Environment International 122 (2019) 256–262

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Cross-sectional associations between urinary triclosan and serum thyroid function biomarker concentrations in women

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ARTICLE INFO

Handling Editor: Lesa Aylward

Keywords:
Endocrine disrupting chemicals
Triclosan
Thyroid hormones
Women
Epidemiology

ABSTRACT

Introduction: Exposure to the antimicrobial agent triclosan is ubiquitous. Research in animals shows that triclosan can cause decreases in thyroxine concentrations. However, the potential effects of triclosan on thyroid function in humans are unclear.

Objective: To estimate the association between urinary triclosan concentrations and serum thyroid function biomarkers in women seeking assisted reproduction treatment in the Environment and Reproductive Health (EARTH) Study.

Methods: We conducted a cross-sectional study of 317 women enrolled in the EARTH Study, a prospective preconception cohort that recruits Boston area couples. Using samples collected at study entry, we quantified urinary triclosan and serum thyroid function biomarker concentrations, specifically free and total thyroxine and triiodothyronine, thyroid-stimulating hormone (TSH), and thyroid antibodies. We estimated covariate-adjusted differences in thyroid function biomarkers per 10-fold increase in triclosan using linear regression models. We examined effect modification by body mass index (BMI) and infertility diagnosis.

Results: The median urinary triclosan concentration was 7.8 μg/L (IQR: 3.0–59 μg/L). Each 10-fold increase in triclosan was inversely associated with free triidothyronine (T_3) (β: −0.06 pg/mL; 95% CI: −0.1, −0.01), thyroperoxidase antibody (TPOAb) (−10%; 95% CI: −19, −0.4), and thyroglobulin antibody (TgAb) (−12%; 95% CI: −23,0.9) concentrations. BMI and infertility diagnosis modified the association of triclosan with free T_3 and TPOAb, respectively.

Conclusion: Urinary triclosan concentrations were inversely associated with specific serum thyroid function biomarkers in this cohort, suggesting that triclosan may affect thyroid homeostasis and autoimmunity.

1. Introduction

In 2016 and 2017, the U.S. Food and Drug Administration (FDA) banned the antimicrobial agent triclosan from over-the-counter hand and body washes. The ruling was the result of health concerns raised about the adverse impacts of triclosan on human health, including allergy risk, antimicrobial resistance, developmental toxicity, and endocrine disruption (Braun, 2016; FDA, 2016; FDA, 2017). However, triclosan is still found in other products, including some toothpastes and personal care products, and the Environmental Protection Agency's (EPA) risk assessment of triclosan in household products continues to be updated (EPA, 2017). Biomonitoring data suggest that triclosan exposure is ubiquitous in many countries, including the United States (Han et al., 2016; Yin et al., 2015; CDC, 2018).

Due to its endocrine-disrupting properties, triclosan may alter thyroid homeostasis (Paul et al., 2009). This is particularly important for women trying to conceive as thyroid hormones are essential for normal female reproduction (Korevaar et al., 2018a). Previous results from studies in rodents indicate that concentrations of triclosan above those experienced by humans reduce thyroxine concentrations across the lifespan (Johnson et al., 2016). Paul et al. (2009) showed that this may partly be due to triclosan mediated upregulation of thyroid

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https://doi.org/10.1016/j.envint.2018.11.015
Received 5 August 2018; Received in revised form 7 November 2018; Accepted 7 November 2018
Available online 23 November 2018
0160-4120/

hormone sulfation and glucuronidation, leading to increased thyroid hormone excretion. The findings in animal studies are consistent with results from some studies in humans. In pregnant women, Aker et al. (2016), Wang et al. (2017), and Berger et al. (2018) observed inverse associations of triclosan with maternal total T_3, free T_4, and total T_4 concentrations, respectively. However, null associations were found in both a randomized intervention where pregnant women were assigned to use triclosan-containing products (Ley et al., 2017) and two other pregnancy cohorts (Aker et al., 2018; Braun et al., 2017). In neonates, Wang et al. (2017) and Braun et al. (2017) reported inverse associations of maternal triclosan concentrations with free T_3 and total T_4 concentrations, respectively. In children and adolescents, Braun et al. (2017) and Koeppe et al. (2013) observed positive associations of triclosan with total T_4 and total T_3, respectively.

Thyroid antibody positivity, which reflects thyroid autoimmunity, is an important risk factor for thyroid dysfunction. However, there is limited literature on triclosan exposure and thyroid autoimmunity, but previous research suggests negative associations between bisphenol A and thyroid autoimmunity (Chailurkit et al., 2016). Thyroid antibody positivity has been associated with adverse reproductive outcomes such as ovarian reserve (Korevaar et al., 2018a, 2018b), miscarriage (Liu et al., 2014), and premature delivery (Korevaar et al., 2013).

Given that existing epidemiology studies to date are inconclusive on the potential impact of triclosan exposure on thyroid function, we aimed to estimate the association between urinary triclosan concentrations and thyroid function biomarkers concentrations in women seeking assisted reproduction treatment in the Environment and Reproductive Health (EARTH) Study.

2. Materials and methods

2.1. Study participants

Since 2004, the EARTH Study has recruited women and their partners from the Massachusetts General Hospital (MGH) Fertility Center into a prospective preconception cohort that examines how the environment, diet, and other factors impact human reproduction. Further details on study design, participant retention, and follow-up have been published (Messerlian et al., 2018). Briefly, study staff invited women and their partners seeking medically assisted reproductive treatment to participate in the study and if enrolled, study staff followed them through the course of their fertility evaluation and treatments. Study staff administered questionnaires, collected biospecimens, and abstracted clinical information longitudinally during the course of their fertility evaluations and treatments (Messerlian et al., 2018).

For this cross-sectional study, we analyzed the baseline serum samples collected between 2004 and 2015 for thyroid biomarkers of the 558 women who enrolled in the EARTH Study during that time period. Of those selected, we excluded the women whose urine was not previously analyzed for triclosan (n = 219). We then excluded women who were previously diagnosed with hypothyroidism or hyperthyroidism (n = 13), reported thyroid medication use in the past three months (n = 7), or had missing covariate information (n = 1). All participants provided written informed consent. The Institutional Review Boards of MGH and Harvard T.H. Chan School of Public Health and of the Centers for Disease Control and Prevention (CDC) approved this study.

2.2. Urinary triclosan concentrations

At enrollment, trained study staff collected a single spot urine sample from each woman in a sterile polypropylene cup. Our staff analyzed each sample for specific gravity using a handheld refractometer, divided it into aliquots, and then froze samples at −80 °C. Study staff then shipped the samples on dry ice to the CDC (Atlanta, GA, USA) to quantify triclosan concentrations using online solid phase extraction-isotope-dilution high performance liquid chromatography-tandem mass spectrometry (Ye et al., 2008). The limit of detection (LOD) for triclosan was 2.3 μg/L or 1.0 μg/L, depending on the year the sample was analyzed, and values below the LOD were replaced with the LOD divided by the square root of two (Hornung and Reed, 1990). Triclosan concentrations were standardized by specific gravity (SG) to account for urine dilution using the following formula: $Tc = T_i[(1.013 - 1) / (SG_i - 1)]$, where 1.013 was the median specific gravity of these participants, T_i is the measured triclosan concentration of the ith participant, SG_i is the specific gravity of the ith participant, and Tc is the SG-standardized triclosan concentration (μg/L).

2.3. Serum thyroid measures

Our study staff collected a single non-fasting blood sample from each woman via venipuncture on the same day of urine sample collection. After being centrifuged, staff stored the serum at −80 °C before sending it on dry ice to the Department of Clinical Chemistry, Máxima Medical Center (Veldhoven, The Netherlands) for analysis of thyroid function biomarkers. There, staff quantified concentrations of thyroid stimulating hormone (TSH), free and total thyroxine (T_4) and triiodothyronine (T_3), and thyroperoxidase antibody (TPOAb) and thyroglobulin antibodies (TgAb), using electrochemoluminescence assays (Cobas® e601 platform; Roche Diagnostics, Mannheim, Germany). Between-run coefficients of variation were 2.1%, 3.5%, 3.8%, 3.8%, and 7.7% for TSH, free T_4, total T_4, free T_3 and total T_3, respectively. Coefficients of variation were 12.4% and 7.1% for TPOAb at 33 or 100 IU/L, respectively, 10.9% and 8.6% for TgAb at 76 and 218 IU/L, respectively.

2.4. Covariates

Using a directed acyclic graph, we identified factors expected to be associated with both concentrations of triclosan and thyroid function biomarkers, which we then adjusted for in our analysis (Supplemental Fig. 1). We assessed age, race, and smoking status from the baseline questionnaire. At study entry, study staff measured weight and height, and we used these to calculate Body Mass Index (BMI, kg/m^2).

2.5. Statistical methods

We calculated descriptive statistics on participant demographics and the distribution of urinary triclosan and serum thyroid function biomarkers concentrations according to these characteristics. We used linear regression to estimate the unadjusted and covariate-adjusted relations between specific-gravity standardized urinary triclosan and serum thyroid function biomarker concentrations. Triclosan concentrations were right-skewed so we $\log_{10}$-transformed them to reduce the influence of outliers. The distributions of TSH, TPOAb, and TgAb were right-skewed and we $\log_{10}$-transformed them to approximate normality assumptions of linear regression. The concentrations of free and total T_3 and T_4 were approximately normal and we did not transform them for the analysis. Our final multivariable regression model included age and BMI as continuous variables, while race was categorized as White or non-White, and smoking status as current, former, or never. We ran another multivariable model that also adjusted for infertility cause, using the Society for Assisted Reproductive Technology classifications: male cause, female cause, or unknown (Messerlian et al., 2017). However, we acknowledge that infertility cause could be a potential mediator of thyroid function.

To improve interpretability, the regression coefficients for the $\log_{10}$-transformed thyroid function biomarkers were back-transformed and expressed as a percent differences per 10-fold increase in urinary triclosan concentration. We assessed the dose-response relations between triclosan and thyroid function biomarkers using two methods: a generalized additive model (GAM), allowing a flexible dose-response relationship, and by categorizing triclosan into quartiles, with the lowest

quartile serving as the reference group. From the GAM, we calculated the p-value for the difference in the fit of models where triclosan was non-linear vs. linear using a likelihood ratio test. From the models using quartiles, we determined p-values for a linear trend from a regression where we assigned each participant the median value of their corresponding triclosan quartile. We used R version 3.3.3 to perform the data analysis.

2.6. Secondary analyses

BMI is a well-known determinant of thyroid function and autoimmunity, and likely affects these endpoints via different pathways than those that triclosan would (Laurberg et al., 2012). Thus, we examined possible effect modification by BMI category (BMI $< 25\ kg/m^2$ vs BMI $\geq 25\ kg/m^2$). Since the underlying causes of infertility may increase vulnerability to the effects of triclosan, potentially through hampered autoimmunity, we examined infertility diagnosis as an effect modifier (Liu et al., 2014). We determined whether associations between triclosan and thyroid function biomarkers differed across the strata of both of these modifiers by using a product interaction term between triclosan and BMI or infertility diagnosis category. We then estimated strata-specific covariate-adjusted associations. We considered modification to be present when the p-value for the interaction term was < 0.10 because our power to detect interactions was likely low (Greenland, 1983).

3. Results

The 317 women included in our analysis had a median age of 34 years and BMI of $23.2\ kg/m^2$. Most participants were white (82%) and never smoked (73%) (Table 1). We detected triclosan in 79% of the participants with a median specific gravity standardized urinary concentration of 7.8 μg/L and an IQR from 3.0–59 μg/L. The full range of triclosan values covered 3.6 orders of magnitude. Median urinary triclosan concentration did not vary considerably across categories of each covariate with the exception of infertility diagnosis. Women whose infertility cause was unknown (n = 143) had a median urinary triclosan concentration of 5.5 μg/L while those who were female-related (n = 97) or male related (n = 75), had median concentrations of 12.1 μg/L and 11.3 μg/L, respectively (Table 1).

When compared to clinical reference thresholds for free (0.8–1.7 ng/dL) and total T_4 (5.1–14.1 μg/dL), free (2.4–4.2 pg/mL) and total T_3 (80–200 ng/dL), and TSH (0.30–4.0 mU/L), 2%, 3%, 1%, 3%, and 5% of women in our study had values outside of the reference range, respectively (Table 1). The median thyroid function biomarkers did not differ considerably across strata of each covariate (Table 1).

After adjustment for covariates, each 10-fold increase in urinary triclosan concentrations was associated with a −0.06 pg/mL lower free T_3 concentrations (95% CI: −0.10, −0.01), 10% lower TPOAb concentrations (95% CI: −19, −0.4), and 12% lower TgAb concentrations (95% CI: −23, 0.9) (Table 2). These associations were fairly linear across the range of urinary triclosan concentrations (Fig. 1, non-linearity p-values ≥ 0.29). Generally, concentrations of free T_3, TPOAb, and TgAb decreased across increasing triclosan quartiles, with the largest decrease in the fourth quartile (Supplemental Table 1). Our results for these thyroid function biomarkers were essentially identical in unadjusted and covariate-adjusted models (Table 2). After adjusting for infertility cause, we still observed inverse associations between urinary triclosan concentrations and serum free T_3 (β: −0.06 pg/mL; 95% CI: −0.11, −0.01), TPOAb (−12%; 95% CI: −21, −2.5), and TgAb (−14%; 95% CI: −26, −1.4) concentrations (Table 2). Triclosan was not associated with TSH, free or total T_4, or total T_3.

3.1. Secondary analyses

The association between urinary triclosan concentrations and serum

Table 1
Percentiles of specific gravity standardized urinary triclosan concentrations and serum thyroid function biomarker concentrations by covariates among women in EARTH Study women at baseline.

Participants Overview	N (%)	Triclosan μg/L	TSH mU/L	Free T_4 ng/dL	Total T_4 μg/dL	Free T_3 pg/mL	Total T_3 ng/dL	TgAb IU/mL	TPOAb IU/mL
		Median (25th–75th)	Median (25th–75th)	Median (25th–75th)	Median (25th–75th)	Median (25th–75th)	Median (25th–75th)	Median (25th–75th)	Median (25th–75th)
All	317 (100)	7.8 (3.0–59)	1.9 (1.4–2.6)	1.2 (1.1–1.3)	7.5 (6.8–8.5)	3.1 (2.9–3.4)	118 (102–135)	0.02 (0.01–0.02)	0.12 (0.10–0.16)
Race									
White	260 (82)	7.8 (3.0–60)	1.8 (1.4–2.5)	1.2 (1.1–1.3)	7.4 (6.7–8.4)	3.1 (2.9–3.5)	118 (102–136)	0.02 (0.01–0.02)	0.12 (0.10–0.16)
Other	57 (18)	6.9 (3.4–25)	2.2 (1.6–2.9)	1.3 (1.1–1.3)	7.9 (7.0–8.7)	3.1 (2.9–3.3)	115 (103–128)	0.01 (0.01–0.02)	0.12 (0.10–0.17)
Age									
≤ 29	33 (10)	7.4 (2.3–34)	2 (1.5–2.4)	1.2 (1.1–1.3)	7.7 (6.6–8.5)	3.1 (3.0–3.4)	119 (107–130)	0.01 (0.01–0.02)	0.12 (0.10–0.14)
30–34	139 (44)	11.2 (3.7–63)	1.8 (1.4–2.5)	1.2 (1.1–1.3)	7.5 (6.9–8.6)	3.1 (2.9–3.4)	118 (101–137)	0.02 (0.01–0.03)	0.12 (0.10–0.16)
35–39	102 (32)	6.4 (3.0–36)	1.9 (1.4–2.8)	1.2 (1.1–1.3)	7.4 (6.7–8.4)	3.1 (2.9–3.4)	116 (102–131)	0.01 (0.01–0.02)	0.12 (0.09–0.15)
≥ 40	43 (14)	5.3 (2.6–151)	1.9 (1.4–2.8)	1.2 (1.1–1.3)	7.4 (6.7–8.4)	3.1 (2.8–3.5)	120 (109–136)	0.02 (0.01–0.02)	0.12 (0.10–0.18)
Smoker									
Never	232 (73)	8.2 (3.1–76)	1.9 (1.4–2.7)	1.2 (1.1–1.3)	7.5 (6.8–8.5)	3.1 (2.9–3.4)	117 (103–134)	0.02 (0.01–0.02)	0.12 (0.10–0.16)
Former	79 (25)	7.0 (2.6–37)	1.8 (1.3–2.4)	1.2 (1.1–1.3)	7.6 (6.6–8.6)	3.1 (2.9–3.4)	121 (101–137)	0.02 (0.01–0.02)	0.12 (0.10–0.15)
Current	6 (2)	12.2 (7.1–16)	2.1 (1.7–2.5)	1.3 (1.2–1.3)	7.4 (7.3–8.0)	3.1 (3.0–3.5)	120 (111–132)	0.01 (0.01–0.02)	0.12 (0.12–0.13)
BMI category									
≤ 24.9	205 (65)	7.8 (3.0–73)	1.8 (1.4–2.6)	1.2 (1.1–1.3)	7.5 (6.7–8.5)	3.1 (2.9–3.4)	116 (100–133)	0.02 (0.01–0.02)	0.12 (0.10–0.16)
≥ 25	112 (35)	7.8 (3.1–37)	2 (1.4–2.6)	1.2 (1.1–1.3)	7.5 (6.8–8.5)	3.2 (3.0–3.5)	122 (107–137)	0.02 (0.01–0.02)	0.12 (0.10–0.15)
Infertility cause									
Female	97 (30)	12.1 (4.1–89)	1.8 (1.2–2.5)	1.2 (1.1–1.3)	7.3 (6.7–8.4)	3.1 (2.9–3.5)	124 (104–137)	0.02 (0.01–0.03)	0.12 (0.11–0.16)
Male	75 (24)	11.3 (4.2–142)	2.1 (1.6–2.9)	1.2 (1.1–1.3)	7.7 (7.0–8.6)	3.1 (2.9–3.4)	116 (104–134)	0.02 (0.01–0.02)	0.12 (0.10–0.16)
Unknown	142 (45)	5.5 (2.4–21)	1.9 (1.4–2.6)	1.2 (1.1–1.3)	7.4 (6.7–8.5)	3.1 (2.9–3.4)	117 (101–132)	0.02 (0.01–0.02)	0.11 (0.09–0.15)
Missing	3 (1)								

Table 2
Unadjusted and adjusted difference in serum thyroid function biomarker concentrations for each 10-fold increase in urinary triclosan concentrations among EARTH Study women.[a,b]

Thyroid function biomarker (units)	Unadjusted model β (95% C.I.)	Adjusted model[a] β (95% C.I.)	Adjusted model with infertility cause[b] β (95% C.I.)
TSH (% difference)	−4.3 (−10, 1.9)	−4.4 (−10, 1.8)	−4.8 (−11, 1.5)
Free T_4 (ng/dL)	0.00 (−0.02, 0.02)	0.00 (−0.02, 0.02)	0.00 (−0.02, 0.02)
Total T_4 (μg/dL)	0.05 (−0.15, 0.24)	0.04 (−0.15, 0.25)	−0.04 (−0.16, 0.24)
Free T_3 (pg/mL)	−0.06 (−0.10, −0.01)	−0.06 (−0.10, −0.01)	−0.06 (−0.11, −0.01)
Total T_3 (ng/dL)	−1.9 (−5.5, 1.6)	−1.7 (−5.3, 1.8)	−2.1 (−5.7, 1.6)
TPOAb (% difference)	−10 (−19, −0.4)	−9.8 (−19, −0.1)	−12 (−21, −2.5)
TgAb (% difference)	−12 (−23, 0.9)	−12 (−23, 0.9)	−14 (−26, −1.4)

[a] Adjusted for race (white vs. other), age (continuous), smoking status (current, former, vs. never), and BMI (continuous), n = 317.
[b] Adjusted for race (white vs. other), age (continuous), smoking status (current, former, vs. never), BMI (continuous), and infertility cause (male, female, vs. unknown), n = 314.

free T_3 concentrations differed by BMI category (interaction p-value = 0.03) (Fig. 2, Supplemental Table 2). Urinary triclosan concentrations were inversely associated with free T_3 concentrations (β: −0.08 pg/mL; 95% CI: −0.14, −0.03) among women with a BMI < 25 kg/m^2, but not associated among women with a BMI ≥ 25 kg/m^2 (β: 0.04 pg/mL; 95% CI: −0.04, 0.11).

The association between urinary triclosan concentrations and TPOAb concentrations differed by infertility cause (interaction p-value = 0.02) (Supplemental Table 3, Supplemental Fig. 2). Urinary triclosan concentrations were associated with 32% lower TPOAb concentrations (95% CI: −46, −14) among women whose infertility cause was male-related, while urinary triclosan concentrations were associated with 3% (95% CI: −20, 16) or 5% (95% CI: −17, 9) lower TPOAb concentrations if the infertility cause was female-related or unknown, respectively. BMI and infertility cause did not modify the association between triclosan and any other thyroid function biomarkers (Supplemental Tables 2, 3).

4. Discussion

In this cross-sectional study of women from the EARTH study, urinary triclosan concentrations were inversely associated with serum free T_3, TPOAb, and TgAb concentrations. Urinary triclosan concentrations were not associated with concentrations of TSH, total T_3, or free or total T_4. The association between urinary triclosan concentrations and serum free T_3 concentrations was modified by BMI, where the inverse association was observed among women with a BMI < 25 kg/m^2, but not among women with a BMI ≥ 25 kg/m^2. The association between urinary triclosan concentrations and TPOAb concentrations was modified by infertility cause; the inverse association was observed among women whose infertility cause was male-related, but not if the cause was female-related or unknown.

While there is limited literature on the association between urinary triclosan and serum thyroid function biomarker concentrations in women prior to conception, our results are consistent with some previous epidemiological research in other populations. Similar to our findings, Wang et al. (2017) reported an inverse association between urinary triclosan concentrations measured in the 3rd trimester of pregnancy and free T_3 in neonates. Aker et al. (2018) reported a decrease in total T_3 with increasing urinary triclosan concentrations among pregnant women. However, in a different pregnancy cohort, Aker et al. (2016) found no associations between urinary triclosan concentrations and free T_3, free T_4, or TSH concentrations. Ley et al. (2017) reported no effect of triclosan containing product use on TSH, T_3, and T_4, concentrations in pregnant women from a randomized intervention with exposure occurring from 16 weeks until the end of gestation. While we did not see any associations between triclosan and T_4, others have reported such findings. Wang et al. observed an inverse association between urinary triclosan concentrations and free T_4 among pregnant women in the 3rd trimester (Wang et al., 2017). In a pregnancy cohort in Northern California, Berger et al. (2018) found that triclosan was associated with lower maternal total T_4 concentrations, although the 95% confidence interval for this association included the null after adjusting for other environmental phenols. Braun et al. (2017) reported that urinary triclosan concentrations during the 2nd and 3rd trimesters of pregnancy and at delivery were associated with lower neonatal cord serum total T_4 concentrations, while childhood urinary triclosan concentrations were positively associated with total T_4 concentrations at age 3 years. In an analysis of NHANES data of adult participants, Koeppe et al. (2013) reported no associations between triclosan and thyroid function biomarkers. However, in the adolescent participants, they reported a positive relationship between triclosan and total T_3 concentrations (Koeppe et al., 2013).

The different patterns of associations between this and previous studies could be explained by the study participant characteristics, thyroid hormone measurements, or triclosan exposure misclassification. Six of the prior studies examined the association between triclosan exposure and thyroid function in pregnant women. During pregnancy, thyroid activity changes in many ways, including higher production of T_3 and T_4 (Korevaar et al., 2017). The study participants for Koeppe et al. (2013) included men as well as adolescents. Furthermore, Braun et al. (2017), Aker et al. (2016), Aker et al. (2018), and Berger et al. (2018) collected three, two, four, and two urine samples, respectively, while the present and several prior studies used one sample, which may have resulted in triclosan exposure misclassification (Perrier et al., 2016).

Previous studies in rats show that triclosan can increase sulfotransferase and type 3 deiodinase expression, which are responsible for T3 conjugation and metabolism of T3 to diiodothyronine, respectively (Zhang et al., 2018). This mechanism is consistent with the inverse association we observed between triclosan and free T3 concentrations. However, these two processes are also responsible for metabolizing thyroxine (T4). Yet, we did not observe an inverse association of triclosan with free or total T4. Future studies could examine different mechanisms through which triclosan may affect thyroid hormone homeostasis.

Unlike other studies, we did not observe an association of triclosan with either serum total or free T_4. Rodent studies have consistently reported an association between triclosan and T_4 (Johnson et al., 2016). Paul et al. (2009) hypothesized that this association may be due to triclosan enhancing metabolism of T_4 via upregulation of hepatic enzymes. One potential challenge to translating findings from animal studies to human data is that triclosan exposures in animal studies were up to 13,000-fold higher than estimated average exposures in humans (Paul et al., 2009). In addition, triclosan could affect thyroid homeostasis via mechanisms other than glucuronidation or sulfation, such as by inhibition of type 2 deiodinase (thyroid hormone activating enzyme) or activation of type 3 deiodinase (thyroid hormone inactivating

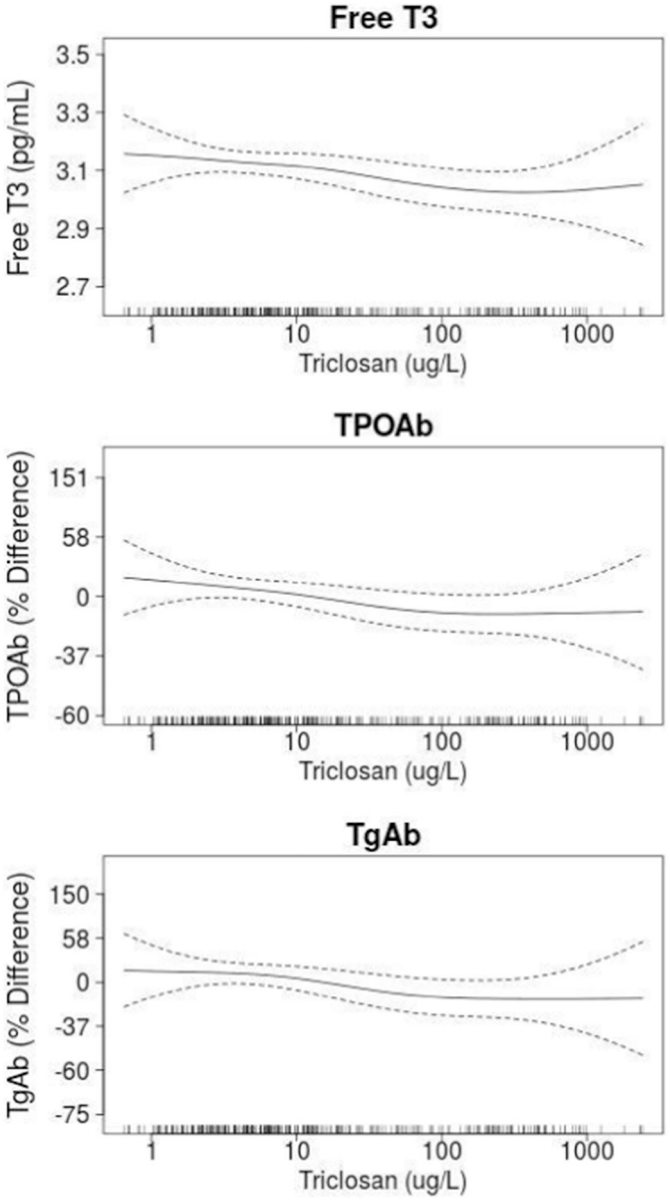

Fig. 1. Adjusted mean serum free T_3, TgAb, and TPOAb concentrations according to log_{10}-transformed urinary triclosan concentrations from a generalized additive model (GAM): EARTH Study Women[a,b]

a- The solid line shows the covariate-adjusted mean values from a GAM that regressed free T_3, TgAb, or TPOAb on log_{10} triclosan. The non-linearity p-values for free T_3, TgAb, and TPOAb were 0.49, 0.53, and 0.64, respectively, indicating that the association was linear.

b- Adjusted for race (white vs. other), age (continuous), smoking status (current, former, vs. never), and BMI (continuous).

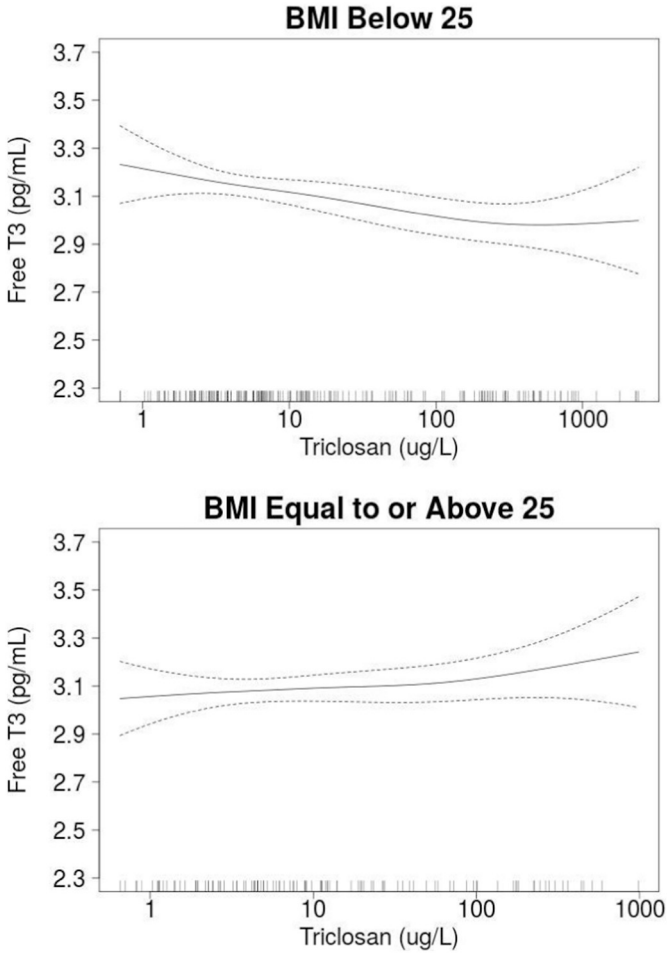

Fig. 2. Generalized additive model of the relationship between free T_3 and log-transformed triclosan among women with a BMI $< 25\,kg/m^2$ (n = 205) and BMI $\geq 25\,kg/m^2$ (n = 112): EARTH Study Women[a,b]

a- The solid line shows the covariate-adjusted fitted values from a GAM that regressed free T_3 for women with a BMI < 25 or a BMI ≥ 25; non-linearity p-values were 0.37 and 0.38, respectively.

b- Adjusted for race (white vs. other), age (continuous), smoking status (current, former, vs. never), and BMI (continuous).

enzyme) (Paul et al., 2009; Butt et al., 2011; Shimizu et al., 2013).

We are only aware of one prior randomized clinical trial in humans examining the associations between triclosan-containing toothpaste use and thyroid antibodies (TPOAb and TgAb), which reported null findings (Cullinan et al., 2012). In general, thyroid antibodies are indicative of thyroid autoimmunity and associated autoimmune thyroid diseases (Deroux et al., 2016). We speculate that the inverse association of triclosan with TPOAb and TgAb concentrations may be due to the ability of triclosan to influence the immune system (Bertelsen et al., 2013). In mice, triclosan can impair T-helper cell type 2 (TH2) cytokine response (Marshall et al., 2017). Thus, triclosan may reduce thyroid autoantibody production. However, further studies are necessary to investigate these potential mechanisms.

In our effect modification analysis, we observed that the inverse association between triclosan and free T_3 concentrations was only present among women with a BMI $< 25\,kg/m^2$. Previous research on obesity and thyroid function is inconclusive with studies reporting both positive and inverse associations between serum free T_3 and BMI (Laurberg et al., 2012). We can only speculate as to why we saw this effect modification, but it may be that women with higher BMI have poorer homeostatic regulation of thyroid function, which may result in us not being able to observe effects of triclosan on thyroid hormone function biomarkers among these women. We also observed that the association between triclosan and TPOAb concentrations was modified by infertility cause; the inverse association was only present among women who were from a couple where the infertility cause was male-related. We speculate that we observed this association because women with subfertility may be more likely to be susceptible to thyroid autoimmunity, and thus there is less chance for other determinants (such as triclosan) to have an effect on thyroid autoimmunity biomarkers. However, we acknowledge that these findings could be spurious given that we examined multiple associations.

This study has some strengths and limitations. Most notably is its cross-sectional design, which limited our ability to establish the directionality of the observed associations. Second, since triclosan has a half-

life of approximately 21 h, a single urinary concentration only reflects exposure over the last several days (Sandborgh-Englund et al., 2006). Thus, a single spot urine sample may not accurately classify a woman's long-term triclosan exposure and might result in triclosan exposure misclassification, which we would expect to attenuate our results towards the null (Perrier et al., 2016). While we assessed triclosan exposure using a specific biomarker, 21% of our participants had triclosan concentrations below the limit of detection. Third, we were able to measure the full spectrum of clinically relevant thyroid function biomarkers, where other studies were not able to consider both free and total concentrations of T_4 and T_3, as well as thyroid antibodies. Fourth, women in our cohort were predominantly white, college educated, and seeking fertility treatment, which might limit the generalizability of our findings to other populations. However, the distribution of urinary triclosan concentration among women in our study was similar to that for women from the NHANES (Woodruff et al., 2011). Finally, there is the potential for residual confounding by socioeconomic status, iodine status, and exposure to other endocrine disrupting chemicals. Prior studies suggest that associations between triclosan exposure and health outcomes may be negatively confounded given that triclosan exposure tends to be higher among higher socioeconomic status groups who also tend to have better health outcomes (Stacy et al., 2017; Jackson-Browne et al., 2018). It is also possible that other factors associated with both higher urinary triclosan concentrations and poorer thyroid function biomarkers could positively confound the associations. However, the homogeneity of certain characteristics in our participants may reduce the potential for confounding. Furthermore, adjustment for our set of covariates did not meaningfully change our results.

5. Conclusions

The results of this cross-sectional study should be interpreted cautiously, but our findings provide evidence that triclosan exposure may affect some aspects of thyroid homeostasis and autoimmunity. Given the importance of proper thyroid function across the lifespan and potential for even subtle changes in thyroid function to affect human health, our results raise concerns about the potential toxicity of triclosan exposure.

Funding

This work was supported by grants from the National Institute of Environmental Health Sciences (R01 ES024381 and R01 ES027408).

Disclosure

JMB was financially compensated for serving as an expert witness for plaintiffs in litigation related to tobacco smoke exposures.

Acknowledgements

The authors gratefully acknowledge all members of the EARTH study team, specifically the Harvard T. H. Chan School of Public Health research staff Myra Keller, and Ramace Dadd, physicians and staff at Massachusetts General Hospital fertility center. A special thank you to all of the study participants. We also acknowledge Xiaoyun Ye, Xiaoliu Zhou, and Tao Jia for their technical assistance measuring urinary triclosan and thank Antonia Calafat for her assistance with preparing the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.11.015.

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