-Infant Research on Environmental Chemicals (MIREC) Study

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Objective: To assess the potential effect of bisphenol A (BPA), triclosan (TCS), and phthalates on women's fecundity, as measured by time to pregnancy (TTP).
Design: Pregnancy-based retrospective TTP study.
Setting: Not applicable.
Patient(s): A total of 2,001 women during the first trimester of pregnancy recruited between 2008 and 2011 (the Maternal-Infant Research on Environmental Chemicals (MIREC) Study), with 1,742 women included in the BPA analysis, 1,699 in the TCS analysis, and 1,597 in the phthalates analysis.
Intervention(s): None.
Main Outcome Measure(s): Fecundability odds ratios (FORs) estimated using the Cox model modified for discrete time data.
Result(s): The BPA concentrations were not statistically significantly associated with diminished fecundity either in crude or adjusted models. Women in the highest quartile of TCS (>72 ng/mL) had evidence of decreased fecundity (FOR 0.84; 95% confidence interval, 0.72–0.97) compared with the three lower quartiles as the reference group. Exposure to phthalates was suggestive of a shorter TTP, as indicated by FORs greater than 1, although the 95% confidence interval always included 1.
Conclusion(s): Elevated TCS exposure may be associated with diminished fecundity. BPA and phthalates showed no negative impact; on the contrary, some phthalates might be associated with a shorter time to pregnancy. A major limitation of the study was that only one measurement of exposure was available for each woman after conception. Further research is necessary to test these findings. (Fertil Steril® 2015;103:1011–20. ©2015 by American Society for Reproductive Medicine.)
Key Words: Bisphenol A, fecundity, phthalates, reproduction, triclosan

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Endocrine-disrupting chemicals (EDCs) have the potential to interfere with hormone functions. Their ubiquitous presence in the environment coupled with the detection of several EDCs in large biomonitoring surveys (1, 2) has raised concern about their possible adverse health effects. Because the endocrine system is essential for sexual development and reproductive functions, research is emerging about the effect of EDCs on human fecundity, defined as the biologic capacity for

reproduction (3). Some of these chemicals have long half-lives, allowing bioaccumulation and persistence in the environment. On the opposite end of the spectrum are those chemicals with short elimination half-lives, considered nonpersistent, though their high-volume production makes them a common source of human exposure. Bisphenol A (BPA), triclosan (TCS), and phthalates belong in this latter group.

Exposure to BPA is common, with more than 90% of the populations of the United States and Canada having detectable urinary concentrations (1, 2). Although BPA has recognized endocrine disrupting properties in animals (4), there is limited information regarding the effect of BPA exposure on human fecundity. Several studies conducted in infertile couples seeking assisted reproductive technology (ART) have suggested reproductive effects (5–7), but only one study has assessed the impact of BPA on couple fecundity in a population-based setting, the U.S. LIFE Study (8). This prospective cohort of 501 couples, recruited upon discontinuing contraception to become pregnant, reported no association between female or male BPA urinary concentrations and time to pregnancy (TTP), an epidemiologic metric widely used for the study of human fecundity (9).

Triclosan, a broad-spectrum phenolic biocide with activity against bacteria and fungi, is used in personal care products (10). Triclosan was detectable in about 75% of the urine samples collected as part of NHANES survey of the U.S. population (11, 12) and the 2009–2011 Canadian Health Measures Survey (2). It has a similar structure to known EDCs, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and BPA, and to thyroid hormones (13). These structural similitudes, coupled with some limited evidence from experimental studies of effects on diverse hormones, suggest that TCS may influence endocrine function and possibly the reproductive axis (13). Epidemiologic studies on TCS have been scarce. Two studies reported no significant impact of prenatal exposure to TCS on birth size (14, 15). A recent analysis of urine samples from NHANES 2003–2008, reported a positive association between TCS and body mass index (BMI) (16). No studies assessing the effect of TCS on TTP have been conducted to date.

There is evidence suggesting that several phthalates may be endocrine disruptors (17) and may affect development and reproduction (18, 19). A large number of phthalate metabolites are detectable in more than 95% of the populations in the United States and Canada (2, 20), and in women of reproductive age (21). Nonetheless, there is a paucity of studies assessing the effect of phthalates on women's fecundity. In Generation R, a large pregnancy cohort study conducted in the Netherlands, occupational exposure to phthalates was assessed using a job-exposure matrix, and was reported to be suggestive of longer TTP (22, 23). In Italy, concentrations of several phthalate metabolites were assessed in 56 infertile couples from an ART center, and they were found to be significantly higher than in the control group of fertile couples (24). Recently, in the LIFE Study, no phthalate metabolite in female urine was statistically associated with a longer TTP, although one metabolite [mono (3-carboxypropyl) phthalate] was associated with a shorter TTP. In men, urinary concentrations of monomethyl, mono-*n*-butyl, and monobenzyl phthalates were associated with a longer TTP (8). Of note, although most of the literature assessing the adverse health effects of phthalates has been focused on the effect of individual metabolites; some studies have suggested that simultaneous exposure to multiple phthalates may have a cumulative impact (25).

There is limited research exploring the effects of nonpersistent EDCs on TTP. Most studies to date have focused on ART outcomes and male exposures, despite the fact that female reproductive function is also susceptible to hormonally active chemicals (26). To this end, data from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, a Canadian pregnancy and birth cohort, was analyzed to assess the effect of BPA, TCS, and phthalates on women's fecundity, as measured by TTP.

MATERIALS AND METHODS

Population and Study Design

The MIREC Study is a pregnancy cohort of 2,001 women recruited in 10 cities across Canada between 2008 and 2011 (27). Women were approached during the first trimester of pregnancy at participating hospitals and clinics and were observed for a total of five visits up to 10 weeks postpartum. A detailed questionnaire was administered during the first study visit (<14 weeks' gestation) that included information on demographics, present medical and obstetric history, and lifestyle characteristics.

To determine the TTP, women were asked, "How long did it take you to get pregnant with this pregnancy?" (in months). Women were further asked about the last type of birth control method the couple had used before this pregnancy. Those who had used some method (75% of the cohort) were asked if they had stopped it before the index pregnancy (89%) or if the pregnancy was the result of a birth control failure (11%). In this way, we assumed that if it was not a birth control failure the index pregnancy was from unprotected intercourse.

The exclusion criteria were as follows. Eighteen participants withdrew from the study, and all their data and samples were destroyed. We excluded women who had missing data for the specific compound/group studied (46 for BPA, 96 for TCS, and 211 for phthalates), TTP (14 for BPA and TCS, and 15 for phthalates), or specific gravity (n = 3). We also excluded women who required egg donation (n = 4) or reported male factor infertility (n = 26), as well as women whose index pregnancy was the result of a birth control failure (148 for BPA, 141 for TCS, and 154 for phthalates). Thus, 1,742 women were included in our BPA analysis, 1,699 in the TCS analysis, and 1,597 in the phthalates analysis.

The study was approved by ethics committees at Health Canada and Sainte-Justine University Hospital Center, as well as the hospitals affiliated to the study across Canada. Written informed consent was obtained from all participants.

Analytic Methods

As part of the biomonitoring component of MIREC, a spot urine sample was collected in polypropylene cups during

the first trimester visit. These samples were aliquotted into 30-mL Nalgene tubes, frozen at −20°C within 2 hours of collection, and shipped on dry ice to the MIREC coordinating center in Montreal where they were stored at −30°C. Urine samples were shipped in batches to the laboratory for analysis. Chemical analyses were performed by the Toxicology Laboratory located in the Institut national de santé publique du Québec (http://www.inspq.qc.ca/ctqenglish/), which is accredited by the Standards Council of Canada under ISO 17025 and CAN-P-43. The accuracy and precision of the analyses are evaluated on a regular basis through the laboratory's participation in external quality assessment programs (27).

As part of the initial MIREC protocol, urine samples were analyzed for bisphenol A (BPA) and 11 phthalate metabolites (those for which the laboratory had a method available at the time of the study design): low molecular weight [mono-*n*-butyl phthalate (MnBP), mono-ethyl phthalate (MEP), mono-benzyl phthalate (MBzP), mono-methyl phthalate (MMP)]; intermediate molecular weight [mono-cyclo-hexyl phthalate (MCHP)]; and high molecular weight [mono-isononyl phthalate (MiNP), mono-*n*-octyl phthalate (MnOP), mono-(3-carboxypropyl) phthalate (MCPP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), and mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP)] (28). Subsequently, additional research funds were obtained for the triclosan (TCS) analysis as part of formative research for the U.S. National Children's Study. This analysis was restricted to those women who agreed to participate in the MIREC Biobank (98% of the cohort).

Urinary total BPA (free plus conjugated) concentrations were quantified using an established protocol. Samples were analyzed by gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) (INSPQ method E-454) (28). Phthalate metabolites were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) (INSPQ method E-453). Further details are described in Arbuckle et al. (28).

For the TCS analysis, sensitive LC-MS/MS methods were developed for the analysis of free and conjugated forms TCS in urine. Detailed quality assurance/quality control (QA/QC) procedures are described in Provencher et al. (29). To account for urine dilution, the specific gravity was measured in thawed urine samples by a refractometer (UG-1, Atago 3461; Atago U.S.A.).

Field blanks were included 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. Results did not show any evidence of contamination (28, 30).

Statistical Analysis

Descriptive statistics, including the percentage detected, the median, and the geometric mean, were computed for all chemicals. Concentrations below the limit of detection (LOD) were set to the LOD divided by 2. The total TCS was calculated by summing the free and conjugated forms. We considered the effect of total BPA, total TCS, and each individual phthalate metabolite independently. In the case of phthalate metabolites, we also categorized them into low and high molecular weight (LMW and HMW) and calculated the sum of their molecular weights in each category as a measure of total LMW and HMW phthalates. In addition, we calculated the estrogenicity equivalency factor (EEF) as proposed by Braun et al. (31).

We also considered different alternatives for modeling exposure. First, biomarker concentrations were log transformed and divided by its standard deviation (32). Second, concentrations were categorized a priori into quartiles.

Fecundability odds ratios (FORs) were estimated using the Cox model modified for discrete time data, which allows for a cycle-varying intercept (33). The FORs estimate the odds of becoming pregnant each cycle, given exposure to the specific compound conditional on not being pregnant in the previous cycle. A FOR <1 denotes a reduction in fecundity or longer TTP, and a FOR >1 denotes a shorter TTP. The TTP was censored at month 13. Linearity and proportional hazard assumptions were verified (33).

The potential confounders were maternal age, smoking, education, and household income, which have been identified as predictors of exposure to BPA, TCS, and phthalates in the MIREC cohort (28, 30). In addition to the covariates that also impact fecundity, body mass index (BMI) was included in the adjusted models (3). Maternal and paternal age were highly correlated ($r = 0.73$), which precluded the inclusion of paternal age into the model. We did not include parity in our model because its adjustment can induce overadjustment bias (34).

To account for urine dilution, specific gravity was included as a covariate in the regression model (35). We also evaluated the possible interactions between specific gravity and the time of urine collection, as was evidenced in previous analyses conducted by our group (28). Nonetheless, we did not include interactions in our final models because none were observed. Statistical analysis was performed using STATA 10.0 (Stata Corporation), and SAS 9.3 (Statistical Analysis System) specifically for the discrete-time Cox proportional models.

RESULTS

The characteristics of the study population are presented in Table 1. The distributions of demographic and lifestyle characteristics were similar for the three compounds/group studied (i.e., BPA, TCS, and phthalate metabolites). The mean maternal age was 32.8 years (standard deviation [SD] ± 5.0) years, and the mean paternal age was 34.7 (SD ± 5.6). The median gestational age at interview was 12 weeks, ranging from 6 to 14 weeks. Most participants included in the analysis (81%) were born in Canada; about two-thirds had a university degree, and more than one-third reported a household income higher than $100,000 CAD. More than half the women had had at least one prior pregnancy with a live birth, and about 15% were obese or active smokers during the preconception period. Maternal and paternal age, parity, and prepregnancy BMI were associated with TTP.

TABLE 1

Association of study population characteristics with TTP by chemical measured: the MIREC Study.

	BPA (n = 1,742)		TCS (n = 1,699)		Phthalates (n = 1,597)	
Characteristic	**N (%)[a]**	***P* value[b]**	**N (%)[a]**	***P* value[b]**	**N (%)[a]**	***P* value[b]**
Maternal age (y), mean (SD)	32.84 (4.95)	<.001	32.84 (4.91)	<.001	32.85 (4.96)	<.001
Paternal age (y), mean (SD)[c]	34.76 (5.68)	<.001	34.74 (5.58)	<.001	34.74 (5.69)	<.001
Gestational age (wk), median (min–max)[d]	12 (6–14)	.04	12 (6–14)	.03	12 (6–14)	.05
Education		.38		.31		.40
Some college or less	237 (13.6)		229 (13.5)		211 (13.2)	
College diploma	398 (22.8)		389 (22.9)		365 (22.9)	
Undergraduate	644 (37.0)		631 (37.1)		591 (37.0)	
Graduate (M.Sc., Ph.D.)	463 (26.6)		450 (26.5)		430 (26.9)	
Country of birth		.92		.93		.81
Canada	1,408 (80.8)		1,376 (81.0)		1,294 (81.0)	
United States	27 (1.6)		27 (1.6)		24 (1.5)	
Mexico	8 (0.5)		8 (0.4)		6 (0.4)	
China	16 (0.9)		15 (0.9)		16 (1.0)	
Other	283 (16.2)		273 (16.1)		257 (16.1)	
Household income		.18		.16		.21
<$60,000	363 (20.8)		353 (20.8)		327 (20.5)	
$60,001–100,000	614 (35.3)		602 (35.4)		566 (35.4)	
>$100,000	685 (39.3)		671 (39.5)		633 (39.6)	
No response	80 (4.6)		73 (4.3)		71 (4.5)	
Parity conditional on gravidity		<.001		.002		.001
No prior pregnancy	499 (28.7)		485 (28.5)		459 (28.6)	
Prior pregnancy						
Without live birth(s)	270 (15.5)		266 (15.7)		253 (16.0)	
With live birth (s)	972 (55.8)		947 (55.8)		884 (55.4)	
Maternal smoking		.77		.78		.54
Never	1,078 (61.9)		1,049 (61.8)		993 (62.2)	
Former	398 (22.9)		390 (23.0)		360 (22.6)	
Current[e]	264 (15.2)		258 (15.2)		242 (15.2)	
Prepregnancy BMI		.01		.01		.01
<24.9	1,031 (63.5)		1003 (63.3)		960 (64.4)	
25–29.9	354 (21.8)		346 (21.9)		326 (21.8)	
>30	238 (14.7)		234 (14.8)		205 (13.8)	
Paternal smoking		1.00		1.00		.98
No	1,219 (83.0)		1,191 (83.0)		1,117 (82.7)	
Yes	249 (17.0)		244 (17.0)		233 (17.3)	

Note: BMI = body mass index.
[a] Values are n (%), unless otherwise stated.
[b] *P* values for the association with time to pregnancy: likelihood ratio for continuous variables, log rank test for categorical variables.
[c] Paternal age was missing in 234, 224, and 210 participants for BPA, TCS, and phthalates, respectively.
[d] Gestational age was missing in two participants.
[e] Includes women who quit smoking during pregnancy or 1 y before.

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The distribution of TTP was similar across chemicals, with a median time of 2 months for the three compounds/group, and a 25th percentile of 1 month. Although the 75th percentile was 5 months in the case of BPA and TCS, it was 4 months in the case of phthalates. Detectable urinary concentrations of total BPA were found in 87% of the samples, and total TCS was detectable in more than 99% (Table 2). As for phthalates, six metabolites were detectable in more than 98% of the samples (MnBP, MEP, MBzP, MEHP, MEOHP, and MEHHP), and MCPP was detectable in 82%. However, four metabolites (MMP, MCHP, MiNP, and MnOP) were detectable in fewer than 14% of the samples, and for this reason they were excluded from further analyses.

As Table 3 reflects, BPA concentrations were not significantly associated with diminished fecundity either in crude or adjusted models, independent of the way in which concentrations were considered (i.e., continuous, quartiles of BPA, or comparing the highest quartile with the three lower quartiles).

As for TCS, 1 standard deviation increase in the log transformed concentrations of TCS was associated with longer TTP, but the 95% confidence interval (CI) included 1 (FOR 0.94; 95% CI, 0.88–1.01). The same pattern was observed for the highest TCS quartile of exposure compared with the lowest quartile (FOR 0.89; 95% CI, 0.74–1.07). It is noteworthy that when we considered the three lower quartiles as the reference group, women in the highest quartile of TCS (>72 ng/mL) had evidence of decreased fecundity (FOR 0.84; 95% CI, 0.72–0.97) (Fig. 1; Table 3).

All phthalate metabolites had a similar pattern of association with TTP independent of the variable transformation or the variables included in the statistical models. In general, exposure to phthalates was suggestive of a shorter TTP, as indicated by FOR >1, although the 95% CI always included 1 (Table 3; Supplemental Table 1, available online). Total LMW and HMW metabolites were positively associated with TTP, although the values were not statistically significant

TABLE 2

Bisphenol A, triclosan, and phthalate metabolites (ng/mL) in maternal urine.

Analyte	LOD	< LOD, n (%)	Median	Minimum	Maximum	GM (95% CI)
Bisphenol A (BPA), n = 1,742	0.2	226 (13)	0.8	<LOD	130	0.78 (0.73–0.82)
Triclosan (TCS), n = 1,699	0.12	20 (0.1)	8.3	<LOD	6,784	11.93 (10.67–13.34)
Phthalate metabolites (n = 1,597)						
Low molecular weight						
Mono-*n*-butyl phthalate (MnBP)	0.20	4 (0.25)	12	<LOD	3,100	11.44 (10.78–12.15)
Mono-ethyl phthalate (MEP)	0.50	2 (0.13)	28	<LOD	13,000	32.09 (29.67–34.70)
Mono-benzyl phthalate (MBzP)	0.20	10 (0.63)	5	<LOD	420	5.10 (4.79–5.44)
Mono-methyl phthalate (MMP)	5.0	1,375 (86.1)	2.5	<LOD	1,000	3.03 (2.95–3.11)
Intermediate molecular weight						
Mono-cyclo-hexyl phthalate (MCHP)	0.20	1,482 (92.8)	0.1	<LOD	77	0.12 (0.11–0.12)
High molecular weight						
Mono-isononyl phthalate (MiNP)	0.40	1,574 (98.6)	0.2	<LOD	6.2	0.21 (0.20–0.21)
Mono-*n*-octyl phthalate (MnOP)	0.70	1,568 (98.2)	0.35	<LOD	7.9	0.36 (0.36–0.36)
Mono-(3-carboxypropyl) phthalate (MCPP)	0.20	290 (18.2)	0.93	<LOD	370	0.87 (0.81–0.93)
Mono-(2-ethylhexyl) phthalate (MEHP)	0.20	24 (1.5)	2.2	<LOD	340	2.27 (2.14–2.40)
Mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP)	0.20	5 (0.31)	6.5	<LOD	980	6.42 (6.06–6.81)
Mono-(2-ethyl-5-hydroxy-hexyl)phthalate (MEHHP)	0.40	14 (0.88)	9.4	<LOD	1,200	9.21 (8.65–8.79)

Note: CI = confidence interval; GM = geometric mean; LOD = limit of detection.

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(Supplemental Table 2, available online). Moreover, their FORs were of similar magnitude to those of the individual metabolites. The FORs according to the EEF were also of similar magnitude (data not shown).

We conducted a sensitivity analysis including the women with birth control failure. In the case of BPA, the concentrations were higher in women with birth control failures. The mean difference for the log transformed concentrations was statistically significant (P=.02). Including these women in the analysis did not change our results (adjusted FOR = 1.00; 95% CI, 0.93–1.07). In the case of TCS, concentrations were similar between women with birth control failures versus those without (P=.92), leading to similar results using the continuous (adjusted FOR = 0.96; 95% CI, 0.91–1.02) and dichotomized transformations (adjusted FOR = 0.85; 95% CI, 0.75–0.98). As for phthalate metabolites, concentrations were higher in women with birth control failures, reaching the statistical significance in the case of MBP and MBzP (P=.03). Including these women in the analyses did not change our results (MBP: adjusted FOR = 1.04; 95% CI, 0.96–1.14; MBzP: FOR = 1.04; 95% CI, 0.96–1.12).

DISCUSSION

The MIREC Study is the largest study to have assessed the effect of ubiquitous plasticizers such as BPA and phthalates on women's fecundity as measured by TTP, and it is the first to examine the potential effect of TCS. We found that urinary concentrations of TCS at the highest quartile of exposure were associated with a 16% reduction in fecundity. In addition, although BPA was not associated with TTP, it is noteworthy that in the case of phthalates the FORs were almost universally >1, suggesting a shorter TTP, although the 95% CI included 1.

Compared with the few studies available worldwide that have assessed concentrations of TCS in pregnant women, MIREC reported the highest urinary concentration of TCS (6,784 μg/L) but had considerably lower median concentrations than the other international studies (30). Higher socioeconomic class and older age were determinants of TCS exposure in the MIREC cohort (30). The MIREC population tended to be more highly educated than the population of women giving birth in Canada (27). Higher education may be associated with postponed childbirth, hence increasing age at the time of pregnancy attempt. Despite accounting for all these factors in our statistical models, decreased fecundity at the highest quartile of TCS exposure was maintained. Indeed, maternal and paternal age and smoking status, recognized determinants of fecundity, were similar through the quartiles of TCS exposure (data not shown).

Because this is the first study conducted at the population level assessing the impact of TCS on TTP, interpreting our findings in the context of the available literature is difficult. As recently reviewed by Dann and Hontela (13), TCS may have endocrine-disrupting effects. Several in vitro human cell-based assays have demonstrated the potential for TCS to act as an antiestrogen and/or antiandrogen (36–38). Animal studies with male rats (39, 40) have shown that TCS decreases serum levels of testosterone and the activity of several important steroidogenic enzymes. In addition, TCS has been shown to be a powerful inhibitor of estrogen sulfonation in sheep placental tissue (41), which could impair the maintenance of pregnancy.

Finally, the homeostasis of thyroid hormones, critical for reproductive success (42, 43), might also be a target of TCS. The structural similarity of TCS to thyroid hormones has prompted experimental studies on this domain (13). In vitro, TCS was capable of inhibiting sulfation of thyroid hormones (44). In animals, TCS exposure was associated with decreased levels of thyroxine (T_4) in female (45) and male rats (39). However, the human relevance of the rat thyroid studies has been questioned in the Health Canada

TABLE 3

Fecundability odds ratios (95% confidence intervals) for bisphenol A, triclosan, and phthalate metabolites.

Compound	N[a]	Unadjusted	Adjusted[b]	Adjusted[c]
Bisphenol A (BPA)				
BPA (ng/mL)[d]	1,742	0.99 (0.93–1.05)	0.99 (0.92 1.06)	1.0 (0.92–1.07)
BPA quartiles (ng/mL)				
0.1–0.3	436	1	1	1
0.4–0.8	448	0.99 (0.84–1.17)	0.98 (0.83–1.16)	0.98 (0.82–1.18)
0.81–1.7	450	1.02 (0.87–1.20)	1.00 (0.83–1.21)	0.96 (0.79–1.17)
≥1.8	408	0.93 (0.79–1.10)	0.91 (0.74–1.12)	0.95 (0.77–1.17)
BPA dichotomized (ng/mL)[e]				
<1.8	1,334	1	1	1
≥1.8	408	0.93 (0.81–1.07)	0.92 (0.79 1.07)	0.97 (0.83–1.14)
Triclosan (TCS)				
TCS (ng/mL)[d]	1,699	0.96 (0.91–1.02)	0.96 (0.90–1.02)	0.94 (0.88–1.01)
TCS quartiles (ng/mL)				
0.01–2.14	425	1	1	1
2.14–8.28	425	1.16 (0.98–1.37)	1.16 (0.98–1.39)	1.10 (0.91–1.31)
8.33–71.6	425	1.13 (0.95–1.33)	1.14 (0.95–1.36)	1.10 (0.92–1.32)
≥71.7	424	0.94 (0.79–1.11)	0.95 (0.79–1.13)	0.89 (0.74–1.07)
TCS dichotomized (ng/mL)[e]				
<71.7	1,275	1	1	1
≥71.7	424	0.86 (0.75–0.99)	0.86 (0.75–0.99)	0.84 (0.72–0.97)
Phthalate metabolites				
Continuous (ng/mL)[d]	1,597			
Mono-*n*-butyl phthalate (MnBP)		1.04 (0.97–1.10)	1.04 (0.95–1.14)	1.02 (0.93–1.12)
Mono-ethyl phthalate (MEP)		1.02 (0.96–1.08)	1.01 (0.94–1.08)	1.00 (0.93–1.08)
Mono-benzyl phthalate (MBzP)		1.05 (0.99–1.12)	1.06 (0.98–1.14)	1.02 (0.94–1.11)
Mono-(3-carboxypropyl) phthalate (MCPP)		1.04 (0.98–1.11)	1.05 (0.97–1.13)	1.08 (0.99–1.18)
Mono-(2-ethylhexyl) phthalate (MEHP)		1.04 (0.98–1.10)	1.04 (0.97–1.13)	1.04 (0.96–1.13)
Mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP)		1.04 (0.98–1.10)	1.04 (0.96–1.13)	1.07 (0.98–1.17)
Mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP)		1.03 (0.97–1.09)	1.02 (0.94–1.11)	1.06 (0.97–1.16)
Quartiles (ng/mL)				
Mono-*n*-butyl phthalate (MnBP)				
0.1–5.1	405	1	1	1
5.2–12	409	1.02 (0.86–1.21)	1.02 (0.85–1.22)	1.01 (0.84–1.22)
13–25	397	1.06 (0.89–1.26)	1.06 (0.86–1.31)	1.08 (0.87–1.35)
≥26	386	1.08 (0.91–1.28)	1.08 (0.84–1.38)	1.03 (0.80–1.33)
Mono-ethyl phthalate (MEP)				
0.25–11	416	1	1	1
12–28	388	0.93 (0.79–1.11)	0.92 (0.76–1.10)	0.89 (0.74–1.08)
29–89	397	0.95 (0.80–1.13)	0.93 (0.77–1.12)	0.88 (0.72–1.07)
≥90	396	1.09 (0.92–1.30)	1.06 (0.87–1.29)	1.01 (0.82–1.24)
Mono-benzyl phthalate (MBzP)				
0.1–2.2	402	1	1	1
2.3–5.0	403	0.98 (0.83–1.17)	0.99 (0.83–1.19)	0.90 (0.75–1.09)
5.1–12	405	1.10 (0.93–1.31)	1.11 (0.91–1.36)	1.03 (0.84–1.26)
≥13	387	1.10 (0.93–1.31)	1.12 (0.90–1.39)	1.00 (0.80–1.26)
Mono-(3-carboxypropyl) phthalate (MCPP)				
0.1–0.3	404	1	1	1
0.31–0.92	394	1.13 (0.95–1.35)	1.13 (0.94–1.35)	1.09 (0.91–1.31)
0.93–2.1	400	1.12 (0.94–1.33)	1.11 (0.91–1.35)	1.08 (0.88–1.33)
≥2.2	399	1.09 (0.92–1.29)	1.07 (0.86–1.34)	1.10 (0.87–1.38)
Mono-(2-ethylhexyl) phthalate (MEHP)				
0.1–1.0	402	1	1	1
1.1–2.2	414	1.08 (0.91–1.28)	1.08 (0.91–1.30)	1.07 (0.89–1.29)
2.3–4.4	386	1.08 (0.90–1.28)	1.09 (0.88–1.34)	1.06 (0.86–1.32)
≥4.5	395	1.13 (0.95–1.35)	1.15 (0.92–1.43)	1.13 (0.90–1.43)
Mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP)				
0.1–2.9	400	1	1	1
3.0–6.5	418	1.09 (0.92–1.29)	1.08 (0.90–1.30)	1.08 (0.89–1.30)
6.6–13	382	1.04 (0.88–1.25)	1.04 (0.84–1.29)	1.10 (0.87–1.37)
≥14	397	1.11 (0.93–1.31)	1.10 (0.86–1.40)	1.18 (0.92–1.53)

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TABLE 3

Continued.

Compound	N[a]	Unadjusted	Adjusted[b]	Adjusted[c]
Mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP)				
0.2–4.1	403	1	1	1
4.2–9.4	401	1.07 (0.90–1.27)	1.06 (0.88–1.27)	1.08 (0.89–1.31)
9.5–20	409	1.04 (0.88–1.24)	1.02 (0.82–1.26)	1.09 (0.87–1.36)
≥21	384	1.07 (0.90–1.28)	1.04 (0.82–1.33)	1.14 (0.89–1.47)

[a] Total numbers for unadjusted and specific gravity adjusted models.
[b] Adjusted for specific gravity.
[c] Adjusted for specific gravity, maternal age, maternal smoking, education, income, BMI. Due to missing values in some covariates, the N for BPA, TCS, and phthalate metabolites were 1,623, 1,583, and 1,491 respectively.
[d] Log transformed and rescaled by their standard deviation.
[e] Dichotomized as <75th percentile versus ≥75th percentile.

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assessment of TCS, which concludes that the overall database does not currently support effects of triclosan on thyroid function as a critical effect for risk characterization in humans (10). Our finding of decreased fecundity warrants additional epidemiologic studies at the population level as well as further work related to the possibility of a disrupting endocrine effect of TCS, as supported by some of these experimental studies, to elucidate the potential impact of TCS on human reproduction.

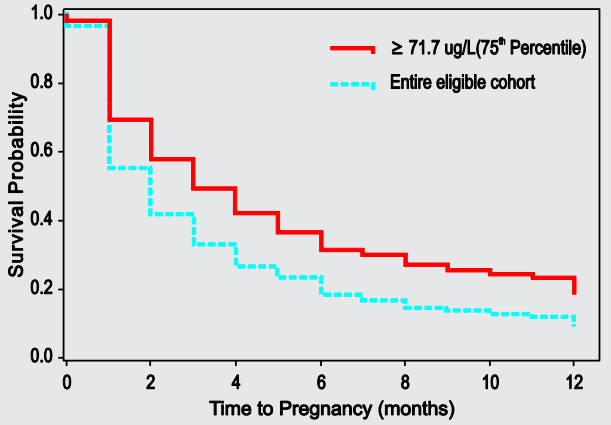
As for BPA, the only study that has assessed the effect of BPA on couple's fecundity reported similar results to ours. Neither female nor male BPA concentrations were associated with TTP (8). Our sample size is almost three times larger than the LIFE Study, so the lack of an association in the LIFE Study was likely not due to limited statistical power. However, low BPA exposure in both studies might explain the absence of association, if there truly is one. The geometric mean in our study (0.80 μg/L) and in the LIFE Study (0.63 μg/L) were lower than those reported in NHANES 2003–2004 for females ≥6 years of age (2.41 μg/L) (20) as well as in the Canadian Health Measures Surveys (CHMS) of 2007–2009 (1.26 μg/L) (46) and 2009–2011 (1.2 μg/L) for women 20–39 years of age (2), although caution should be taken when comparing these results because the substitution methods for concentrations below the LOD may differ among them. Because animal studies have suggested that BPA has endocrine-disruption capacity, additional epidemiologic studies in populations having higher exposures to BPA should be conducted before making firm conclusions about the absence of its effect on fecundity.

Altogether, it is interesting that although the experimental evidence of BPA being an endocrine modulator appears to be much stronger than for TCS, no effect of BPA was observed on TTP whereas an effect was observed for TCS. This finding may suggest another mechanism for impaired fecundity other than endocrine disruption or that the experimental models evaluated to date are not a good reflection of human fecundity. Alternatively, the association observed with triclosan may not be causal and due to other unknown factors.

In regards to phthalates, the interpretation of our results is even more challenging. Most of the FORs exceeded 1, suggesting a shorter TTP, although not statistically significant. In the LIFE Study, 9 out of the 14 metabolites assessed in women had FOR >1 in the adjusted models, however with the exception of MCPP, the CI also included 1 (5). Further, men's urinary concentrations of MMP, MnBP, and MBzP were associated with a longer TTP (FOR 0.80; 95% CI, 0.70–0.93; FOR 0.82; 95% CI, 0.70–0.97; and FOR 0.77; 95% CI, 0.65–0.92, respectively). In general, median phthalate metabolite concentrations in maternal urine in MIREC were comparable to those reported for women 20–39 years of age in cycle 2 of the CHMS (2009–2011) (2). Some phthalates may have estrogenic activity, although it is weak compared with 17β-estradiol (47).

In addition, experimental studies have reported antiandrogenic activity of some phthalates in vitro (48, 49) and in male rats (50). This antiandrogenic effect has been the focus of recent epidemiologic studies. For example in men, MEHP and diisononyl phthalate (DiNP) have been associated with decreased testosterone production (51); in women, MnBP, mono-isobutyl phthalate, MBzP, and the sum of metabolites of DEHP and of DiNP have been associated with delayed pubarche (52). Furthermore, a recent case control study reported

FIGURE 1



Time-to-pregnancy distribution for the cohort and those women with urinary triclosan concentrations ≥71.7 μg/L (75th percentile), adjusting for specific gravity, age, smoking, education, income, and body mass index.

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a decreased likelihood of polycystic ovary syndrome, a condition characterized by hyperandrogenemia, in women with higher concentrations of MBzP and MnBP (53). In pregnant women, concentrations of DEHP metabolites were associated with decreased testosterone among all women and between MnBP and testosterone among women carrying a female fetus (54). Thus, our finding of a shorter TTP at higher concentrations of phthalates is consistent with this previous evidence of a potential antiandrogenic effect of some of these compounds.

Our study has important limitations that need to be considered. Because this is a pregnancy-based TTP study, women who were infertile and/or did not have access to infertility treatment were excluded by design from our study. Thus, if BPA or phthalates have a negative impact on TTP, women with the highest exposures would have been excluded from our study. In addition, we measured the concentrations of chemicals only in women, and the process of reproduction involves not only the female and male partner individually, but also many factors that are couple mediated. Furthermore, we are assuming that the concentrations measured during the first trimester of pregnancy represent the concentrations that were present during the preconception period. In this regard, Braun et al. (55) evaluated the variability of urinary phthalate metabolites and BPA concentrations before and during pregnancy in a cohort of women receiving infertility treatment. The study found that the absolute differences in urinary concentrations for these chemicals were relatively small, which according to the authors might suggest that women did not change their preconception behaviors to reduce exposure to these chemicals during pregnancy (55). Nonetheless, using the intraclass correlation coefficients, the reliability of a spot urine sample to predict exposure over a few months is limited for repeated measures of BPA (56, 57) and several phthalate metabolites (55, 58–60). In regards to TCS, the reliability seems to be better (57, 61, 62). Another possibility is that concentrations of these chemicals could be metabolized differently before or during pregnancy due to the physiologic changes occurring during this period (63). Braun et al. (55) did not observe a consistent change in most phthalate metabolites or BPA during pregnancy, suggesting that urinary concentrations of these compounds might not be influenced by pregnancy-induced changes in pharmacokinetics, assuming that sources of exposure remained constant over the pregnancy.

Digit preference reporting is another limitation of pregnancy-based TTP studies (32); however, it is estimated that stable estimates of the TTP distribution can be obtained with approximately 200 values per exposure group (64), a number that, due to our large sample size, was always attained in the different categories of exposure. Furthermore, to evaluate whether digit preference had any effect on our results, we applied the method recently proposed in McLain et al. (65). We estimated a piecewise exponential model with three separate knot scenarios, each using seven knots; the locations for knot scenarios were $\{1,2,4,9,18,30,\infty\}$, $\{1,2,4,9,15,27,\infty\}$, and $\{1,2,4,10,17,29,\infty\}$. The estimates showed little bias (data not shown), suggesting that digit preference had little impact in our results.

Additional potential limitations in the exposure assessment need to be considered. First, no exposure data were available for 2% of the eligible women for BPA, 5% for TCS, and 10% for phthalates. It is considered that complete case analysis is unlikely to introduce bias when the incomplete cases are less than about 5% (66). In most of the cases, there was no laboratory result because the woman did not provide sufficient urine for all the chemical analyses that were done. More phthalate results were missing because they were analyzed in the second aliquot of urine, whereas BPA was analyzed in the first. We analyzed TCS in the first aliquot, but we lost 2% of women who did not consent to further analyses of the biobanked specimens. We consider that the missing values for phthalates as consequence of being measured in the second aliquot of urine are independent of both observed and unobserved data, which is defined in the literature as "missing completely at random" (MCAR), in which case, complete case analysis is an acceptable approach (67).

Another limitation is that concentrations below the LOD were set to the LOD divided by 2. It has been suggested that this practice may lead to increased bias and an underestimation of the error variance, which results in lowered power for statistical hypothesis testing (68, 69). However, simulation studies using alternative methods to account for exposures below the LOD, have demonstrated that the LOD divided by 2 worked fairly well in simulations with $\leq$50% exposure data below the LOD (68). Methods have also been proposed for Cox regression models with covariates subject to a lower LOD, but they have not provided much improvement over the LOD divided by 2 (69).

In our study, TCS and five phthalates metabolites (MnBP, MEP, MBzP, MEHP, MEOHP, and MEHHP), were detected in more than 98% of the samples, which suggests that the probability of bias due to our substitution approach is very low for these particular chemicals. In the case of BPA and MCPP, the detection rates were also high (87% and 82%, respectively), which is reassuring. On the other hand, four phthalate metabolites were detectable in less than 14% of the samples (MMP, MCHP, MiNP, and MnOP). These metabolites were excluded from further analyses, an approach used in large biomonitoring surveys when the proportion of results below the LOD is greater than 40% (20). However, an analysis of these metabolites as continuous variables showed that the adjusted FORs were approximately 1, although not statistically significant for MMP (FOR 1.03; 95% CI, 0.96–1.10), MiNP (FOR 1.0; 95% CI, 0.94–1.06), and MnOP (FOR 1.02; 95% CI, 0.96–1.09) using the continuous scale. In the case of MCHP, the FOR was $<$1, but the 95% CI was large and not statistically significant (FOR 0.93; 95% CI, 0.72–1.18).

In summary, our data suggest that elevated TCS exposure ($>$72 ng/mL) may be associated with diminished fecundity, as suggested by a longer TTP. In regards to phthalates and BPA, we found no evidence of a negative impact on TTP and even some suggestion that exposure to some phthalates might be associated with a shorter TTP. Further studies are necessary to test our findings and elucidate the potential impact of nonpersistent environmental contaminants on human fecundity.

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SUPPLEMENTAL TABLE 1

Fecundability odds ratios (95% confidence intervals) for phthalate metabolites (dichotomized).

Quartiles dichotomized (ng/mL)[d]	N[a]	Unadjusted	Adjusted[b]	Adjusted[c]
Mono-*n*-butyl phthalate (MnBP)				
<26	1,211	1	1	1
≥26	386	1.05 (0.91–1.21)	1.03 (0.87–1.23)	0.98 (0.82–1.17)
Mono-ethyl phthalate (MEP)				
<90	1,201	1	1	1
≥90	396	1.14 (0.99–1.31)	1.13 (0.97–1.31)	1.11 (0.95–1.30)
Mono-benzyl phthalate (MBzP)				
<13	1,210	1	1	1
≥13	387	1.07 (0.93–1.24)	1.06 (0.91–1.24)	1.02 (0.87–1.21)
Mono-(3-carboxypropyl) phthalate (MCPP)				
<2.2	1,198	1	1	1
≥2.2	399	1.01 (0.87–1.16)	0.98 (0.83–1.15)	1.03 (0.87–1.21)
Mono-(2-ethylhexyl) phthalate (MEHP)				
<4.5	1,202	1	1	1
≥4.5	395	1.08 (0.93–1.24)	1.07 (0.91–1.25)	1.07 (0.91–1.26)
Mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP)				
<14	1,200	1	1	1
≥14	397	1.06 (0.92–1.22)	1.04 (0.88–1.23)	1.09 (0.92–1.30)
Mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP)				
<21	1,213	1	1	1
≥21	384	1.04 (0.90–1.20)	1.01 (0.86–1.20)	1.10 (0.89–1.26)

[a] Total numbers for unadjusted and specific gravity adjusted models.
[b] Adjusted for specific gravity.
[c] Adjusted for specific gravity, maternal age, maternal smoking, education, income, BMI. Due to missing values in some covariates, the N was 1,491.
[d] Dichotomized as <75th percentile versus ≥75th percentile.

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SUPPLEMENTAL TABLE 2

Fecundability odd ratios (95% confidence intervals) for total phthalate metabolites (μmol/L).

Variable	N[a]	Unadjusted	Adjusted[b]	Adjusted[c]
Continuous variable (μmol/L)[d]	1,597			
Low molecular weight		1.03 (0.97–1.09)	1.02 (0.95–1.10)	1.02 (0.94–1.10)
High molecular weight		1.03 (0.97–1.10)	1.03 (0.95–1.13)	1.07 (0.98–1.17)
Quartiles (μmol/L)				
Low molecular weight				
0.005–0.12	400	1	1	1
0.12–0.28	399	0.92 (0.77–1.09)	0.90 (0.75–1.08)	0.90 (0.74–1.09)
0.28–0.69	399	0.92 (0.76–1.10)	0.90 (0.73–1.09)	0.86 (0.70–1.06)
≥0.69	399	1.06 (0.89–1.27)	1.02 (0.82–1.26)	0.98 (0.78–1.23)
High molecular weight				
0.002–0.03	400	1	1	1
0.03–0.07	399	1.03 (0.87–1.23)	1.03 (0.86–1.24)	1.02 (0.85–1.24)
0.07–0.14	399	1.06 (0.89–1.26)	1.05 (0.85–1.31)	1.12 (0.90–1.41)
≥0.14	399	1.08 (0.91–1.29)	1.08 (0.85–1.37)	1.17 (0.91–1.51)
Quartiles dichotomized (μmol/L)[e]				
Low molecular weight				
<0.69	1,198			
≥0.69	399	1.13 (0.98–1.30)	1.12 (0.96–1.31)	1.10 (0.94–1.29)
High molecular weight				
<0.14	1,198			
≥0.14	399	1.03 (0.88–1.22)	1.04 (0.88–1.22)	1.08 (0.91–1.29)

[a] Total numbers for unadjusted and specific gravity adjusted models
[b] Adjusted for specific gravity.
[c] Adjusted for specific gravity, maternal age, maternal smoking, education, income, BMI. Due to missing values in some covariates, the N was 1,491.
[d] Log transformed and rescaled by their standard deviation.
[e] Dichotomized as <75th percentile versus ≥75th percentile.

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