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Time-varying associations of gestational and childhood triclosan with pubertal and adrenarchal outcomes in early adolescence

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Background: Triclosan is an endocrine-disrupting chemical, but associations with pubertal outcomes remain unclear. We examined associations of gestational and childhood triclosan with adolescent hormone concentrations and pubertal stage.

Methods: We quantified urinary triclosan concentrations twice during pregnancy and seven times between birth and 12 years in participants recruited from Cincinnati, OH (2003–2006). We averaged concentrations across pregnancy and childhood and separately considered individual exposure periods in multiple informant models. At 12 years, we measured serum hormone concentrations (males [n = 72] and females [n = 84]—dehydroepiandrosterone-sulfate, luteinizing hormone, follicle-stimulating hormone; males—testosterone; females—estradiol). Also at age 12 years, participants self-reported physical development and menarchal timing. We estimated associations (95% confidence interval) of triclosan with hormone concentrations, more advanced physical development, and age at menarche.

Results: For females, each doubling of childhood triclosan was associated with 16% lower estradiol concentrations (–29%, 0%), with stronger associations for measures closer to adolescence. We found suggestive evidence that higher triclosan at any age was associated with ~10% (for gestational triclosan: –18%, –2%) lower follicle-stimulating hormone concentrations among males and early postnatal (1–3 years) triclosan was associated with 63% (5%, 96%) lower odds of advanced pubic hair development in females. In multiple informant models, each doubling of gestational triclosan concentrations was associated with 5% (0%, 9%) earlier age at menarche, equivalent to 5.5 months.

Conclusion: Gestational and childhood triclosan concentrations were related to some pubertal outcomes including hormone concentrations and age at menarche. Our findings highlight the relevance of elucidating potential sex-specific and time-dependent actions of triclosan.

Keywords: Endocrine disruption; Triclosan; Puberty; Adrenarche

Introduction

Pubertal timing is critical for lifelong health. Among females, earlier age at menarche or thelarche has been linked to breast cancer, cardiometabolic disease, gastrointestinal disorders, and depression.[1–4] In contrast, delayed puberty in females is associated with lower bone mass accrual, elevating the risk for osteoporosis and fracture, and adverse psychosocial outcomes, and cardiovascular outcomes.[3,5,6] Among males, early puberty is associated with adverse cardiovascular outcomes, depression, and gastrointestinal disorders, whereas delayed puberty is associated with anxiety and depression.[3] Factors affecting pubertal timing include genetics, nutritional status, and emotional well-being.[7,8] Additionally, some environmental contaminants, referred to as endocrine-disrupting chemicals (EDCs), are known or suspected to affect endocrine system function and sexual maturation.[9–11]

Triclosan is an EDC detected in approximately three-quarters of the United States population.[12,13] Recognizing its potential to disrupt hormones and contribute to antibacterial

What this study adds:
Prior research suggests that exposure to triclosan, an endocrine-disrupting compound, is associated with pubertal outcomes, but it is unknown whether there are windows of heightened susceptibility to its effects. We aimed to clarify the prospective and concurrent associations of serial measures of urinary triclosan concentrations during gestation and the first 12 years of life with pubertal and adrenarchal outcomes including hormone concentrations, attained pubertal stage at follow-up, and age at menarche. Our findings suggest that triclosan concentrations were associated with differences in self-reported adrenarchal and pubertal development in a sex-specific manner, with windows of heightened susceptibility to exposure.

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Data are not publicly available due to their sensitive and identifiable nature, but may be available upon reasonable request. Code is available upon request from Hannah E. Laue (Hannah.E.Laue@Dartmouth.edu).

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resistance, the US Food and Drug Administration banned the use of triclosan in soaps and washes in 2016[14] and the United States Environmental Protection Agency regulates its use as a pesticide[15] By 2019 it was voluntarily removed from toothpastes in the United States. However, exposure continues through consumer products not regulated by the Food and Drug Administration including toys, clothing, and kitchenware.[16,17] Some evidence indicates that triclosan could affect pubertal development. For instance, in vivo, in vitro, and in silico models suggest that triclosan is a partial androgen receptor agonist and decreases circulating luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone.[18,19] Similarly in vivo and in vitro models indicate triclosan has direct estrogenic effects, with increased vitellogenin and decreased sperm counts in male mosquito fish and increased uterine weight in female rats.[20] However, the few studies in humans are inconclusive as to whether triclosan alters hormone concentrations or related phenotypes.[21–30] A major limitation of prior studies is that they considered a single period of triclosan exposure, often concurrent with outcome assessment, which leaves open the possibility of reverse causation. Additionally, most prior studies have assessed either hormone concentrations or phenotypic endpoints, rather than both, which would provide a more comprehensive understanding of the association between triclosan exposure and pubertal development.

In this study, we aimed to clarify the prospective and concurrent association of serial measures of triclosan concentrations during gestation and the first 12 years of life with pubertal and adrenarchal outcomes including hormone concentrations, attained pubertal stage at follow-up, and age at menarche. Serial measures of urinary triclosan concentrations from gestation through age 12 years, allowed us to identify periods of heightened susceptibility to triclosan in sexual maturation.

Methods

Study participants

The Health Outcomes and Measures of the Environment (HOME) Study is a longitudinal pregnancy and birth cohort in Cincinnati, OH, which has previously been described in depth.[31] Briefly, individuals 18 years or older were recruited in 2003–2006 during the second trimester of pregnancy, and their children participated in extensive follow-up with collection of biospecimens and health and neurobehavioral outcomes. All pregnant people provided written informed consent for themselves and their children. Children provided informed assent at the 12-year study visit.[31,32] Participants followed through 12 years of age had similar sociodemographic, perinatal, and infant characteristics to those originally recruited.[32] The Institutional Review Boards (IRBs) at Cincinnati Children's Hospital Medical Center (CCHMC) and participating delivery hospitals approved this study. The Centers for Disease Control and Prevention (CDC), Dartmouth College, and Brown University deferred to the CCHMC IRB as the IRB of record.

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Triclosan quantification

As previously described, triclosan was quantified up to two times during gestation (~16 and ~26 weeks) and at delivery in urine from pregnant participants, and up to seven times in children (annual samples from 1 to 5 years of age, 8 years, and 12 years; Supplemental Table 1; http://links.lww.com/EE/A270).[33–36] Using online solid phase extraction coupled to high-performance liquid chromatography-isotope dilution with tandem mass spectrometry, trained laboratory technicians at the CDC quantified total (conjugated and free) triclosan urinary concentrations.[37] The limit of detection (LOD) was 2.3 ng/ml for all samples collected before 8 years of age and 1 ng/ml for samples collected at or after the 8-year visit due to technological advances. Samples with triclosan concentrations below the LOD were assigned LOD/$\sqrt{2}$.[38] Urine creatinine concentrations were measured at the CDC to account for urine dilution.[39] Concentrations were $\log_{10}$-transformed to reduce the influence of outliers.

Hormone quantification

As previously described, we quantified adrenarchal (dehydroepiandrosterone-sulfate [DHEA-S]) and pubertal hormones (FSH and LH) in both male and female participants.[40] We collected a morning (median time 9:58, interquartile range 9:44, 10:16)[40] fasting blood samples and stored serum samples at −80 °C until analysis. DHEA-S was quantified at the endocrinology laboratory at CCHMC using a competitive binding immunoenzymatic assay.[40] Gonadotropins LH and FSH were also measured at CCHMC using a sequential two-step immune-enzymatic assay. FSH and LH were not quantified in a subset of males (n = 28) due to financial constraints. Estradiol was measured in females by quantitative chemiluminescent immunoassay at Associated Regional and University Pathologist Laboratories in Salt Lake City, UT. Testosterone was quantified in males by liquid chromatography-tandem mass spectrometry at CCHMC. All laboratories are CLIA-certified and follow standard protocols for quality assurance and quality control. To better approximate normal distributions, each hormone was $\log_{10}$-transformed.

Pubertal staging assessment

During the 12-year study visit, participants were asked to self-assess their sexual maturity according to Tanner stage criteria when they were changing clothes for other study procedures.[41] Females assessed their breast (i.e., pubertal) development males and females assessed their pubic hair (i.e., adrenarchal) development. A trained research assistant reviewed a series of images with written descriptions representing each of the 5 Tanner stages with each participant, after which participants examined their body in a full-length mirror and endorsed the picture they felt was the best representation of their physical development. Two females did not complete the Tanner staging. Stages range from 1 (no development) to 5 (mature). Due to small sample sizes in Tanner stages 1 and 5, we combined stages 1 and 2 (no to early development) and stages 4 and 5 (mature development; Supplemental Table 2; http://links.lww.com/EE/A270). As a sensitivity analysis, we separated stages 1 and 2 for males and excluded females reporting stage 1 development (Supplemental Table 2; http://links.lww.com/EE/A270). Female participants and their caregivers each reported whether the participant had reached menarche, and, if so, at what age. Menarchal status was missing for one participant.

Covariate assessment

We selected covariates a priori based on a directed acyclic graph created with Dagitty (Supplemental Figure 1; http://links.lww.com/EE/A270).[42] We assessed child race/ethnicity, household

income, and age at the 12-year study visit with standardized interviews. Due to sample size limitations, we dichotomized race/ethnicity as non-Hispanic white and underrepresented racial/ethnic groups (non-Hispanic Black, American Indian, Asian/Pacific Islander, Hispanic) as a proxy for systemic racism and cultural factors related to the exposure and outcome. We evaluated maternal smoking during pregnancy by quantifying cotinine, a metabolite of nicotine, in serum collected during pregnancy. Continuous $\log_{10}$-transformed serum cotinine concentrations were used in statistical analyses, but for descriptive analyses, we categorized pregnant participants as not exposed to cigarette smoke (<0.015 ng/ml), exposed to secondhand smoke (0.015–3 ng/ml), or active smokers (≥3 ng/ml).[43] Females who had reached menarche reported time since their last period, but we did not include this in our analysis due to the limited sample size. We did not include child body mass index (BMI) in our primary models because obesity may lie on the causal pathway between triclosan exposure and pubertal development.[23,24] We conducted a sensitivity analysis adjusting for BMI *Z*-scores that were calculated from child weight and height measured at the 12-year study visit. We also conducted unadjusted analyses to assess the influence of the selected confounders and covariates.

Statistical methods

We calculated descriptive statistics of triclosan concentrations, outcomes, and covariates in males and females separately. We visually examined triclosan concentration distributions by study visit and Tanner stage and compared average hormone concentrations by age at the 12-year study visit and Tanner stage. Due to triclosan's short half-life (~21 hours) and episodic nature of exposure, urinary concentrations may vary within individuals, leading to measurement error. To account for this exposure misclassification we applied a regression calibration approach that was previously described.[34] Briefly, for gestational concentrations, which showed no time trends across measurements,[36] we used linear mixed effects models to estimate a weighted average of concentrations across the gestation visits for each participant. Geometric mean triclosan concentrations in childhood had a quadratic time trend.[36] Thus, we estimated subject-specific triclosan trajectories for each participant before estimating the average childhood concentration by calculating the area under the trajectory and dividing by the length of exposure for each subject (i.e., age at follow-up). This resulted in a single estimate of gestational (16 and 26 weeks) and childhood (1, 2, 3, 4, 5, 8, and 12 years) triclosan concentrations.

We first investigated associations of average gestational and childhood triclosan concentrations from regression calibration models with serum hormone concentrations using linear models adjusted for covariates to determine whether there were consistent associations between triclosan concentrations and outcomes in either exposure window.[34] Additional analyses were then conducted to identify more discrete periods of heightened susceptibility during childhood. We fit models using each period of exposure assessment individually and co-adjusting for both windows of exposure. For hormones measured in both males and females, we quantified sex-specific associations by including an interaction term between sex and triclosan concentration. Sex-specific $P < 0.05$ and interaction $P < 0.1$ were used as evidence of sex-specific associations. Estimates were expressed as the relative percent difference in hormone concentration per doubling of urinary triclosan concentration calculated as $100\ (2^{\beta}-1)$, where β was the effect estimate from models.[44] To assess the association between triclosan and Tanner stage, we employed cumulative logit ordinal regression models, which assume the odds between each level of the outcome are proportional to one another (i.e., the odds ratio comparing mid to early development is proportional to the odds ratio comparing mature to mid-development).[45] Finally, to assess whether triclosan concentrations were associated with age at menarche, we used accelerated failure time (AFT) models, modeling maternal- and self-report separately.[46,47] AFT models use all observations and estimate time to event (menarche) or censoring (follow-up) without an event. Primary AFT models used maternal report of age at menarche. For these models $P = 0.05$ was considered statistically significant.

To examine periods of heightened susceptibility to triclosan, we employed multiple informants models using linear regression with generalized estimating equations (GEEs) adjusting for the same covariates as previously described[48,49] and applied to studies of triclosan in this cohort.[33–35,50] Briefly, all observations are modeled in a set of estimating equations with no overall intercept, a random intercept for each participant, and interactions between exposure window and all variables in the model (exposure and covariates). This results in embedded separate linear regressions for each exposure window. Using this approach, we simultaneously estimated associations between $\log_{10}$-transformed urinary triclosan concentrations at eight time points (measurement error corrected average gestational concentrations, 1Y, 2Y, 3Y, 4Y, 5Y, 8Y, and 12Y) and $\log_{10}$-transformed serum hormone concentrations. The regression calibration method allows for imputation of missing exposure values if a participant has at least one measurement. For Tanner stage outcomes we employed ordinal GEEs and AFT GEEs for age at menarche. For each of these models, we used the omnibus interaction test to identify outcomes for which there was suggestive evidence ($P < 0.2$) that associations differed by exposure window. This lenient *P* value was selected due to the limited power of the omnibus interaction test.[51]

Results

Among 156 participants contributing to our analysis, 84 were female (Table 1). Females were more likely than males to have mothers who actively smoked and were exposed to secondhand smoke during pregnancy. Urinary triclosan concentrations were lower in 12-year-olds than concentrations measured earlier in childhood before creatinine standardization (Supplemental Figure 2; http://links.lww.com/EE/A270). Hormone concentrations were positively correlated with age at assessment and Tanner stage (Supplemental Figures 3 and 4; http://links.lww.com/EE/A270).[40] As expected,[52] females reported more advanced Tanner stage than males (Supplemental Table 2; http://links.lww.com/EE/A270).

In models of gestational and childhood triclosan concentrations adjusting for covariates, each doubling of childhood triclosan was associated with 16% lower (95% confidence interval [CI] = –29, –0) estradiol concentrations; no association was observed for gestational triclosan (Table 2). The associations between triclosan and estradiol concentrations were stronger for triclosan measures later in childhood (e.g., at 12 years $\beta = -18\%$ per doubling of triclosan [95% CI = –32, –1], $P = 0.04$; Figure 1, Supplemental Table 3; http://links.lww.com/EE/A270), with suggestive evidence of a negative linear age trend in the associations (–12% [95% CI = –26, 5] for each successive window of exposure, $P = 0.17$). No significant associations were found for other hormones in females. Estimates from models co-adjusting for gestational and childhood exposure were similar to models without the co-adjustment (Supplemental Table 4; http://links.lww.com/EE/A270).

We found that the association between triclosan and FSH differed by child's sex, with a negative association among males and a null association among females. Among males, triclosan concentrations during gestation and in childhood were associated with lower FSH concentrations (Figure 1, Supplemental Table 3; http://links.lww.com/EE/A270). For example, each doubling

Table 1.

Characteristics of participants in the Health Outcomes and Measures of the Environment (HOME) study contributing to analysis of association of triclosan concentrations with adrenarchal and pubertal development (n [%], mean ± standard deviation, or median [25 percentile, 75 percentile])

	Males (n = 72)	Females (n = 84)
Maternal pregnancy serum cotinine		
Not exposed (<0.015 ng/ml)	26 (36.1)	18 (21.4)
Secondhand smoke (0.015–3 ng/ml)	43 (59.7)	57 (67.9)
Active smoker (≥3 ng/ml)	3 (4.2)	9 (10.7)
Child's race		
Non-Hispanic white	40 (55.6)	45 (53.6)
Not-non-Hispanic white	32 (44.4)	39 (46.4)
Family annual income (USD)	59,000 ± 44,000	57,000 ± 43,000
Child's age at 12-year assessment (year)	12.4 ± 0.7	12.2 ± 0.6
Child's BMI *Z*-score at 12-year assessment	0.3 ± 1.1	0.7 ± 1
Average urinary gestational triclosan concentration (μg/g creatinine)	23.2 (9.7, 47.5)	19.8 (9.8, 34.6)
Average urinary childhood triclosan concentration (μg/g creatinine)	30.7 (13.2, 71.3)	21.8 (10.7, 41.9)
Serum hormone concentrations		
Dehydroepiandrosterone-sulfate (μg/dl)	126.6 ± 71.8	109.9 ± 52.7
Luteinizing hormone (mIU/ml)	2.9 ± 2.4	5.9 ± 2.8
Follicle-stimulating hormone (mIU/ml)	1.8 ± 1.4	3.9 ± 3.5
Testosterone (ng/dl)	191.4 ± 196.4	–
Estradiol (pg/dl)	–	54.5 ± 56.8
Age at menarche[a] (year)	–	11.5 ± 0.7

[a]Average of maternal- and self-report for n = 35 females with either maternal or self-report of menarche. Menarchal data missing for one participant.

Table 2.

Adjusted percent difference in serum hormone concentrations per doubling in average gestational and childhood urinary triclosan concentrations from linear models for individual windows of exposure

	Males (n = 72)		Females (n = 84)		
Exposure window	**Estimate (95% CI)[a]**	***P*[b]**	**Estimate (95% CI)[a]**	***P*[b]**	**Interaction *P*[c]**
Dehydroepiandrosterone-sulfate					
Gestational	5 (–3, 12)	0.21	–1 (–9, 7)	0.78	0.28
Childhood	–2 (–9, 6)	0.65	–2 (–10, 5)	0.53	0.85
Follicle-stimulating hormone[d]					
Gestational	–10 (–18, –1)	0.03	6 (–3, 17)	0.21	0.01
Childhood	–10 (–19, 0)	0.04	3 (–7, 13)	0.60	0.03
Luteinizing hormone[d]					
Gestational	–3 (–16, 13)	0.73	5 (–10, 22)	0.55	0.49
Childhood	3 (–12, 21)	0.68	–1 (–15, 15)	0.87	0.62
Testosterone					
Gestational	–7 (–25, 16)	0.53			
Childhood	1 (–20, 29)	0.91			
Estradiol					
Gestational			–4 (–18, 13)	0.60	
Childhood			–16 (–29, –0)	0.05	

[a]Models are adjusted for child's race/ethnicity (non-Hispanic white, not-non-Hispanic white), age at follow-up visit (continuous), household income (continuous), and maternal gestational serum cotinine (continuous). For hormones measured in males and females (follicle-stimulating hormone, luteinizing hormone, dehydroepiandrosterone-sulfate) models include child's sex (male, female) and an interaction between child's sex and triclosan.

[b]Sex-specific *P* value.

[c]Interaction *P* value between child's sex and exposure.

[d]Sample size for males: n = 44.

of gestational triclosan concentrations was associated with 10% lower FSH concentrations ([95% CI = –18, –2], *P* = 0.02). We found no association of triclosan with LH or DHEA-S among males. There was no observable association between triclosan and testosterone among males. The inclusion of BMI *Z*-scores in the model did not meaningfully alter results (Supplemental Tables 5–7; http://links.lww.com/EE/A270). Similarly, our conclusions from unadjusted models were not notably different (Supplemental Tables 8–10; http://links.lww.com/EE/A270).

Average urinary gestational or childhood triclosan concentrations were not associated with adrenarchal or pubertal stage at age 12 years, although estimates were imprecise (Table 3, Supplemental Table 11; http://links.lww.com/EE/A270). However, in multiple informant models, each doubling of triclosan concentrations at ages 12 and 24 months was associated with 63% lower odds (OR = 0.37, [95% CI = 0.14, 0.95]) of having attained more advanced pubic hair development among females, but not males (i.e., triclosan was associated with less mature development; Figure 2, Supplemental Table 12; http://links.lww.com/EE/A270). However, the omnibus *P* value suggested that the association did not vary across windows of exposure assessment. The inclusion of BMI *Z*-scores in the model did not meaningfully alter results (Supplemental Tables 13–15; http://links.lww.com/EE/A270). Sensitivity analyses separating Tanner stages 1 and 2 males and excluding Tanner stage 1 females had similar results

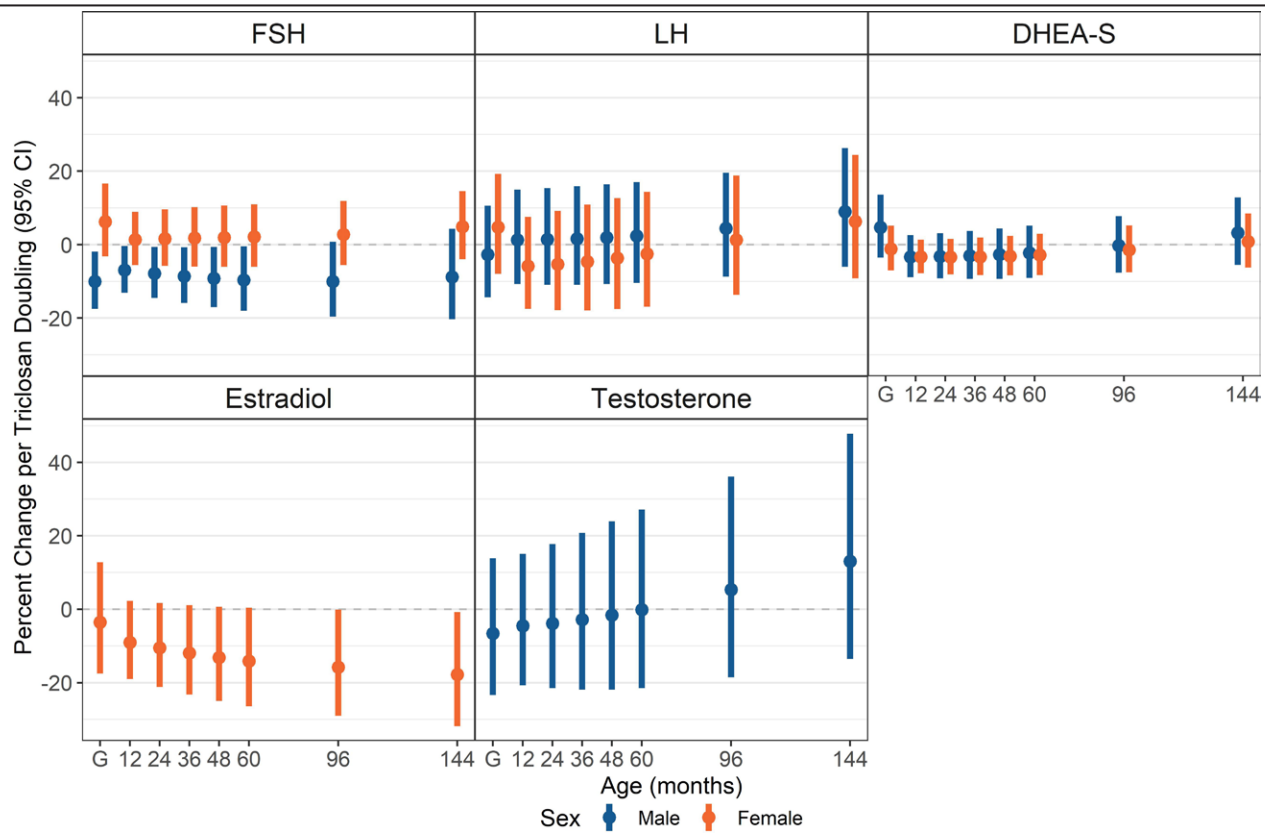

Figure 1. Adjusted percent difference in serum hormone concentrations per doubling in creatinine-normalized urinary triclosan concentrations across gestation and childhood from linear generalized estimating equations. Models are adjusted for child's race/ethnicity (non-Hispanic white, not-non-Hispanic white), age at follow-up visit (continuous), household income (continuous), and maternal gestational serum cotinine (continuous). For hormones measured in males and females follicle-stimulating hormone (FSH), luteinizing hormone (LH), dehydroepiandrosterone-sulfate (DHEA-S) models include child's sex (male, female) and an interaction between child's sex and triclosan. Age along the *x*-axis is shown in months, with "G" representing average gestational concentrations. Number of female observations = 672 (84 individuals); number of male observations = 576 (72 individuals), except for LH and FSH = 352 (44 individuals).

Table 3.

Adjusted odds ratios for each more advanced development stage per doubling in gestational and childhood urinary triclosan concentrations from cumulative logit ordinal models for individual windows of exposure

		Males (n = 72)		Females (n = 82)	
Exposure window		**Odds ratio (95% CI)[a]**	***P***	**Odds ratio(95% CI)[a]**	***P***
Pubic hair development					
	Gestational	1.08 (0.38, 3.06)	0.88	1.70 (0.56, 5.12)	0.35
	Childhood	0.79 (0.24, 2.59)	0.70	0.50 (0.16, 1.63)	0.25
Breast development					
	Gestational			1.29 (0.41, 4.1)	0.67
	Childhood			1.63 (0.48, 5.6)	0.44

[a]Models are adjusted for child's race/ethnicity (non-Hispanic white, not-non-Hispanic white), age at follow-up visit (continuous), household income (continuous), and maternal gestational serum cotinine (continuous). Pubic hair development models were run separately in males and females.

(Supplemental Tables 16–18; http://links.lww.com/EE/A270). In unadjusted models, average childhood urinary triclosan was associated with lower odds of more advanced pubic hair development in males (OR = 0.39 [0.17, 0.90], *P* = 0.03, Supplemental Table 19; http://links.lww.com/EE/A270), with no evidence of variation in the estimate across the postnatal period (Supplemental Tables 20 and 21; http://links.lww.com/EE/A270). The difference in estimates between unadjusted and adjusted models was possibly driven by negative confounding by race/ethnicity.

Maternal- and self-report of age at menarche were highly correlated (Spearman rho = 0.8; Supplemental Figure 5; http://links.lww.com/EE/A270). Each doubling of average gestational triclosan concentrations was associated with 4% earlier age at menarche (time ratio [TR] = 0.96 [0.9, 1.01], *P* = 0.13), equivalent to 5.5 months (95% CI = 14 months earlier, 1.5 months later) (Table 4). Estimates were similar in models co-adjusting for gestational and childhood concentrations (Supplemental Table 22; http://links.lww.com/EE/A270) In multiple informant models, the estimate for gestational concentrations was similar (TR = 0.96 per doubling [0.92, 1], *P* = 0.07; Figure 3, Supplemental Table 23; http://links.lww.com/EE/A270). Adjusting for BMI *Z*-scores enhanced the association from multiple informant models (TR = 0.9 [95% CI = 0.86, 0.95]), and models for average gestational and childhood concentrations produced a similar estimate to models without BMI *Z*-scores (Supplemental Tables 24–26; http://links.lww.com/EE/A270). Estimates were similar using self-report of age at menarche (Figure 3, Table 4, Supplemental Tables 22–26; http://links.lww.com/EE/A270). In

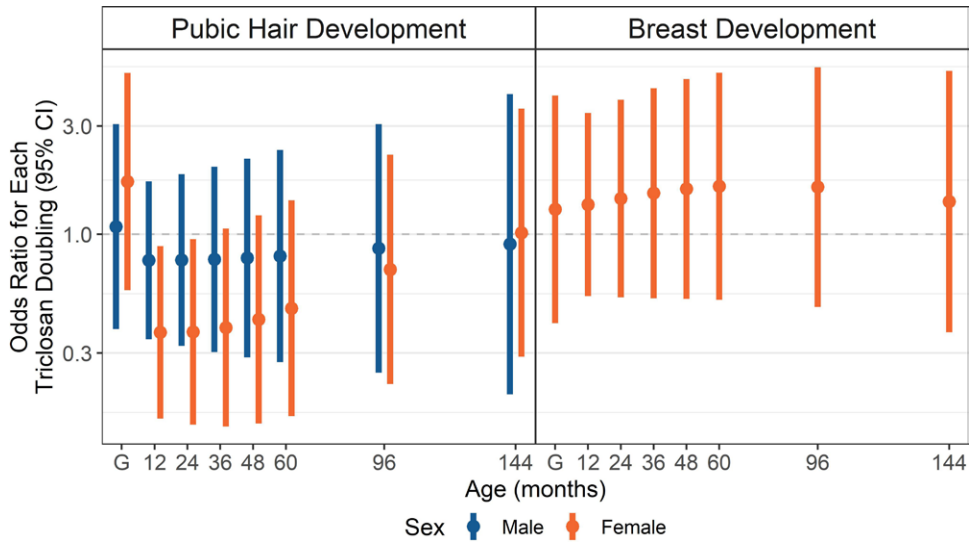

Figure 2. Adjusted odds ratios for more advanced development per doubling in creatinine-normalized urinary triclosan concentrations across gestation and childhood from cumulative link generalized estimating equations. Models are adjusted for child's race/ethnicity (non-Hispanic white, not-non-Hispanic white), age at follow-up visit (continuous), household income (continuous), and maternal gestational serum cotinine (continuous). Models for pubic hair development were run separately in males and females. Age along the *x*-axis is shown in months, with "G" representing average gestational concentrations. Number of female observations = 656 (82 individuals); number of male observations = 576 (72 individuals).

Table 4.

Adjusted ratios for time until menarche per doubling in gestational and childhood urinary triclosan concentrations from accelerated failure time models for individual windows of exposure (n = 83)

	Maternal report		Self-report	
Exposure window	**Time ratio (95% CI)[a]**	***P***	**Time ratio (95% CI)[a]**	***P***
Gestational	0.96 (0.9, 1.01)	0.13	0.95 (0.90, 1.00)	0.07
Childhood	1.03 (0.96, 1.1)	0.39	1.04 (0.97, 1.11)	0.27

[a]Models are adjusted for child's race/ethnicity (non-Hispanic white, not-non-Hispanic white), age at follow-up visit (continuous), household income (continuous), and maternal gestational serum cotinine (continuous).

unadjusted models, average childhood urinary triclosan concentrations were associated with increased time to menarche (TR = 1.05 per doubling [1.01, 1.09], P = 0.02, Supplemental Table 27; http://links.lww.com/EE/A270), with differences between the unadjusted and adjusted estimates likely driven by race/ethnicity. There was no evidence of variation in the estimate across the postnatal period (Supplemental Tables 28 and 29; http://links.lww.com/EE/A270).

Discussion

In this United States cohort, urinary triclosan concentrations were associated with differences in self-reported adrenarchal and pubertal development and hormone concentrations in an age-dependent and sex-specific manner. Females with higher postnatal triclosan concentrations had lower estradiol and later adrenarche, whereas females born to mothers with higher triclosan concentrations during pregnancy had earlier menarche. Males born to mothers with higher gestational and childhood triclosan concentrations had lower FSH concentrations. We did not find evidence of associations between urinary triclosan concentrations and other measures of sexual maturation.

Results of previous studies that examined the association of triclosan with gonadotropin and sex steroid hormones in a general population are generally consistent with our findings. One study in premenopausal adult females (18–44 years of age) examined triclosan and other EDCs at the beginning of a menstrual cycle in relation to blood hormone concentrations across the following two cycles.[53] While triclosan was not associated with estradiol, FSH, or LH in single chemical models, a principal component analysis revealed that a component correlated with high phenol concentrations, including triclosan, was associated with lower concentrations of these hormones. In a cross-sectional study using data from the US National Health and Nutrition Examination Survey and the Canadian Health Measures Survey triclosan was positively associated with levels of estradiol in females aged 6–11 years, but there was no association among females aged 12–19 years, who are more similar in age to the participants in our study when estradiol was quantified.[21] Triclosan was also associated with lower testosterone levels in males aged 12–19 years. However, this may be a statistical artifact due to truncating measured triclosan concentrations, as other National Health and Nutrition Examination Survey-based studies have found little evidence of an association between triclosan and testosterone concentrations.[21,22]

Prior research also supports our findings related to phenotypic outcomes (Tanner stage and age at menarche). In the Breast Cancer and Environmental Research Program study, mid-childhood (6- to 8-year-old) urinary triclosan was associated with earlier age of thelarche (i.e., advancing from Tanner breast stage 1 to 2+)[23,25] but not menarche.[24] Because most females in our population were Tanner breast stage 2+, we were unable to estimate the odds of reaching thelarche. Nevertheless, we observed greater odds of more advanced breast development with higher postnatal triclosan concentrations, although the confidence interval for this estimate encompassed the null. In contrast, in the CHAMACOS cohort gestational and mid-childhood (~9-year-old) triclosan concentrations were not associated with onset of

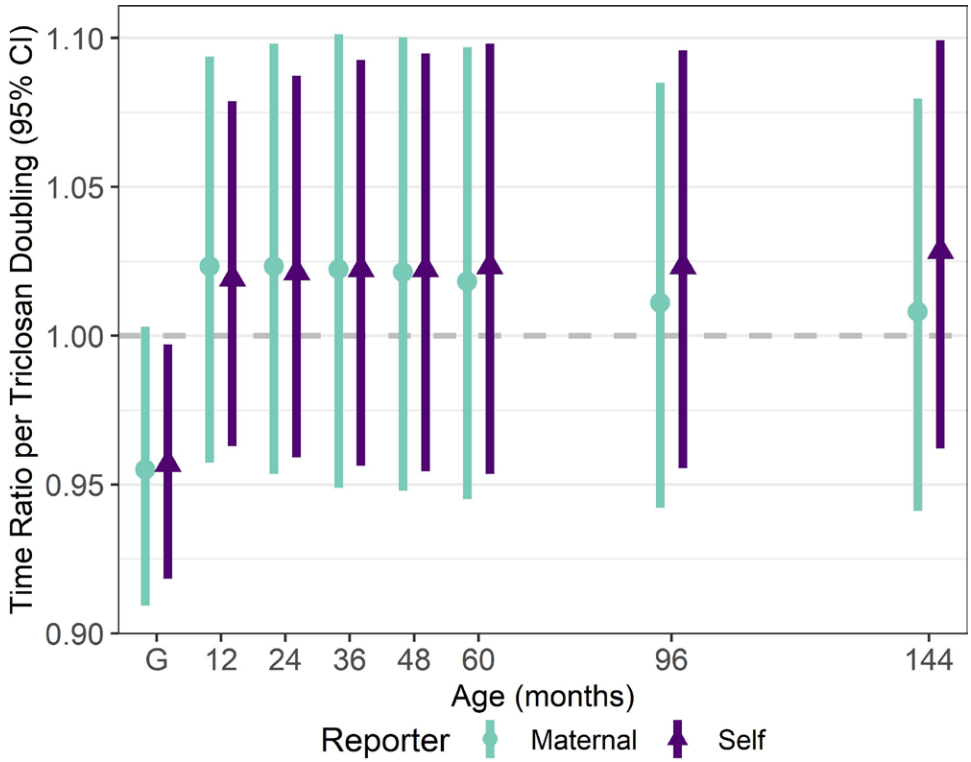

Figure 3. Time ratio for age at menarche per doubling in creatinine-normalized urinary triclosan concentrations across gestation and childhood from accelerated failure time generalized estimating equations. Models are adjusted for child's race/ethnicity (non-Hispanic white, not-non-Hispanic white), age at follow-up visit (continuous), household income (continuous), and maternal gestational serum cotinine (continuous). Age along the *x*-axis is shown in months, with "G" representing average gestational concentrations. Number of observations = 664 (83 individuals, 35 of whom reached menarche by follow-up).

puberty or onset of adrenarche, but gestational concentrations were associated with earlier menarche (0.7 months per doubling);[26] we also observed an association between gestational triclosan concentrations and earlier menarche, but our estimate was larger (5.5 months per doubling) with a wide confidence interval encompassing the CHAMACOS estimate. In cross-sectional studies, triclosan has not been associated with differences in age at menarche.[27–30] Taken together, these studies indicate there may be sensitive developmental windows when triclosan exposure has the greatest impact on pubertal development (e.g., gestation for age at menarche and mid-childhood for breast development).

Our findings pertaining to hormones and pubertal outcomes were not always aligned. For example, we observed a negative association between postnatal triclosan and estradiol concentrations in adolescence, but associations with breast development or age at menarche had 95% CIs including the null. Hormone concentrations are correlated with pubertal and adrenarchal self-staging assessed concomitantly,[40] but hormone concentrations measured before onset of breast development may provide additional insight into the observed associations. Additionally, we did not account for variability in estradiol concentrations related to the menstrual cycle due to the low proportion (42%) of participants having reached menarche, which may have decreased precision in our estimates. Similarly, we observed early postnatal triclosan concentrations were associated with less advanced pubic hair development among females, but we found no association with DHEA-S concentrations. Our analysis may be limited by the low proportion of prepubertal females in our population. Our finding that triclosan was associated with FSH but not LH or testosterone among males raises the possibility of disrupted regulation of FSH production.[54,55] Given the role of FSH in spermatogenesis, our findings are in line with prior reports of associations between triclosan and abnormal sperm morphology.[56–58]

Our results have different downstream implications. For example, higher gestational triclosan exposure, which was associated with earlier menarche in our study, may increase the risk of health effects related to earlier puberty, including depression and breast cancer.[59–61] However, replication and additional longitudinal studies are required to support this. Our estimate that doubling gestational urinary triclosan concentrations was associated with 5.5 months earlier menarche is clinically relevant, and larger than prior estimates,[26] although the confidence interval encompassed the null and should be interpreted with caution. We also found suggestive evidence of an association between early childhood postnatal triclosan concentrations and delayed adrenarche among females, which may have implications for bone health.[5,62]

Our study should be interpreted in the context of its limitations. Although correlated, triclosan concentrations were significantly lower at the 12-year visit than at earlier ages. This finding may reflect regulatory changes regarding the use of triclosan in consumer products[14] or may relate to the collection of a fasting urine at this later visit, which could temporarily reduce ingested triclosan exposure and artificially increase creatinine concentrations.[63] We did not account for diurnal variation in hormones, which may have led to outcome measurement error, biasing results toward the null assuming nondifferential misclassification. We scheduled morning study visits and collected blood samples at the beginning of each visit to minimize variability in the timing of collection. By age 12 years, most HOME Study participants, particularly females, had initiated adrenarche and all but one had reached thelarche. Thus, our ability to determine the impact of triclosan on these clinical outcomes was limited. Further, self-reporting of pubertal development may not be accurate;[64] we anticipate this would lead to nondifferential misclassification and therefore bias our estimates toward the null. Our sample size was constrained, especially for FSH and LH among males, preventing us from examining effect modification by characteristics like BMI,[23,24] and possibly leading to null findings. Although participants followed through 12 years of age

were similar to those recruited at baseline, loss to follow-up may have led to selection bias. Finally, triclosan is one of many EDCs to which children are exposed and may act synergistically with other EDCs in influencing sexual maturation. Future studies would benefit from considering mixtures of exposures.

Our prospective cohort design and serial assessment of triclosan concentrations allowed us to examine windows of sensitivity for hormones and pubertal endpoints. We used a regression calibration approach to minimize the impact of triclosan measurement error.[34] By considering multiple pubertal endpoints (hormone concentrations, developmental self-staging) we were able to discern subtle differences in their associations with triclosan. HOME Study participants' urinary triclosan concentrations[65] and pubertal development[61] are similar to the general U.S. population, enhancing the transportability of these findings.

This study contributes to the epidemiologic evidence of triclosan's capacity to disrupt the endocrine system, particularly the hypothalamic-pituitary-gonadal axis. We found gestational triclosan concentrations were associated with earlier menarche, albeit not significantly, but childhood concentrations were associated with lower hormone concentrations and delayed development. Our findings highlight the relevance to better elucidate potential sex-specific and time-dependent actions of triclosan during these key developmental periods.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

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