Human Reproduction, Vol.38, No.1, pp. 139–155, 2023
Advance Access Publication on November 8, 2022 https://doi.org/10.1093/humrep/deac230

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Urinary phenol concentrations and fecundability and early pregnancy loss

Ana K. Rosen Vollmar [1,*], Clarice R. Weinberg[2], Donna D. Baird[3], Allen J. Wilcox[3], Antonia M. Calafat [4], Nicole C. Deziel[1], Caroline H. Johnson [1], and Anne Marie Z. Jukic [3]

[1]Environmental Health Sciences, Yale School of Public Health, New Haven, CT, USA [2]Biostatistics & Computational Biology Branch, National Institute of Environmental Health Sciences, Research Triangle, NC, USA [3]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle, NC, USA [4]Organic Analytical Toxicology Branch, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

*Correspondence address. Environmental Health Sciences, Yale University School of Public Health, 60 College Street, New Haven, CT 06520, USA. E-mail: ana.rosenvollmar@aya.yale.edu https://orcid.org/0000-0002-8009-0258

Submitted on June 02, 2022; resubmitted on September 22, 2022; editorial decision on October 05, 2022

STUDY QUESTION: Are urinary phenol concentrations of methylparaben, propylparaben, butylparaben, triclosan, benzophenone-3, 2,4-dichlorophenol or 2,5-dichlorophenol associated with fecundability and early pregnancy loss?

SUMMARY ANSWER: 2,5-dichlorophenol concentrations were associated with an increased odds of early pregnancy loss, and higher concentrations of butylparaben and triclosan were associated with an increase in fecundability.

WHAT IS KNOWN ALREADY: Phenols are chemicals with endocrine-disrupting potential found in everyday products. Despite plausible mechanisms of phenol reproductive toxicity, there are inconsistent results across few epidemiologic studies examining phenol exposure and reproductive function in non-fertility treatment populations.

STUDY DESIGN, SIZE, DURATION: Specimens and data were from the North Carolina Early Pregnancy Study prospective cohort of 221 women attempting to conceive naturally from 1982 to 1986. This analysis includes data from 221 participants across 706 menstrual cycles, with 135 live births, 15 clinical miscarriages and 48 early pregnancy losses (before 42 days after the last menstrual period).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Participants collected daily first-morning urine specimens. For each menstrual cycle, aliquots from three daily specimens across the cycle were pooled within individuals and analyzed for phenol concentrations. To assess sample repeatability, we calculated intraclass correlation coefficients (ICCs) for each phenol. We evaluated associations between phenol concentrations from pooled samples and time to pregnancy using discrete-time logistic regression and generalized estimating equations (GEE), and early pregnancy loss using multivariable logistic regression and GEE.

MAIN RESULTS AND THE ROLE OF CHANCE: ICCs for within-person variability across menstrual cycles in pooled phenol concentrations ranged from 0.42 to 0.75. There was an increased odds of early pregnancy loss with 2,5-dichlorophenol concentrations although the CIs were wide (5th vs 1st quintile odds ratio (OR): 4.79; 95% CI: 1.06, 21.59). There was an increased per-cycle odds of conception at higher concentrations of butylparaben (OR: 1.62; 95% CI: 1.08, 2.44) and triclosan (OR: 1.49; 95% CI: 0.99, 2.26) compared to non-detectable concentrations. No associations were observed between these endpoints and concentrations of other phenols examined.

LIMITATIONS, REASONS FOR CAUTION: Limitations include the absence of phenol measurements for male partners and a limited sample size, especially for the outcome of early pregnancy loss, which reduced our power to detect associations.

WIDER IMPLICATIONS OF THE FINDINGS: This study is the first to use repeated pooled measures to summarize phenol exposure and the first to investigate associations with fecundability and early pregnancy loss. Within-person phenol concentration variability underscores the importance of collecting repeated samples for future studies. Exposure misclassification could contribute to differences between the findings of this study and those of other studies, all of which used one urine sample to assess phenol exposure. This study also contributes to the limited literature probing potential associations between environmental exposures and early pregnancy loss, which is a challenging outcome to study as it typically occurs before a pregnancy is clinically recognized.

STUDY FUNDING/COMPETING INTEREST(S): This research was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health (award number F31ES030594), the Intramural Research Program of the National Institutes of Health, the National Institute of Environmental Health Sciences (project numbers ES103333 and ES103086) and a doctoral fellowship at the Yale School of Public Health. The authors declare they have no competing interests to disclose.

TRIAL REGISTRATION NUMBER: N/A.

Key words: phenols / fecundability / time to pregnancy / early pregnancy loss / reproductive function

Introduction

Phenols are a class of chemicals with endocrine disrupting potential. Both *in vitro* and *in vivo* studies suggest phenols can influence reproductive function through estrogenic (Karpuzoglu *et al.*, 2013; Kim and Choi, 2014; Du *et al.*, 2021; Hu *et al.*, 2021), antiestrogenic (Dann and Hontela, 2011; Kim and Choi, 2014), antiandrogenic (Dann and Hontela, 2011; Hu *et al.*, 2021) and thyroid disruptive effects (Dann and Hontela, 2011; Wolff *et al.*, 2015; Aker *et al.*, 2016). This study focuses on seven phenols, methylparaben, propylparaben, butylparaben, triclosan, benzophenone-3, 2,4-dichlorophenol and 2,5-dichlorophenol, which have been used in the manufacture of everyday items for decades, and to which exposure is widespread (Kutz *et al.*, 1992; Calafat *et al.*, 2008, 2010; Ye *et al.*, 2014; Hines *et al.*, 2015; Han *et al.*, 2016).

Parabens have been added as antimicrobial preservatives to foods, cosmetics, personal care products and pharmaceuticals since the 1930s (CIR Expert Panel, 2008; Vandenberg and Bugos, 2021). Triclosan is used as an antibacterial ingredient in soaps, cosmetics, toothpastes and other personal care products (Dann and Hontela, 2011). Benzophenone-3 is an ultraviolet radiation filter added to sunscreens, cosmetics and plastics since the 1970s (Dann and Hontela, 2011; Kim and Choi, 2014; Ghazipura *et al.*, 2017). 2,4-dichlorophenol is a metabolite of the common herbicide 2,4-dichlorophenoxyacetic acid, which has been in use since the 1940s (Kutz *et al.*, 1992). 2,5-dichlorophenol is a metabolite of 1,4-dichlorobenzene, which is used in mothballs, mold/mildew fumigants and room deodorizers (Ye *et al.*, 2014). Although many phenols are rapidly metabolized and excreted, intermittent and repeated exposures, including throughout the day, may lead to endocrine disruption (Karpuzoglu *et al.*, 2013; Hipwell *et al.*, 2019). While the reproductive toxicity of bisphenol A has received considerable attention, the reproductive impact of exposure to other phenols has been less studied (Diamanti-Kandarakis *et al.*, 2009; Zoeller *et al.*, 2012; Jukic *et al.*, 2016; Minguez-Alarcon and Gaskins, 2017; Hipwell *et al.*, 2019; Green *et al.*, 2021).

Despite plausible mechanisms by which phenols could impact fecundability and pregnancy loss, epidemiologic studies of the phenols included in this study are scarce (Chen *et al.*, 2013; Buck Louis *et al.*, 2014; Vélez *et al.*, 2015; Wang *et al.*, 2015; Smarr *et al.*, 2017). In non-fertility treatment populations, suggestive findings include reduced fecundability with methylparaben (Smarr *et al.*, 2017) and triclosan exposure (Vélez *et al.*, 2015), and increased odds of mid-pregnancy miscarriage with triclosan exposure (Wang *et al.*, 2015). Other studies reported no association between fecundability and women's exposure to benzophenone-3, triclosan, propylparaben or butylparaben (Buck Louis *et al.*, 2014; Vélez *et al.*, 2015; Smarr *et al.*, 2017), and no association between benzophenone-3 exposure and miscarriage (Chen *et al.*, 2013). An important limitation of all of these studies is the use of a single biospecimen to assess exposure for each person. A measure at one point in time could cause measurement error that might attenuate estimates of association. Such error would be expected because of the intermittent nature of exposures and the rapid metabolism and excretion of phenols, leading to high temporal variability of the chemical biomarker concentrations, and possible mis-timing of exposure assessment in relation to health outcomes (Smith *et al.*, 2012; Bertelsen *et al.*, 2014; Engel *et al.*, 2014; Johns *et al.*, 2015; Weiss *et al.*, 2015; Calafat, 2016; Cox *et al.*, 2016; Pollack *et al.*, 2016; Aylward *et al.*, 2017).

Subfertility can have major physical, emotional and financial consequences (Bloom *et al.*, 2016; Hipwell *et al.*, 2019; Green *et al.*, 2021). Approximately 30% of cases of infertility are unexplained (Bitler and Schmidt, 2006; Jain, 2006; Nachtigall, 2006; Bloom *et al.*, 2016). While delayed childbearing, obesity, smoking, sexually transmitted infections and stress are all known contributors to declines in fertility (Petraglia *et al.*, 2013), environmental exposures are also hypothesized to be of importance and are included in public health strategies for addressing infertility (Centers for Disease Control and Prevention, 2014; Bloom *et al.*, 2016). However, research in humans on the effects of endocrine disruptors on fertility and fecundability remains limited (Rattan *et al.*, 2017; Hipwell *et al.*, 2019; Green *et al.*, 2021). If phenols contribute to decreased fecundability or early pregnancy loss, then minimizing preconception exposure to common phenol-containing consumer products could have substantial public health benefits.

This study uses daily preconception urine specimens to assess the phenol exposure of individuals across each menstrual cycle. The study estimates the intra-individual and inter-individual variability of longitudinal, menstrual cycle-specific urinary phenol concentrations and prospectively examines associations between preconception urinary phenol concentration and fecundability and early pregnancy loss. To our knowledge, two of the phenols we examine here, 2,4-dichlorophenol and 2,5-dichlorophenol, have not been previously studied in relation to fecundability, and there are no published studies of early pregnancy loss considering the phenols included in this study.

Materials and methods

Study sample

This analysis was conducted within the Early Pregnancy Study (EPS), a prospective cohort study of early pregnancy loss that enrolled 221 women, in North Carolina from 1982 to 1986, who were attempting to conceive. That study has been described previously in detail (Wilcox *et al.*, 1985, 1988b). Participants reported no known fertility problems, and entered the study when they stopped using contraception in order to conceive. They collected daily first-morning urine

samples for 6 months if they did not conceive a clinical pregnancy, or until 8 weeks after the start of their last menstrual period (LMP) if they had a recognized clinical pregnancy. Participants completed an in-person intake interview, which gathered information on demographic, medical, reproductive and behavioral characteristics. During follow-up, they kept a daily log of menstrual bleeding and unprotected sexual intercourse (Wilcox *et al.*, 1985). The urine samples were later used to determine participants' phenol exposure status. The study protocol and the measurement of phenols for this study were approved by the National Institute of Environmental Health Sciences (NIEHS) institutional review board (IRB #000058). The analysis of de-identified specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research, and the analyses for this study were determined to be not human subjects research by the Yale University institutional review board (ID 2000022845).

Identification of pregnancy

Pregnancy was defined as a human chorionic gonadotropin (hCG) level of >0.025 ng/ml for three or more consecutive days (Wilcox *et al.*, 1988b). When a pregnancy was identified, the day of ovulation (and presumed conception) was determined by an algorithm using the ratio of estrone-3-glucuronide, an estrogen metabolite, and pregnanediol-3-glucuronide, a progesterone metabolite (Baird *et al.*, 1991; Ecochard *et al.*, 2001). The day of implantation was defined as the first day of the pregnancy where hCG was ≥0.01 ng/ml, and persisted above this level.

Menstrual cycles and conception attempts

The 221 participants contributed a total of 740 menstrual cycles. After excluding 7 anovulatory cycles and 27 cycles without unprotected intercourse during the week ending on the identified day of ovulation, this analysis included 706 cycles: 508 non-conception and 198 conception cycles. Of the conception cycles, 135 resulted in a live birth, 15 in a clinical miscarriage (onset of bleeding at 6–25 gestational weeks, including one ectopic and one molar pregnancy) and 48 in an early pregnancy loss (loss-associated bleed beginning before 42 days post-LMP) (Wilcox *et al.*, 1988b, 1990). These 706 cycles included 264 pregnancy attempts, defined as a series of menstrual cycles ending in conception or the conclusion of participation if a participant did not conceive. Most participants contributed just one pregnancy attempt (n = 180), but 41 who had an early loss continued in the study without realizing that they had conceived, and consequently contributed more than one pregnancy attempt.

Urine specimen collection, storage and pooling

Daily first-morning urine specimens were collected in preservative-free, polypropylene containers and stored in home freezers for up to 2 weeks. Study personnel transported the containers on ice to a central storage location, where they were kept at −20°C. After initial hormone assays, specimens were moved to −80°C for long-term, temperature-controlled storage (Jukic *et al.*, 2016). In 2010, an equal aliquot was drawn from each of three daily specimens collected approximately one week apart across a given menstrual cycle and pooled to allow estimates of environmental exposures over the course of the full menstrual cycle (Jukic *et al.*, 2016). In most cases, aliquots were drawn from Monday urine specimens since participants collected two vials on Mondays, resulting in more urine available for analysis. In addition to being spaced about one week apart, other constraints on specimen selection included that urine specimens be from after the end of menstrual bleeding, and reflect both the follicular and luteal phases (Jukic *et al.*, 2016). All pooled samples are from either non-conception menstrual cycles, or the conception cycle. Non-conception pooled samples include two specimens from after the end of menses but before ovulation, and one specimen from after ovulation. Conception cycle pooled samples contain two specimens from after the end of menses and before ovulation, and one specimen from after ovulation/conception but before implantation, which typically occurs 8–10 days after ovulation (Wilcox *et al.*, 1999).

Phenol exposure assessment

Phenol measurement

Because phenols are rapidly metabolized and urinary concentrations exhibit relatively high intra-individual variability (Smith *et al.*, 2012; Engel *et al.*, 2014; Perrier *et al.*, 2016; Pollack *et al.*, 2016; Aylward *et al.*, 2017; Vernet *et al.*, 2018), pooled rather than single urine samples were analyzed to better represent phenol exposure over the course of a menstrual cycle. Total urinary concentrations (ng/ml) of methylparaben, propylparaben, butylparaben, triclosan, benzophenone-3, 2,4-dichlorophenol and 2,5-dichlorophenol were quantified in pooled samples at the CDC in 2010 using a modification of the high-performance liquid chromatography-isotope dilution-tandem mass spectrometry method reported in Ye *et al.* (2005). Results on other chemicals evaluated at the same time have been published previously (Nepomnaschy *et al.*, 2009; Baird *et al.*, 2010; Jukic *et al.*, 2016; Chin *et al.*, 2019). Creatinine concentration (mg/dl) was measured in the same pooled samples using a spectrophotometric procedure (Jaffe reaction) on an Olympus AU400e chemistry analyzer, and phenol concentrations were creatinine-adjusted (μg/g creatinine) to account for varying urinary dilution by dividing the measured phenol concentration (μg/l) by the creatinine concentration (mg/dl) of a given sample and multiplying by 100 (Barr *et al.*, 2005; MacPherson *et al.*, 2018). Compared to adjustment using creatinine measured in the individual samples comprising a pool and then averaged, such direct measurement of creatinine in pooled samples for adjustment of pooled assessment of an analyte produces minimal bias (Rosen Vollmar *et al.*, 2021).

Quality control

At the time of the analysis at the CDC, a total of ten random individual (non-pooled) quality control samples from this study were used to evaluate the integrity of the specimens by measuring the free and total concentrations of all seven phenols. Because these phenols excrete mainly as urinary conjugates, relatively high percentages of unconjugated or free phenols are suggestive of specimen degradation or contamination during collection, storage or handling practices (Guidry *et al.*, 2015). To our knowledge, the samples experienced at least two freeze-thaw cycles before measurement. When the free phenol concentration is <20% of the total amount detected, specimen degradation or contamination is considered unlikely (Guidry *et al.*, 2015). The percentage of all free biomarkers was below the limit of detection

(LOD) or <20% for 97% of the samples, suggesting systematic specimen degradation or contamination was unlikely. Pooled samples missing a phenol concentration because of other quality control matters (e.g. concentration higher than the highest calibrator, carry-over) were excluded from the analysis (1–6% of samples for each phenol, see Supplementary Table SI). Additionally, a previous study on the EPS cohort reported that bisphenol-A, which was measured in the same samples and at the same time as the phenols included here, was stable during 22–24 years of long-term subfreezing temperature-controlled storage, supporting the validity of the phenol measurements used in this study (Nepomnaschy *et al.*, 2009).

Concentrations below the LOD

We imputed the concentration of phenols <LOD for propylparaben ($LOD_{propylparaben}$ = 0.2 ng/ml, n = 1 (0.1%) pooled sample), benzophenone-3 ($<LOD_{benzophenone\text{-}3}$ = 0.4 ng/ml, n = 105 (15%)) and 2,4-dichlorophenol ($LOD_{2,4\text{-}dichlorophenol}$ = 0.2 ng/ml, n = 4 (0.6%)) with a single imputation using a maximum likelihood procedure assuming a log-normal distribution based on the LOD-truncated distribution detectable of phenol measurements, as has been recommended for applications when ≤30% of the data are non-detectable (Lubin *et al.*, 2004). Because of the relatively large number of non-detectable concentrations for butylparaben ($LOD_{butylparaben}$ = 0.2 ng/ml, n = 320 (45%)) and triclosan ($LOD_{triclosan}$ = 2.3 ng/ml, n = 344 (49%)), we did not impute values <LOD (Lubin *et al.*, 2004). Instead, we *a priori* planned to use a categorical structure to model these phenols, with measurements <LOD comprising the lowest concentration category.

Phenol concentration categories

To determine how best to model each phenol, we looked at unadjusted associations with fecundability, including continuous and categorical (e.g. deciles, quintiles) structures. The final form was chosen based on visual inspection (by selecting forms with as few categories as possible that reflected any unadjusted associations observed in continuous structures or forms with many categories, see Supplementary Figs S1, S2, S3, S4, S5, S6 and S7) and the lowest value of the Akaike Information Criterion (AIC). This procedure led to categorization in quintiles for the following phenols with higher (>85%, Supplementary Table SI) detection frequencies: methylparaben, propylparaben, benzophenone-3, 2,4-dichlorophenol and 2,5-dichlorophenol. For butylparaben and triclosan, more than 44% of concentrations were <LOD and we used a three-level categorical variable to capture potential nonlinear exposure–response relationships, as can be characteristic of endocrine-disruptors (Vandenberg *et al.*, 2012). Concentrations <LOD were used as the reference or low concentration group. Measurements >LOD were modeled in multiple categorical structures to determine medium and high concentration groups, leading to categorization in three groups: <LOD, LOD to median and >median, where the median refers to the median among detectable concentrations.

Covariates

Potentially confounding covariates were selected for inclusion *a priori* based on a directed acyclic graph (Supplementary Fig. S8), previous analyses of the EPS cohort (Wilcox *et al.*, 1988a, 1990; Weinberg *et al.*, 1989, 1994; Jukic *et al.*, 2016) and other literature that examined fecundability or pregnancy loss and phenol exposure in non-fertility treatment settings (Chen *et al.*, 2013; Buck Louis *et al.*, 2014; Vélez *et al.*, 2015; Wang *et al.*, 2015; Smarr *et al.*, 2017). To determine how to parameterize covariates, we looked at unadjusted associations with fecundability (Supplementary Table SII) using continuous structures where possible, or clinically meaningful categories and percentiles (e.g. quartiles, tertiles). Final forms were determined using visual inspection and the lowest value of the AIC. Covariates for the time to pregnancy analysis were age (continuous, linear), BMI (kg/m^2 in three categories defined by the clinical categories of low (<18.5), normal (18.5–24.9) and high (≥25)), race/ethnicity (in two categories of white (n = 212) and non-white (Black, n = 6; Asian/Pacific Islander, n = 2; or other, n = 1)), smoking at enrollment (yes/no), alcohol use at enrollment (drinks/month in three categories based on the 25th and 75th percentiles) and season of a pooled sample's median aliquot date (in four categories). Covariates for the early pregnancy loss model were age, BMI, smoking, alcohol use, caffeine at enrollment (in tertiles) and season. For summary statistics, we calculated medians and interquartile ranges (IQRs) for phenol concentrations for the whole cohort and stratified by participant characteristics, using the first pooled sample (Table I). All available and eligible pooled samples were used for other analyses.

Statistical analysis

All analyses were carried out in SAS 9.4, using PROC GENMOD and PROC LOGISITC for analyses of association.

Phenol concentration distribution and variability

We calculated the percentiles of each urinary phenol (μg/g creatinine) across the cohort, and compared these to those of other non-pregnant reproductive-aged women, including from the National Health and Nutrition Examination Survey (NHANES). We used Spearman correlation coefficients to describe the correlation between phenols, and we described the within-subject correlation across cycles with intraclass correlation coefficients (ICCs). For butylparaben and triclosan, we calculated ICCs restricted to those samples with detectable concentrations. As participants who took longer to conceive could have changed their behavior over time, modifying potential phenol exposures, we used a mixed effects linear regression model with a random intercept for each participant to assess whether there were associations between menstrual cycle number (1–7) and phenol concentration (continuous, μg/g creatinine, natural log-transformed), with no other covariates included.

Time to pregnancy

We used a discrete-time logistic regression model and generalized estimating equations (GEE) to estimate per-cycle fecundability odds ratios (FORs), including 706 menstrual cycles, 264 pregnancy attempts and 198 conceptions. An FOR < 1 indicates reduced per-cycle odds of conception, and an FOR > 1 indicates increased odds. The model included categorical urinary phenol concentrations, covariates and the cycle number of attempt, which reset to 1 after each early loss and was modeled categorically. Cycle numbers 8 and 9 only occurred in a single participant, so that attempt was truncated at cycle 7. Participants had multiple pregnancy attempts (1, 2 or 3), and the model accounted for this clustering using a generalized linear model estimated with GEE and a compound symmetric covariance structure (Williamson *et al.*, 2003). A weight statement was included, with the

Table I Urinary phenol concentration (μg/g creatinine) in pooled samples by participant characteristic.

Characteristic	Participants, *n*	Methylparaben median (IQR)	Propylparaben median (IQR)	Butylparaben median (IQR)	Triclosan median (IQR)	Benzo-phenone-3 median (IQR)	2,4-dichloro-phenol median (IQR)	2,5-dichloro-phenol median (IQR)
Full cohort	221	90.8 (39.5, 185.6)	29.5 (10.3, 75.6)	0.2 (<LOD, 1.8)	<LOD (<LOD, 6.3)	1.7 (0.5, 7.3)	1.2 (0.6, 2.5)	23.5 (13.1, 47.8)
Age (years)								
21–27	88	77.5 (30.5, 185.6)	25.0 (11.0, 95.0)	0.2 (<LOD, 1.7)	<LOD (<LOD, 8.4)	1.4 (0.4, 6.8)	1.4 (0.6, 3.3)	28.1 (14.9, 51.6)
28–30	71	91.7 (47.3, 185.6)	29.0 (9.5, 70.1)	<LOD (<LOD, 1.4)	<LOD (<LOD, 6.3)	2.0 (0.7, 6.5)	1.3 (0.7, 3.4)	24.7 (12.8, 74.6)
31–42	62	111.2 (47.9, 190.6)	30.2 (11.2, 62.0)	0.3 (<LOD, 2.9)	<LOD (<LOD, 6.3)	2.7 (0.6, 12.1)	0.9 (0.6, 1.5)	16.7 (10.7, 35.4)
BMI (kg/m^2)								
Underweight (<18.5)	21	61.7 (25.3, 168.7)	35.2 (4.0, 75.6)	<LOD (<LOD, 1.0)	3.1 (<LOD, 15.2)	1.3 (0.4, 13.0)	0.8 (0.4, 1.1)	16.6 (11.0, 22.7)
Normal weight (18.5–24.9)	175	96.5 (42.0, 195.6)	30.9 (12.9, 82.3)	0.2 (<LOD, 1.7)	<LOD (<LOD, 6.3)	1.7 (0.5, 4.5)	1.3 (0.7, 2.9)	26.0 (13.5, 60.9)
Overweight (≥25.0)	25	63.9 (34.6, 136.3)	20.0 (8.5, 34.9)	0.4 (<LOD, 3.3)	<LOD (<LOD, 3.5)	3.6 (0.8, 20.5)	1.1 (0.5, 1.8)	23.6 (13.1, 39.3)
Race/ethnicity								
White	212	90.8 (40.2, 184.3)	29.6 (11.0, 75.6)	0.2 (<LOD, 1.8)	<LOD (<LOD, 6.4)	1.8 (0.5, 7.5)	1.2 (0.6, 2.5)	23.5 (12.8, 47.8)
Black (n = 6), Asian/Pacific Islander (n = 2), other (n = 1)	9	126.5 (31.7, 308.6)	20.7 (6.0, 53.9)	<LOD (<LOD, 0.2)	<LOD (<LOD, 2.5)	0.5 (0.2, 3.8)	3.8 (1.7, 43.6)	51.2 (27.8, 93.2)
Smoking at intake								
No	208	91.2 (40.2, 185.6)	29.9 (11.0, 79.8)	0.2 (<LOD, 1.8)	<LOD (<LOD, 7.0)	1.9 (0.5, 9.0)	1.2 (0.6, 2.5)	23.8 (13.2, 47.8)
Yes	13	67.2 (39.5, 97.0)	16.1 (6.1, 61.6)	<LOD (<LOD, 3.8)	<LOD (<LOD, <LOD)	0.6 (0.2, 1.4)	1.2 (0.7, 3.1)	20.3 (12.2, 63.7)
Alcohol use at intake (drinks/month)								
0 drinks	41	90.8 (47.9, 214.0)	24.0 (14.9, 65.0)	<LOD (<LOD, 1.1)	<LOD (<LOD, 11.3)	2.5 (0.4, 12.1)	1.6 (0.6, 2.9)	28.3 (14.9, 66.4)
1–17 drinks	128	91.2 (37.2, 185.6)	30.6 (9.7, 84.1)	0.2 (<LOD, 1.6)	<LOD (<LOD, 6.2)	1.7 (0.5, 6.5)	1.2 (0.6, 2.3)	24.1 (12.8, 47.0)
≥18 drinks	52	84.5 (34.0, 165.9)	32.3 (7.9, 67.0)	<LOD (<LOD, 2.7)	<LOD (<LOD, 6.2)	1.7 (0.6, 9.6)	1.2 (0.7, 2.3)	21.8 (13.1, 42.7)
Caffeine at intake (mg/month)								
≤1920 mg	75	100.4 (43.7, 193.7)	31.6 (13.6, 84.8)	0.6 (<LOD, 2.9)	<LOD (<LOD, 6.4)	1.9 (0.4, 7.1)	1.0 (0.5, 2.5)	24.1 (14.0, 51.1)
1921–5999 mg	79	84.0 (31.7, 179.4)	27.4 (6.6, 68.0)	<LOD (<LOD, 1.2)	<LOD (<LOD, 7.8)	1.7 (0.8, 11.7)	1.1 (0.6, 2.8)	19.8 (11.1, 47.7)
≥6000 mg	67	96.7 (40.3, 182.9)	29.0 (10.3, 66.2)	<LOD (<LOD, 0.7)	<LOD (<LOD, 6.1)	1.6 (0.6, 6.6)	1.3 (0.8, 2.2)	29.8 (15.9, 60.9)
Season[a]								
Winter (January–March)	66	114.2 (58.7, 193.7)	40.3 (18.2, 95.7)	0.3 (<LOD, 2.2)	<LOD (<LOD, 7.3)	0.8 (0.3, 2.3)	1.0 (0.5, 1.9)	20.3 (8.3, 51.6)
Spring (April–June)	52	100.4 (46.0, 158.6)	30.0 (9.5, 58.3)	<LOD (<LOD, 1.5)	<LOD (<LOD, 10.2)	3.1 (0.7, 11.8)	1.0 (0.6, 2.1)	21.5 (13.1, 42.7)
Summer (July–September)	55	51.7 (26.3, 200.6)	17.6 (3.7, 35.2)	<LOD (<LOD, 1.2)	<LOD (<LOD, 9.4)	3.0 (0.8, 13.3)	1.7 (0.7, 3.9)	29.7 (16.5, 66.9)
Fall (October–December)	39	77.5 (39.5, 221.8)	44.8 (15.0, 106.1)	0.2 (<LOD, 2.4)	<LOD (<LOD, 4.0)	2.0 (0.6, 4.5)	1.4 (0.8, 2.1)	26.0 (15.8, 44.6)
Cycle outcome								
Non-conception[b]	508	70.7 (30.5, 168.7)	25.6 (7.1, 71.6)	<LOD (<LOD, 1.0)	<LOD (<LOD, 5.6)	1.5 (0.6, 7.0)	1.2 (0.7, 2.8)	24.2 (13.1, 57.4)
All conceptions[b]	198	87.5 (30.3, 177.5)	27.6 (8.4, 79.8)	0.3 (<LOD, 1.4)	<LOD (<LOD, 8.7)	2.0 (0.5, 7.7)	1.3 (0.6, 2.6)	26.5 (13.5, 60.0)
Early loss	48	82.0 (22.0, 166.8)	23.0 (10.1, 67.1)	0.2 (<LOD, 0.9)	<LOD (<LOD, 12.9)	1.2 (0.5, 5.4)	1.2 (0.6, 2.5)	26.4 (16.5, 66.9)
Clinical loss	15	94.1 (20.0, 181.1)	20.8 (4.4, 81.3)	0.4 (<LOD, 1.4)	<LOD (<LOD, 11.2)	10.9 (0.7, 130.9)	2.5 (1.0, 4.5)	31.7 (23.7, 101.6)
Live birth	135	87.5 (31.1, 179.4)	32.3 (7.3, 82.0)	0.3 (<LOD, 1.5)	<LOD (<LOD, 7.7)	2.1 (0.5, 7.0)	1.3 (0.6, 2.4)	24.8 (12.4, 53.1)

To eliminate within-subject correlations across cycles, the pooled samples included here are from the first menstrual cycle contributed by a participant. $LOD_{butylparaben} = 0.2$ ng/ml; $LOD_{triclosan} = 2.3$ ng/ml. IQR, interquartile range; LOD, limit of detection.

[a]Season is based on the season of the date of the median aliquot from a pooled sample.

[b]Non-conception cycles include multiple cycles from study participants; all conceptions include early losses, clinical losses and live births.

weighting variable equal to the inverse of the number of contributed pregnancy attempts. As an example, a participant contributing two attempts had a weight of 0.5 for each attempt (Williamson et al., 2003). Separate models were run for each phenol.

Previous cycle exposures

We also assessed whether phenol concentration in one cycle was associated with conception in the next cycle. This was motivated by previous research in animal models and human fertility treatment settings, which found phenol exposure was associated with abnormal folliculogenesis (Ahn et al., 2012; Smith et al., 2013; Lee et al., 2017; Mínguez-Alarcón et al., 2017; Maske et al., 2018; Santamaría et al., 2019; Jeong et al., 2020). Because follicles begin developing at least one cycle prior to being ovulated, the exposure during the cycle prior to a given ovulation was examined. For this analysis, we excluded the first cycle of each attempt, which was missing previous cycle biomarker measurements by design. This also meant excluding pregnancy attempts that were only one cycle long, where conception occurred in the first cycle; this analysis therefore excluded the most fertile study participants. We recomputed the weights to reflect the updated number of attempts contributed by each participant. This analysis included 152 participants contributing 166 attempts, with 109 conceptions. The same covariates were used as in the main time to pregnancy model, and separate models were run for each phenol.

Early pregnancy loss

We estimated odds ratios (ORs) for early pregnancy loss among conceptions that ended in either early loss or live birth, using multivariable logistic regression with GEE. This analysis included 183 conception cycles from 159 participants, with 48 early pregnancy losses and 135 live births. Clinical miscarriages (n = 15) were excluded from this analysis as urinary phenol concentrations at the time of the clinical loss were not measured. Covariates included were phenol concentrations, age, BMI, smoking, alcohol use, caffeine use and season, and separate models were run for each phenol. The model accounted for clustering within individuals with multiple conceptions with GEE and an independent covariance structure, and included a weight statement with the weighting variable equal to the inverse of the number of conceptions contributed by a participant (Williamson et al., 2003).

Sensitivity analyses

We ran four sensitivity analyses on the main time to pregnancy model. First, we excluded participants who were exposed in-utero to diethylstilbestrol (DES) (n = 7). Second, we excluded smokers (n = 13) from the analysis, as smoking might influence fertility, phenol exposure and phenol metabolism. Third, we did not adjust for season, to assess the influence of seasonal variation on our results. Fourth, we included an indicator variable for participants who had an early loss in the previous cycle, in case an early loss impacts fecundity in the next menstrual cycle. We also ran one sensitivity analysis on the early pregnancy loss model, in which we estimated ORs for any pregnancy loss, combining early pregnancy losses (n = 48) and clinical miscarriages (n = 15). This analysis included 198 conception cycles from 170 participants, with 63 pregnancy losses and 135 live births. Because the etiologies for early losses and clinical miscarriages could be different, and because phenol concentrations were not measured near the time of the clinical losses, we consider this to be a secondary analysis.

Within-individual analysis

We carried out a secondary analysis to assess whether urinary phenol concentrations differed between non-conception and conception cycles within each individual. Because there could be differences in phenol exposure over time, such as for participants who took longer to conceive, in phenol metabolism across participants, or in the timing of the exposure in relation to specimen collection given phenols' rapid metabolism, we thought this might better reflect actual within-individual, cycle-specific differences in phenol concentration. We used conditional logistic regression with case-control matching of conception and non-conception cycles. Conceptions occurring in the first cycle of a conception attempt, or where for some other reason there was no matching non-conception cycle, were excluded, as were participants without a conception cycle. If a participant had multiple eligible conceptions, all conceptions were included in the analysis. For each participant, all available non-conception cycles were grouped with each conception cycle. The conceptions included 48 early pregnancy losses, 9 clinical miscarriages and 85 live births in 114 participants. Creatinine-adjusted phenol concentrations were natural log-transformed to reduce effects of extreme values; no other covariates were included in the model because confounders were matched in a within-individual analysis. As this analysis only included a subset of the study participants, excluding the most and least fertile participants, it had less power to detect potential differences in concentration, and we consider it to be a secondary analysis.

Multipollutant models

We constructed multipollutant models of time to pregnancy and early pregnancy loss. First, we simultaneously included all seven phenols as independent predictors (Cowan-Ellsberry and Robison, 2009; Lazarevic et al., 2019). Second, we created two summed groups of highly correlated phenols that have similar chemical structures: parabens (including methylparaben, propylparaben and butylparaben) with shared sources of exposure, and dichlorophenols (including 2,4-dichlorophenol and 2,5-dichlorophenol) with correlated but distinct sources of exposure, and retained triclosan and benzophenone-3 as individual chemicals because they were distinct in their structures and sources. All other covariates were included in these models. Because any cycles missing one or more phenols due to quality control issues were excluded from the multipollutant analysis, the time to pregnancy analysis included 568 menstrual cycles and 152 conceptions from 186 participants, and the early pregnancy loss analysis included 141 conceptions (36 early pregnancy losses and 105 live births, with clinical miscarriages excluded) from 126 participants.

Results

Phenol concentration distribution and variability

Urinary phenol concentrations were similar across age groups, and across levels of alcohol and caffeine consumption (Table I). Concentrations of benzophenone-3 were higher as BMI increased, but did not differ across BMI for any other phenols. White participants had higher benzophenone-3 concentrations, and lower 2,4-dichlorophenol and 2,5-dichlorophenol concentrations; other phenols did not vary by race/ethnicity. Concentrations of parabens and benzophenone-3 were

higher in non-smokers. Some phenol concentrations varied seasonally: paraben concentrations were higher in the winter and lower in the summer, while benzophenone-3 was higher in the spring/summer and lower in the winter. We also observed that benzophenone-3 and 2,5-dichlorophenol concentrations were higher for participants with clinical miscarriages compared to early pregnancy losses and live births. There was no systematic pattern of increasing or decreasing phenol concentrations within the EPS cohort across the years of sample collection (1982–1986), or associated with menstrual cycle number, suggesting that even if participants who took longer to conceive changed their behavior, this did not modify their phenol exposure profile (Supplementary Table SIII).

We compared urinary concentrations of phenols in the EPS cohort (Supplementary Table SI, samples collected 1982–1986) with those reported in other studies (sampling years 1997–2019) of non-pregnant, reproductive-aged women (Supplementary Table SIV). Triclosan and benzophenone-3 concentrations in EPS were lower than samples collected from 2003 to 2016 (Calafat *et al.*, 2008; Woodruff *et al.*, 2011; Koeppe *et al.*, 2013; Pollack *et al.*, 2016; Arya *et al.*, 2020; Wesselink *et al.*, 2021). 2,4-dichlorophenol and 2,5-dichlorophenol were slightly higher in EPS compared with samples collected 2005–2012 (Engel *et al.*, 2014; Wei *et al.*, 2014; Pollack *et al.*, 2016; Wesselink *et al.*, 2021). Paraben concentrations were variable across all studies without any clear temporal trend; EPS concentrations were within the range of other cohorts, including more contemporary studies with samples collected 2010–2019 (Calafat *et al.*, 2010; Smith *et al.*, 2012; Koeppe *et al.*, 2013; Engel *et al.*, 2014; Pollack *et al.*, 2016; Arya *et al.*, 2020; Jurewicz *et al.*, 2020; Wesselink *et al.*, 2021).

Correlation coefficients between concentrations of different phenols ranged from −0.06 to 0.85, with the strongest correlations observed between 2,4-dichlorophenol and 2,5-dichlorophenol (Spearman's $r = 0.85$), methylparaben and propylparaben ($r = 0.84$), and propylparaben and butylparaben ($r = 0.31$) (Supplementary Table SV). All other correlation coefficients were <0.3, with most <0.1. Because 1,3-dichlorobenzene (a parent compound of 2,4-dichlorophenol) is a minor contaminant of 1,4-dichlorobenzene (the parent compound of 2,5-dichlorophenol), this could explain the high correlation between the dichlorophenols, which has been observed in other studies (Wolff *et al.*, 2008). The inclusion of multiple parabens within a single personal care product to enhance their antimicrobial activity might explain the correlations between the parabens (Nowak *et al.*, 2021).

The ICCs for pooled samples assessed similarity within individuals across menstrual cycles and ranged from 0.42 to 0.75 (Supplementary Table SVI). The highest ICCs were observed for 2,4-dichlorophenol (ICC = 0.75, 95% CI: 0.69, 0.79) and 2,5-dichlorophenol (ICC = 0.75, 95% CI: 0.70, 0.80). The next highest ICCs were found for methylparaben (ICC = 0.66, 95% CI: 0.60, 0.72), propylparaben (ICC = 0.57, 95% CI: 0.50, 0.64) and benzophenone-3 (ICC = 0.53, 95% CI: 0.45, 0.61). For triclosan and butylparaben, where nearly half of samples had concentrations that were <LOD, we restricted analysis to samples with detectable concentrations, and ICCs were lower (triclosan ICC: 0.52, 95% CI: 0.41, 0.63; butylparaben ICC: 0.42, 95% CI: 0.31, 0.55) (Supplementary Table SVI).

Time to pregnancy

There was a reduced odds of conception at the highest concentration category for three phenols (methylparaben, propylparaben and 2,4-dichlorophenol), though all CIs for the FOR were wide and centered close to 1 (Table II, Fig. 1). There was an increased odds of conception across concentration categories for three phenols (butylparaben, triclosan and 2,5-dichlorophenol). For the highest category of butylparaben concentration (>1.09 μg/g creatinine), compared with butylparaben <LOD (0.2 ng/ml), the per-cycle odds of conception were 62% higher (FOR: 1.62; 95% CI: 1.08, 2.44), and results were consistent with a dose-response pattern. For the highest category of triclosan concentration (>6.6 μg/g creatinine), compared with triclosan <LOD (2.3 ng/ml), the per-cycle odds of conception were 49% higher (FOR: 1.49; 95% CI: 0.99, 2.26), but there was no dose–response pattern across the three categories.

The estimated per-cycle odds of conception were reduced across all quintiles compared to the lowest quintile for both methylparaben and 2,4-dichlorophenol, but in all cases CIs were wide. The estimated odds of conception were reduced across most concentration categories for propylparaben, but were imprecise. There was no clear dose–response pattern for these phenols. At the highest concentration category of benzophenone-3 and 2,5-dichlorophenol, the relative odds of conceiving were close to 1, with wide CIs and no dose–response pattern.

Previous cycle exposures

There was no clear association between urinary phenol concentration and odds of conception in the next menstrual cycle (Supplementary Table SVII). Although the odds of conception were estimated as increased for most concentration categories of methylparaben, propylparaben, butylparaben and triclosan, there was no dose-response pattern for any phenol, and all estimates had wide CIs.

Early pregnancy loss

Six out of the seven phenols (methylparaben, propylparaben, butylparaben, triclosan, benzophenone-3 and 2,4-dichlorophenol) were not associated with early pregnancy loss (Table III, Fig. 2). Although increasing methylparaben and propylparaben concentrations were associated with a reduced odds of early loss, all CIs were wide. For 2,5-dichlorophenol there was an increased estimated odds of early loss across all elevated concentration categories, but the CIs were wide: at the highest concentration (>90 μg/g creatinine), the odds of early loss were 379% higher (Q5 vs Q1 OR: 4.79; 95% CI: 1.06, 21.59) compared to the lowest concentration (<11.5 μg/g creatinine). Although the fifth quintile had the highest estimated odds of early loss, there was no clear dose-response pattern. In other cases where there was an elevated OR for a given concentration stratum, suggesting increased odds of early loss, there was no pattern across concentrations that would suggest a dose–response relationship, and the CIs were wide.

Sensitivity analyses

Results from the four sensitivity analyses of the primary time to pregnancy model were all similar to those from the main analysis (Supplementary Table SVIII). Excluding DES-exposed participants resulted in a minimal change to FORs, so these participants were included in the main model. Excluding smokers changed the estimated FORs for all phenols, although there was no consistent direction of change; this confirmed our decision to adjust for smoking. Excluding season from the model resulted in minimal change to FORs. Finally,

Table II Per-cycle odds ratios of conception associated with urinary phenol concentration (μg/g creatinine) from a discrete-time logistic-binomial model.

Phenol concentration (μg/g creatinine)	Participants (conceptions), *n*	OR (95% CI)
Methylparaben		
Q2 (23–51)	42 (35)	0.89 (0.55, 1.43)
Q3 (52–107.8)	47 (36)	0.79 (0.49, 1.29)
Q4 (107.9–213)	52 (40)	0.83 (0.50, 1.37)
Q5 (>213)	43 (41)	0.87 (0.49, 1.55)
		$P = 0.92$[a]
Propylparaben		
Q2 (5–17.7)	42 (35)	0.90 (0.55, 1.46)
Q3 (17.8–42.3)	51 (39)	0.88 (0.54, 1.45)
Q4 (42.4–91)	46 (48)	1.38 (0.84, 2.27)
Q5 (>91)	44 (38)	0.89 (0.52, 1.54)
		$P = 0.25$[a]
Butylparaben[b]		
LOD to median (LOD to 1.08)	52 (51)	1.33 (0.94, 1.88)
>median (>1.09)	62 (55)	1.62 (1.08, 2.44)
		$P = 0.05$[a]
Triclosan[b]		
LOD to median (LOD to 6.5)	50 (53)	1.58 (1.07, 2.34)
>median (>6.6)	51 (52)	1.49 (0.99, 2.26)
		$P = 0.05$[a]
Benzophenone-3		
Q2 (0.41–1.03)	42 (31)	0.73 (0.47, 1.16)
Q3 (1.04–2.6)	40 (37)	1.07 (0.66, 1.75)
Q4 (2.7–11.4)	42 (47)	1.50 (0.87, 2.59)
Q5 (>11.4)	49 (40)	1.08 (0.65, 1.78)
		$P = 0.09$[a]
2,4-dichlorophenol		
Q2 (0.6–0.953)	37 (35)	0.77 (0.48, 1.25)
Q3 (0.954–1.61)	45 (38)	0.85 (0.51, 1.53)
Q4 (1.62–3.7)	42 (41)	0.94 (0.56, 1.57)
Q5 (>3.7)	41 (39)	0.90 (0.53, 1.52)
		$P = 0.87$[a]
2,5-dichlorophenol		
Q2 (11.5–20.1)	43 (38)	1.32 (0.84, 2.08)
Q3 (20.2–32.9)	44 (44)	1.68 (1.02, 2.79)
Q4 (33.0–90.0)	45 (41)	1.62 (0.98, 2.67)
Q5 (>90.0)	34 (34)	1.13 (0.65, 1.95)
		$P = 0.18$[a]

The OR is the per-cycle odds of conception for a given biomarker concentration category over the per-cycle odds of conception at the lowest concentration category, holding all other variables fixed. An OR < 1 indicates reduced odds of conception, and an OR > 1 to increased odds. The OR can also be interpreted as a percent change: for example, the estimated odds of conception at the highest quintile of methylparaben concentrations are 13% lower (95% CI: −51%, 55%) than the odds of conception for the lowest quintile of methylparaben concentrations. For methylparaben, propylparaben, benzophenone-3, 2,4-dichlorophenol and 2,5-dichlorophenol, the reference group is the lowest quintile of urinary phenol concentrations. For butylparaben and triclosan, the reference group is urinary phenol concentration <LOD. Covariates in the model include: urinary phenol concentration, cycle number of pregnancy attempt, age, BMI, race/ethnicity, smoking status, alcohol use and season. OR, odds ratio; Q1–Q5, quintiles 1 through 5; LOD, limit of detection.

[a]All *P*-values are Wald statistics from a Type 3 generalized estimating equations (GEE) analysis of all the beta estimates for a given exposure, simultaneously, and are not based on a trend test across quantiles.

[b]The median refers to the median of concentrations >LOD. For butylparaben LOD = 0.2 ng/ml, and for triclosan LOD = 2.3 ng/ml.

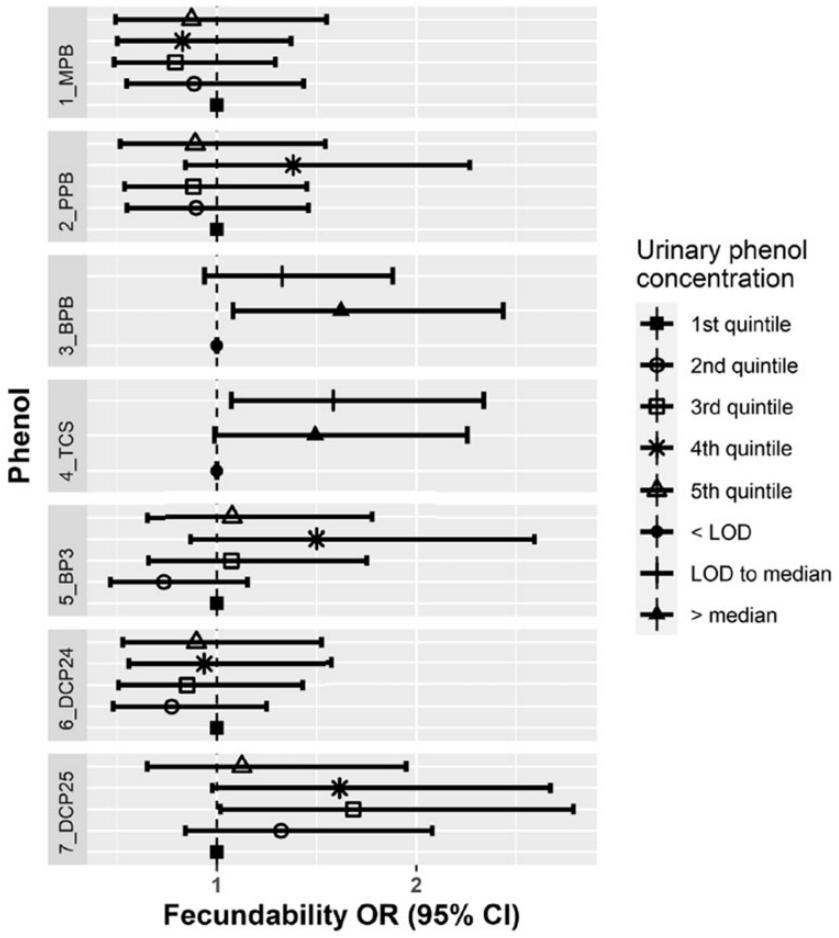

Figure 1. Per-cycle odds ratios of conception associated with urinary phenol concentration (μg/g creatinine). The median refers to the median of detectable concentrations. For butylparaben LOD = 0.2 ng/ml, and for triclosan LOD = 2.3 ng/ml. MPB, methylparaben; PPB, propylparaben; BPB, butylparaben; TCS, triclosan; BP-3, benzophenone-3; DCP-24, 2,4-dichlorophenol; DCP-25, 2,5-dichlorophenol; OR, odds ratio; LOD, limit of detection.

including an indicator variable for an early pregnancy loss in the previous cycle did not substantially alter FORs and there was no association between a recent early loss and fecundability.

Results from the sensitivity analysis of the early pregnancy loss model were consistent with those from the main early loss analysis (Supplementary Table SIX). When both early and clinical pregnancy losses were combined to form a more inclusive pregnancy loss category, the only phenol associated with pregnancy loss was 2,5-dichlorophenol. The increased odds of pregnancy loss at all concentration categories was higher than estimates from the main early loss model, with confidence intervals remaining wide.

Within-individual analysis

There was no systematic difference between non-conception and conception cycle concentrations for any phenol (Supplementary Table SX). For methylparaben, propylparaben, triclosan, benzophenone-3 and 2,4-dichlorophenol, a one-unit increase in phenol concentration (1 μg/g crt) was associated with <10% change in the odds of conception, with wide CIs comfortably including 1. For butylparaben, the odds of conception were 15% lower (OR: 0.85; 95% CI: 0.59, 1.23), and for 2,5-dichlorophenol, the odds of conception were 42% higher (OR: 1.42; 95% CI: 0.90, 2.24), with a one-unit increase in phenol concentration, but CIs were wide.

Multipollutant models

Multipollutant models of time to pregnancy and early pregnancy loss were consistent with single pollutant model results (Supplementary Tables SXI and SXII). In models with all phenols included simultaneously, effect estimates were similar in direction but with increased magnitude and less precise confidence intervals compared to results from single pollutant models, as expected given the high correlations between some of the phenols. In models with summed groups of parabens and dichlorophenols, trends were driven by the phenols with the highest concentrations, methylparaben and 2,5-dichlorophenol, and consistent with single pollutant results. For example, the dichlorophenols were associated with elevated odds of early pregnancy loss across all strata, but confidence intervals were very wide (Supplementary Table SXII).

Table III Odds ratios for early pregnancy loss from a multivariable logistic regression model estimating associations between urinary phenol concentration (μg/g creatinine) and early loss.

Phenol concentration (μg/g creatinine)	Participants (early losses), *n*	OR (95% CI)
Methylparaben		
Q2 (23–51)	35 (8)	0.52 (0.15, 1.78)
Q3 (52–107.8)	30 (7)	0.57 (0.17, 1.99)
Q4 (107.9–213)	36 (12)	1.04 (0.35, 3.07)
Q5 (>213)	40 (8)	0.38 (0.11, 1.28)
		$P = 0.33$[a]
Propylparaben		
Q2 (5–17.7)	34 (10)	0.71 (0.19, 2.65)
Q3 (17.8–42.3)	34 (11)	1.04 (0.32, 3.35)
Q4 (42.4–91)	44 (9)	0.62 (0.19, 1.99)
Q5 (>91)	37 (8)	0.48 (0.13, 1.71)
		$P = 0.69$[a]
Butylparaben[b]		
LOD to median (LOD to 1.08)	46 (14)	1.02 (0.40, 2.63)
>median (>1.09)	50 (11)	0.93 (0.35, 2.46)
		$P = 0.98$[a]
Triclosan[b]		
LOD to median (LOD to 6.5)	51 (11)	0.66 (0.25, 1.73)
>median (>6.6)	48 (14)	1.35 (0.53, 3.39)
		$P = 0.36$[a]
Benzophenone-3		
Q2 (0.41–1.03)	27 (11)	1.92 (0.60, 6.14)
Q3 (1.04–2.6)	37 (9)	1.03 (0.33, 3.19)
Q4 (2.7–11.4)	44 (8)	0.55 (0.18, 1.66)
Q5 (>11.4)	33 (9)	1.01 (0.29, 3.48)
		$P = 0.34$[a]
2,4-dichlorophenol		
Q2 (0.6–0.953)	32 (9)	1.19 (0.39, 3.70)
Q3 (0.954–1.61)	36 (9)	0.73 (0.22, 2.41)
Q4 (1.62–3.7)	37 (9)	0.81 (0.26, 2.54)
Q5 (>3.7)	33 (9)	1.22 (0.40, 3.77)
		$P = 0.86$[a]
2,5-dichlorophenol		
Q2 (11.5–20.1)	36 (12)	3.97 (0.98, 16.08)
Q3 (20.2–32.9)	39 (14)	4.08 (0.94, 17.79)
Q4 (33.0–90.0)	39 (7)	1.20 (0.21, 6.74)
Q5 (>90.0)	30 (10)	4.79 (1.06, 21.59)
		$P = 0.06$[a]

This analysis included 183 conceptions from 159 participants, with 48 early losses and 135 live births. The OR is the odds of early loss for a given biomarker concentration category over the odds of early loss at the lowest concentration category, holding all other variables fixed. An OR < 1 indicates reduced odds of early loss, and an OR > 1 to increased odds. The OR can also be interpreted as a percent change: for example, the estimated odds of early loss at the highest quintile of methylparaben concentrations are 62% lower (95% CI: −89%, 28%) than the odds of conception for the lowest quintile of methylparaben concentrations. For methylparaben, propylparaben, benzophenone-3, 2,4-dichlorophenol and 2,5-dichlorophenol, the reference group is the lowest quintile of urinary phenol concentrations. For butylparaben and triclosan, the reference group is urinary phenol concentration <LOD. Covariates in the model include: urinary phenol concentration, age, BMI, race/ethnicity, smoking status, alcohol use, caffeine use and season. OR, odds ratio; Q1–Q5, quintiles 1 through 5; LOD, limit of detection.

[a]All *P*-values are Wald statistics from a Type 3 generalized estimating equations (GEE) analysis of all the beta estimates for a given exposure, simultaneously, and are not based on a trend test across quantiles.

[b]The median refers to the median of concentrations >LOD. For butylparaben LOD = 0.2 ng/ml, and for triclosan LOD = 2.3 ng/ml.

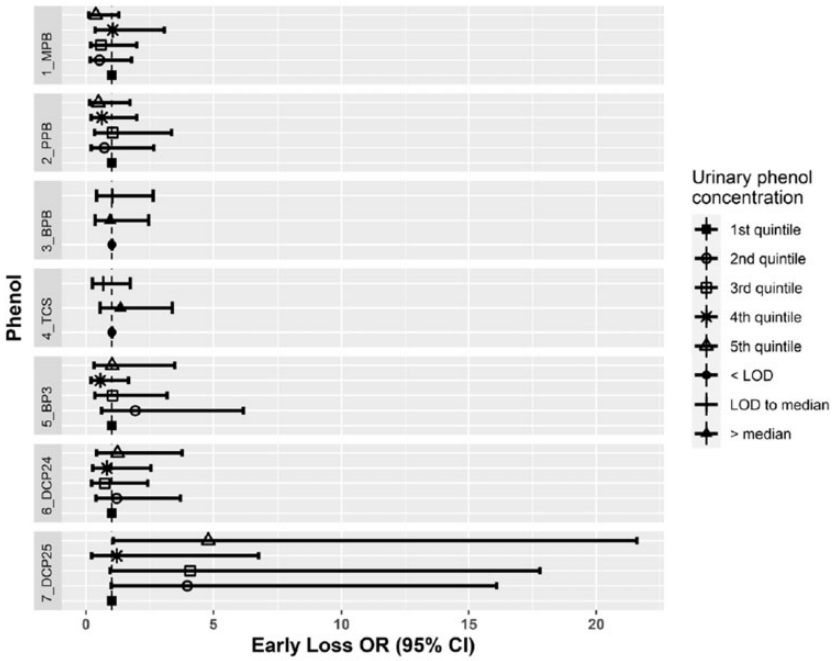

Figure 2. Odds ratios for early pregnancy loss associated with urinary phenol concentration (μg/g creatinine). The median refers to the median of detectable concentrations. For butylparaben LOD = 0.2 ng/ml, and for triclosan LOD = 2.3 ng/ml. MPB, methylparaben; PPB, propylparaben; BPB, butylparaben; TCS, triclosan; BP-3, benzophenone-3; DCP-24, 2,4-dichlorophenol; DCP-25, 2,5-dichlorophenol; OR, odds ratio; LOD, limit of detection.

Discussion

In this prospective study of associations between phenol exposure biomarker concentrations and time to pregnancy and early pregnancy loss, the only adverse association we observed was increased odds of early pregnancy loss with 2,5-dichlorophenol across all concentration categories although the CIs were wide. In contrast, estimates suggested an increased odds of conception at the highest concentration categories of butylparaben and triclosan compared to non-detectable concentrations. There were no associations between these endpoints and concentrations of the other phenols examined in this study, or between previous cycle exposures and the odds of conception.

Three previous studies examined fecundability and exposure to methylparaben, propylparaben, butylparaben, triclosan and benzophenone-3. In the LIFE cohort, no association was reported between female benzophenone-3 concentration and fecundability (Buck Louis *et al.*, 2014). In another study using the LIFE cohort, the odds of conception were estimated as declining by 34% for females in the 4th quartile of methylparaben concentration (≥104 ng/ml; FOR: 0.66; 95% CI: 0.45, 0.97), and there were no associations between fecundability and propylparaben, butylparaben or triclosan concentrations (Smarr *et al.*, 2017). Finally, a retrospective study of time to pregnancy estimated a 16% decline in the odds of conception for women in the 4th quartile of triclosan concentration (>72 ng/ml; FOR: 0.84; 95% CI: 0.72, 0.97) compared to the lower three quartiles, with exposure assessment during pregnancy (Vélez *et al.*, 2015).

Our study's results were consistent with previous findings of no association between benzophenone-3 and propylparaben exposure and fecundability (Buck Louis *et al.*, 2014; Smarr *et al.*, 2017). We also found no association between 2,4-dichlorophenol and 2,5-dichlorophenol concentrations and time to pregnancy. However, in contrast to Smarr *et al.* (2017), we did not find an association between methylparaben and fecundability, and estimated an increase in fecundability at the highest concentrations of butylparaben. Concentrations of methylparaben, propylparaben and butylparaben were similar in the EPS and LIFE cohorts, and there was no temporal trend in paraben concentrations across the cohorts we surveyed (Supplementary Table SIV), which may reflect consistency of use since the 1930s (CIR Expert Panel, 2008; Vandenberg and Bugos, 2021). Parabens are thought to be weakly estrogenic, but repeated exposures throughout the day and to multiple parabens could lead to stronger estrogenic effects (Karpuzoglu *et al.*, 2013). *In vitro* and *in vivo* studies suggest parabens could influence reproductive function through estrogenic, antiandrogenic and thyroid effects, and that exposure to methylparaben and butylparaben impacts rat litter size, live birth rate and pup postnatal survival (Karpuzoglu *et al.*, 2013; Rattan *et al.*, 2017; Hipwell *et al.*, 2019). Previous epidemiologic studies of paraben exposure and human female reproductive function found that butylparaben concentration was associated with shorter menstrual cycle length (Nishihama *et al.*, 2016) and lower estradiol (Aker *et al.*, 2016), and propylparaben concentration was associated with reduced ovarian reserve in a fertility treatment population (Smith *et al.*, 2013), while one study found no associations between paraben exposure and IVF treatment outcomes (Minguez-Alarcon *et al.*, 2016). Together, this literature suggests parabens may affect reproductive function, but does not offer insights into the

differences between this study's findings and those of Smarr et al. (2017), or the biological plausibility of our findings for butylparaben.

We estimated an increase in fecundability at the highest of triclosan concentration category, in contrast to both Vélez et al. (2015) and Smarr et al. (2017), which respectively found decreased fecundability and no association with triclosan (Vélez et al., 2015; Smarr et al., 2017). Based on animal studies, triclosan is thought to disrupt thyroid hormone homeostasis, possibly to have both antiandrogenic and antiestrogenic effects, and to disrupt reproductive hormone synthesis (Dann and Hontela, 2011; Hipwell et al., 2019; Green et al., 2021). Triclosan concentrations were much lower in the EPS cohort (median <LOD (LOD = 2.3 ng/ml), IQR <LOD-6.2 μg/g creatinine) compared to those in both the LIFE (median = 16.8 ng/ml, IQR 5.32–67.5) and MIREC (median = 8.3 ng/ml) cohorts (Vélez et al., 2015; Smarr et al., 2017), which may reflect the fact that triclosan was less commonly used in consumer products in the mid-1980s (National Center for Biotechnology Information, 2022a). Epidemiologic studies of fertility treatment cohorts also generally observed associations between triclosan and reduced fertility at the highest triclosan concentrations (Green et al., 2021). The effect we observed for triclosan occurred at comparatively lower concentrations and together these results are consistent with a nonmonotonic exposure–response pattern.

It is also possible that EPS participants with higher urinary butylparaben and triclosan concentrations represent subgroups with higher reproductive success. For example, triclosan-containing antibacterial soaps were used in healthcare settings in the 1980s, and healthcare workers in the cohort (n = 49) had slightly higher urinary triclosan concentrations (median 2.9 μg/g creatinine) compared to non-healthcare workers (median <LOD). However, there was no association between healthcare occupation and fecundability in unadjusted models (per-cycle OR of conception 1.06 (95% CI: 0.63, 1.78)), suggesting healthcare workers did not have improved reproductive success, and occupation does not explain our findings. We were unable to identify a comparable subgroup related to butylparaben.

Exposure misclassification could contribute to the differences between our study's findings and those of other studies. Buck Louis et al. (2014) and Smarr et al. (2017) used preconception spot urine samples to assess exposure (Buck Louis et al., 2014; Smarr et al., 2017), and Vélez et al. (2015) used first-trimester spot urine samples for retrospective exposure assessment (Vélez et al., 2015), which additionally could be biased by pregnancy-associated nausea and vomiting, and behavior and lifestyle changes. In contrast, we used pooled, repeated preconception samples. For chemicals having urinary concentrations with high intra-individual variability, like the phenols examined here, pooled samples more accurately characterize mean exposure than spot urine specimens (Vernet et al., 2019). The ICCs of the phenols studied here ranged from 0.42 to 0.75, indicating that repeated samples would more accurately characterize an individual's exposure (Deziel et al., 2013). The ICCs we calculated were generally aligned with those of previous studies examining the repeatability of phenol concentrations, with the exception of 2,4-dichlorophenol and 2,5-dichlorophenol, which we found to have higher ICCs than previously reported (Meeker et al., 2013; Pollack et al., 2016; Vernet et al., 2018; LaKind et al., 2019). Because the ICCs in this study were calculated across multiple pooled samples, each representing an average concentration across a menstrual cycle, the ICCs reported here should be higher compared to studies using spot samples.

While two case-control studies have examined clinical pregnancy loss in relation to benzophenone-3 and triclosan exposure (Chen et al., 2013; Wang et al., 2015), none have looked at early pregnancy loss before 6 weeks gestation or exposure to the other phenols that we considered. Chen et al. (2013) reported no association between benzophenone-3 exposure (median benzophenone-3 = 0.1 ng/ml) and pregnancy loss before 20 weeks gestation, with the relatively low median concentration suggesting this cohort had limited benzophenone-3 exposure at the time of sampling (Chen et al., 2013). Wang et al. (2015) found an increased odds of pregnancy loss at 14–24 weeks gestation at medium (OR 2.85, 95% CI: 1.67, 4.85, median triclosan 1.4 mg/ml) and high (OR 2.71, 95% CI: 1.59, 4.63, mean triclosan 11.2 ng/ml) triclosan concentrations compared to women with low concentrations (113 cases (median triclosan 1.4 ng/ml), 339 controls (median triclosan <0.9 ng/ml)) (Wang et al., 2015). Our estimate suggested an association between 2,5-dichlorophenol and early pregnancy loss across all quintiles of urinary concentration compared to the lowest (Table III, Fig. 2). The combination of a large magnitude of association with wide CIs for all the estimates could indicate a strong but imprecise association, but the exact effect estimates should not be over-interpreted. This finding could also be the result of chance: no other phenols were associated with early pregnancy loss, and the small number of pregnancy losses in the EPS cohort reduced our power to detect potential associations, especially those with more modest effect estimates. It is also possible that the outcomes of conception and early loss might sometimes compete with each other. For example, elevated 2,5-dichlorophenol might both enhance fecundability (as suggested by Table II, Fig. 1) and increase the odds of early loss (as suggested by Table III, Fig. 2) through a mechanism where in the presence of 2,5-dichlorophenol, nonviable conceptuses are more likely to implant prior to being maternally discarded. Direct comparison of our findings with Chen et al. (2013) and Wang et al. (2015) is challenging due to differences in study design, such as participant inclusion criteria, timing of exposure assessment and outcome measurements, and data analysis methods and models. Additionally, the etiology of later clinical miscarriage could differ from that of early pregnancy loss, the causes of which are largely unknown apart from chromosomal abnormalities (Creasy et al., 2009).

Although the parent compound of 2,5-dichlorophenol, 1,4-dichlorobenzene, is categorized as a suspected human carcinogen by the International Agency for Research on Cancer and a possible human carcinogen by the US Environmental Protection Agency, there is no literature on exposure to 2,4-dichlorophenol or 2,5-dichlorophenol and reproductive function and fertility in humans. A limited number of studies have examined other endpoints related to endocrine disruption in humans, including reproductive hormone levels, the onset of menarche and breast development in girls, and obesity/adiposity (Wolff et al., 2008, 2015; Buttke et al., 2012; Wei et al., 2014; Aker et al., 2016; Buckley et al., 2016). A study of urinary phenol concentration and reproductive hormone levels in healthy, non-pregnant women reported no association with 2,5-dichlorophenol (Pollack et al., 2018). As an EDC, 2,5-dichlorophenol is estrogenic and theorized to impact thyroid hormone homeostasis (Wolff et al., 2015; Wei and Zhu, 2016). Animal studies found 1,4-dichlorobenzene to be estrogenic, with exposure resulting in offspring with developmental abnormalities in aquatic animal models, and 2,5-dichlorophenol exposure was associated with decreased IVF success in mice (Seyler et al., 1984; Pagano

et al., 1988; Versonnen et al., 2003). The possible effect we observed on early pregnancy loss occurred at relatively higher concentrations of 2,5-dichlorophenol than in the study by Pollack et al. (2018), as 2,5-dichlorophenol concentrations were higher in the EPS cohort in the mid-1980s compared to more recently collected samples (Supplementary Table SIV). This trend is also reflected in declining concentrations in NHANES participants from 2003-2010: the geometric mean concentration of 2,5-dichlorophenol in the EPS cohort was more than twice as high as that of 2003–2004 NHANES participants and four times higher than that seen in 2009–2010 NHANES participants (Ye et al., 2014). Production of 2,5-dichlorophenol has decreased since the mid-1980s, which may account for the higher concentrations detected in the EPS cohort compared to more recent studies, and it has declined across the years of NHANES sampling (Ye et al., 2014; National Center for Biotechnology Information, 2022b).

The results of this study should be considered somewhat exploratory given the limited prior studies of these phenols and the novel early pregnancy endpoint. Although the examined parabens are structurally similar, we did not see any consistent patterns of association with fecundability or early loss. While previous research has not observed triclosan to have a fertility-promoting effect, EDCs can exhibit non-monotonic exposure-response curves even at low concentrations within the range of typical human exposure (Vandenberg et al., 2012). The increased fecundability that we observed was at substantially lower triclosan concentrations than were considered by the other two studies of triclosan and time to pregnancy, which found no association (Smarr et al., 2017) and decreased fecundability (Vélez et al., 2015) with higher triclosan concentrations. Although it is impossible to determine whether triclosan is exhibiting a non-monotonic exposure–response curve across these studies, such patterns are important to consider when evaluating contrasting findings for EDCs (Vandenberg et al., 2012). The associations of increased fecundability with butylparaben and triclosan therefore should be interpreted cautiously, particularly because in the secondary within-individual analysis, we did not observe systematic differences between non-conception and conception cycle concentrations for any phenol, including butylparaben and triclosan (Supplementary Table SX). Finally, it is difficult to provide context for the plausibility of our results related to 2,5-dichlorophenol (an increased odds of early pregnancy loss with 2,5-dichlorophenol) as no other studies of 2,5-dichlorophenol and fecundability or early pregnancy loss have been published. While the possible effect we observed on early pregnancy loss occurred at comparatively higher 2,5-dichlorophenol concentrations, studies in other cohorts are necessary to understand whether there is in fact an association.

The limitations of our study include that we did not have early pregnancy phenol measurements for analyses of pregnancy loss, nor measurements for male partners. Fecundability is a couple-dependent outcome, and evidence suggests that the male partner's exposures can also influence fecundability (Buck Louis et al., 2014, 2016; Smarr et al., 2017). However, concentrations of parabens have been found to be five to ten times higher and benzophenone-3 up to three times higher in women than men (Calafat et al., 2010; Buck Louis et al., 2014; Smarr et al., 2017). We also did not have information about several factors of potential interest, including participants' exercise and physical activity, diet and personal care product use. Because samples were collected from 1982 to 1986, urinary concentrations of some of the phenols examined here, 2,5-dichlorophenol, triclosan and benzophenone-3, differed from current concentrations; however, the other phenols in this study had concentrations comparable to those reported in more recently collected specimens (Supplementary Table SIV). Since the phenols in this study are rapidly metabolized with half-lives of <24 h, and many <2 h (Somani and Khalique, 1982; Sandborgh-Englund et al., 2006; Abbas et al., 2010; Kim and Choi, 2014; Ye et al., 2014), the first-morning urine samples used to estimate exposure may primarily reflect exposures from the previous evening, limiting their capture of exposures occurring during the day. The EPS specimens also were stored for 22–24 years prior to analysis for phenols. Nevertheless, our quality control analysis and results from a previous study of bisphenol-A (Nepomnaschy et al., 2009) suggest that under the subfreezing, temperature-controlled storage conditions used, specimen integrity was not compromised, and confirm the validity of the phenol measurements even after this long-term storage period. Additionally, our sample size was limited, particularly for the outcome of early pregnancy loss, which reduced our power to detect associations. Future analyses could apply additional chemical mixture modeling techniques, but would be constrained by the sample size available and the lack of phenol measurements for certain cycles. Finally, it is possible that the potential associations we observed are chance findings, given the many comparisons we carried out in this study. There is also very limited animal evidence available to contextualize our findings, a lack of other human studies for comparison, and no clear patterns of association across related phenols, such as the parabens and dichlorophenols.

Our study also has a number of strengths, including the use of repeated, pooled measures to summarize phenol exposure across each menstrual cycle. This study was also prospective, with precise dating of critical events including ovulation, conception, implantation and pregnancy loss, using a combination of clinical symptoms and hormone assays. Our analysis leveraged these detailed data to examine the endpoint of early pregnancy loss, a challenging outcome to examine as it typically occurs before a pregnancy is clinically recognized. Apart from chromosomal abnormalities, which have been reported in as many as 60% of first-trimester pregnancy losses, the causes of early pregnancy loss are not well understood, so this study contributes to the limited literature probing potential associations (Wilcox et al., 1988a, 1990; Weinberg et al., 1994; Creasy et al., 2009).

Conclusions

We found that 2,5-dichlorophenol urinary concentrations may be associated with an increased odds of early pregnancy loss, and that higher urinary concentrations of butylparaben and triclosan may be associated with an increase in the per-cycle odds of conception. However, these results should be interpreted with caution. The lack of consistent results across the literature related to EDC exposure and fecundability and pregnancy loss underscores that this is an emerging area of research. This area remains in need of further studies given the endocrine-disruptive potential of these phenols and the substantial public health implications of subfertility.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article cannot be shared publicly to protect the privacy of the individuals who participated in the study. The data may be shared on reasonable request to the corresponding author.

Acknowledgements

We thank Dr D. Robert McConnaughey for his help with data management, Dr Greg Travlos and Ralph Wilson for the assay of creatinine and Drs Kelly Ferguson, Chinaro Kennedy and Danielle Stevens for their comments on an earlier draft of this article.

Authors' roles

A.K.R.V.: conceptualization, methodology, software, formal analysis, writing of original draft, review and editing, visualization, funding acquisition; C.R.W.: conceptualization, methodology, investigation, review and editing, funding acquisition; D.D.B.: conceptualization, methodology, investigation, review and editing, funding acquisition; A.J.W.: conceptualization, methodology, investigation, review and editing, funding acquisition; A.M.C.: investigation, resources, review and editing; N.C.D.: conceptualization, methodology, review and editing; C.H.J.: conceptualization, review and editing; A.M.Z.J.: conceptualization, methodology, software, investigation, review and editing, supervision, funding acquisition.

Funding

This research was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health (award number F31ES030594), the Intramural Research Program of the National Institutes of Health, the National Institute of Environmental Health Sciences (project numbers ES103333 and ES103086) and a doctoral fellowship at the Yale School of Public Health. *Disclaimer:* The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service or the US Department of Health and Human Services.

Conflict of interest

The authors declare they have no conflicts of interest to disclose.

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