RESEARCH ARTICLE

Sex-specific association of exposure to a mixture of phenols, parabens, and phthalates with thyroid hormone and antibody levels in US adolescents and adults

Fang Cheng[2,3] · Xueyu Chen[3] · Jiaxu Fan[3] · Junpeng Qiao[3] · Hongying Jia[1,2,3]

Abstract

Individuals are exposed to multiple phenols, parabens, and phthalates simultaneously since they are important endocrine-disrupting compounds (EDCs) and share common exposure pathways. It is necessary to assess the effects of the co-exposure of these EDCs on thyroid hormones (THs). In this study, data included 704 adolescents and 2911 adults from the 2007–2012 National Health and Nutrition Examination Survey (NHANES). Serum THs measured total triiodothyronine (T3), total thyroxine (T4), free forms of T3 (FT3) and T4 (FT4), thyroid-stimulating hormone (TSH), thyroglobulin (Tg), thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb). And 16 EDCs (3 phenols, 2 parabens, and 11 phthalates) were measured from urine. The relationship between single EDCs and single THs was analyzed using generalized linear regression. And results showed that several EDCs were positively associated with serum T3 and FT3 levels in boys but negatively associated with serum T4 and FT4 levels in girls. And in adults, five EDCs were negatively associated with T3, T4, or FT4. The effects of co-exposure to 16 EDCs on THs were calculated using Bayesian kernel machine regression and quantile-based g-computational modeling, confirmed that co-exposure was related to the increase of T3 in adolescents and the decrease of T4 in both adolescents and adults. Besides, nonlinear and linear relationships were identified between co-exposure and the risk of positive TPOAb and TgAb in girls and adult females, respectively. In conclusion, phenols, parabens, and phthalates as a mixture might interfere the concentrations of THs and thyroid autoantibodies, and the interfering effect varies significantly by sex as well as by age. Further prospective research is warranted to investigate the causal effects and underlying mechanisms of co-exposure on thyroid dysfunction.

✉ Hongying Jia
jiahongying@sdu.edu.cn

1   Shenzhen Research Institute of Shandong University, Shandong University, Shenzhen, China

2   Department of Epidemiology and Health Statistics, School of Public Health, Cheeloo College of Medicine, Shandong University, Jinan 250012, China

3   Center of Evidence-Based Medicine, the Second Hospital of Shandong University, Shandong University, Jinan 250012, China

Introduction

Thyroid is critically involved in the function of multiple biologic systems, including neurologic, metabolic, and reproductive axes (Taylor et al. 2018). The thyroid function is assessed by serum levels of thyroid hormones, including total triiodothyronine (T3), total thyroxine (T4), free forms of T3 (FT3) and T4 (FT4), thyroid-stimulating hormone (TSH), thyroglobulin (Tg), thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb). Of these, most of the circulating T3 and T4 is protein bound, with less than 1% of the thyroid hormones (THs) remaining free forms (FT3 and FT4) and biologically active (Yorita Christensen 2013). TSH is secreted by the hypothalamus to trigger the secretion of FT3 and FT4, and elevated T3 suppresses TSH production, reducing T3 and T4 levels via a classic negative

feedback loop, thus maintaining the physiological level of the hypothalamus–pituitary–thyroid (HPT) axis. Dysregulation of the HPT axis may lead to a range of adverse health effects, including neurodevelopmental disorders, cardiovascular problems, skeletal disorders, and cancer (Murphy et al. 2010).

Environmental factors are associated with disrupted TH levels and increased risk of thyroid disease (Kim et al. 2022). Since the 1970s, exposure to nonpersistent endocrine-disrupting compounds (EDCs), such as phenols, parabens, and phthalates, has become increasingly prevalent and persistent (Gore et al. 2015), and they are often used as plasticizers, fixatives, solvents, antimicrobial agents, and preservatives in personal care products and food contact materials. A survey of US adults found these EDCs in 12 personal care products that women are exposed to daily, as well as in 6 personal care products that men are exposed to daily (Yang et al. 2023). Similarly, dietary consumption leads to co-exposure to these EDCs, with parabens often used as preservatives and antifungals (Muncke et al. 2020), and phenols widely used in epoxy resin lining food and beverage containers, and as monomers for polycarbonate plastics in many consumer products (Lang et al. 2008). Exposure to the aforementioned EDCs may impair TH signaling by interfering with the biosynthesis and metabolic activation/inactivation, as well as influence the HPT axis by disrupting negative feedback loop (Gore et al. 2015; Kahn et al. 2020; Miller et al. 2009).

Humans live in a large exposure pool, with phenols, parabens, and phthalates having multiple co-exposure pathways including food packaging, cosmetics, and other consumer products (Derakhshan et al. 2021; Kim et al. 2017; Martínez et al. 2018; Philippat et al. 2015). Therefore, traditional analytical strategies for single or major chemicals may not be compatible with real-life exposure to multiple exposures simultaneously (Drakvik et al. 2020; Kortenkamp and Faust 2018). Although several studies have shifted from the toxic effects of major compounds to the combined effects of multiple contaminants on human health (Ouidir et al. 2020); only four of these studies have examined the effects of representative phenols, parabens, or phthalates in urine on THs (Kim et al. 2017; Koeppe et al. 2013; Meeker and Ferguson 2011; Przybyla et al. 2018); there is a lack of evidence related to the effects of co-exposures on THs. Also, most studies used multiple linear regression or structural equation modeling to address correlations between mixtures, ignoring chemical interactions and potential nonlinear dose–response relationships. In addition, the distribution of THs was strongly sex- and age-specific (Meeker and Ferguson 2011; Morgenstern et al. 2017; Xing et al. 2023), and the sex-specific effect of co-exposures on both thyroid hormones and thyroid autoantibodies remains uninvestigated.

Hence, the objective of this study included the data from the 2007–012 National Health and Nutrition Examination Survey (NHANES) database to simultaneously consider the association between 16 EDCs (3 phenols, 2 parabens, and 11 phthalates) and thyroid profiles (T3, FT3, T4, FT4, TSH, Tg, TgAb, and TPOAb). Additionally, it introduced three statistical methods — the weighted generalized linear regression model (GLM), Bayesian kernel machine regression (BKMR) (Bobb et al. 2017, 2018), and quantile-based g-computation (QGC) model (Keil et al. 2020) — to better evaluate the total effects of co-exposure of these 16 EDCs on thyroid function. Also, stratified analyses by sex and age were conducted to further explore the effects of co-exposure on thyroid function in different subgroups of adults and adolescents.

Materials and methods

Study population

Three cycles of NHANES data from 2007 to 2012 were selected, as thyroid hormones were measured in total participants in 2007–2008 and in one-third subsamples in 2009–2010 and 2011–2012. Meanwhile, urinary concentrations of environmental phenols, parabens, and phthalates were measured in a randomly selected one-third subset of NHANES participants older than 6 years of age in these three cycles. Eligibility criteria for the analytic sample were (1) complete thyroid hormone profiles ($n = 11{,}638$); (2) complete phenols, parabens, and phthalate metabolites in the study ($n = 3962$); and (3) no missing urinary creatinine measurements, no thyroid-related medication use, and no history of thyroid-related diseases at the point of survey ($n = 312$). Additionally, women who were pregnant at the time of the survey were further excluded ($n = 35$). As a result (Fig. 1), the final analytic sample included 704 adolescents and 2911 adults. The protocol used by NHANES was approved by the Research Ethics Review Board of the National Center for Health Statistics, and all participants provided written informed consent.

Phenols, parabens, and phthalates measured in urine

Spot urine samples were collected at mobile examination centers and stored at $-20\ ^{\circ}$C until shipment for analysis. Phenols and parabens were extracted and determined from urine using a sensitive method of online solid-phase extraction combined with HPLC and tandem mass spectrometry, and phthalate metabolites were quantified in urine using high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry. In this study, we selected chemicals for inclusion based on the metabolite's detectable frequencies ≥ 50%. Thus, as shown in Table S1, the finally chemicals were 3 phenols (bisphenol A (BPA),

Fig. 1 Flowchart of the population included in our final analysis (*N* = 3615) (NHANES, USA, 2007–2012)
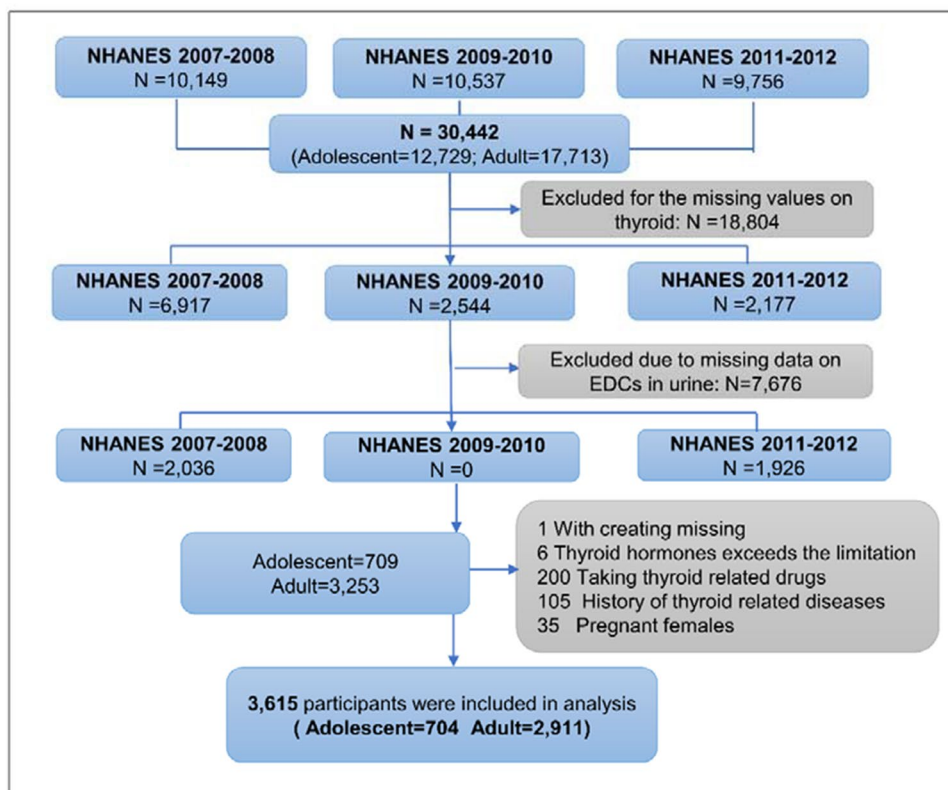
benzophenone-3 (BP-3), triclosan (TCS)), 2 parabens (propyl paraben (PPB), methyl paraben (MPB)), and 11 phthalates (mono-carboxyoctyl phthalate (MCOP), mono-carboxynonyl phthalate (MCNP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-n-butyl phthalate (MnBP), mono-3-carboxypropyl phthalate (MCPP), mono-ethyl phthalate (MEP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-hexyl phthalate (MEHP), mono-isobutyl phthalate (MiBP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-benzyl phthalate (MBzP)). If the concentrations of EDCs metabolites were below LLOD, LLOD divided by the square root of 2 was used to substitute original data. More detailed information on chemical measurement methods and quality control can be obtained from NHANES Laboratory Methods (https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/PHTHTE_G.htm).

Serum thyroid measures

Blood specimens were collected, processed, stored, and shipped to Collaborative Laboratory Services, Ottumwa, Iowa. TSH (0.035–99.564 mIU/L) was quantified with a third-generation two-site immunoenzymatic ("sandwich") hyper-sensitive human TSH assay. T3 (45–297 ng/dL), FT3 (1.9–28.84 pg/mL), and T4 (0.4–16.8 µg/dL) were quantified with competitive binding immunoenzymatic assay. FT4 (0.18–2.54 ng/dL) was quantified with a two-step enzyme

immunoassay. Tg was assessed by a simultaneous one-step sandwich assay. TgAb and TPOAb were quantified with a sequential two-step immunoenzymatic sandwich assay. A sequential two-step immunoenzymatic sandwich assay was used to quantify the levels of anti-TgAb and TPOAb. And TPOAb ≥ 9 IU/mL and TgAb ≥4 IU/mL were considered positive (Yehuda et al. 2017). The detailed description of specimen collection, processing, and laboratory could be obtained at website of NHANES (https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/THYROD_G.htm)

Covariates

Standardized questionnaires were administered to the participants in their home by trained interviewers that collected demographic information regarding age, race/ethnicity, sex, and poverty status. According to previous studies (Kim et al. 2022; Koeppe et al. 2013; Sun et al. 2021), sociodemographic factors included age (continuous variable), sex (male or female), race (Mexican American, non-Hispanic, non-Hispanic black, or other), marital status for adults (married or living with a partner, separated or divorced or widowed, or never married), an education level (below high school, high school graduate, or post-high school), and family income–to-poverty ratio (≤ 1.3, 1.3–3.49 or ≥ 3.5). Health-related status included body mass index (BMI) (underweight, normal

weight, overweight, and obese), hypertension (yes or no), diabetes (yes or no), urinary iodine (continuous variable), and urinary creatinine (continuous variable). Serum cotinine was measured to directly reflect exposure to tobacco smoke ($\geq$10 ng/mL as active smoking, 1–9.9 ng/mL as environmental tobacco smoke exposure, and <1 ng/mL as nonsmoking) (Pirkle et al. 1996).

Statistical analyses

This study included subsamples from two cycles, 2007–2008 and 2011–2012, with a final weight of 0.5 × WTSA2YR + 0.5 × WTSB2YR, and incorporated primary sampling units and stratification. Descriptive and GLM analysis was conducted with STATA version 16.0 (StataCorp, College Station, TX, USA); BKMR and QGC were conducted with R (4.2.1, Package "bkmr" and "qgcomp"). All significance levels were set to 0.05 (two-tailed).

Descriptive analysis

Skewness and kurtosis tests showed a heavily right-skewed distribution of thyroid hormones and EDCs (Table S2). Therefore, the distributions of the THs levels and EDCs concentrations were described using geometric medians (GM) and interquartile ranges (IQR), and natural ln transformations were applied prior to all statistical analysis due to the skewed distribution of these variables. Demographic characteristics were stratified by age and sex, using Student's t-test, χ^2 test, or Mann–Whitney U test for continuous and categorical variables, as needed. Spearman rank correlation analysis was used to evaluate the correlation coefficients.

Weighted generalized linear regression model (GLM)

The associations of urinary concentrations of the 16 EDCs as continuous variables with THs were firstly analyzed by GLM models, where T3, FT3, T4, FT4, TSH, and Tg were applied as continuous outcomes (family = Gaussian) and TPOAb and TgAb as categorical outcomes (family = binomial), respectively. And, the concentrations of these 16 EDCs were also set as categorical variables in quartiles and analyzed using the lowest exposure group as the reference subgroup. Based on previous studies (Barr et al. 2005), adjusted covariates for adolescents included age, race, education, family poverty, BMI, serum cotinine, urinary iodine (ln-transformed), and urinary creatinine (ln-transformed) and, for adults, additional adjusted for marital status, hypertension, and diabetes mellitus.

Bayesian kernel machine regression (BKMR) model

Given the high correlation and potential non-additive dose–response relationship between mixture exposures, the BKMR model allows more flexibility for assessing the joint effect of multiple pollutants while identifying the potential interactions and accounting for the exposure–response association for each component of a mixture (Bobb et al. 2018). In the BKMR model, Gaussian and Probit link functions were used to fit exposure–response relationships between continuous and categorical outcomes, respectively. Specifically, the overall effect was assessed by the change in thyroid hormones when all chemicals were fixed at the 50th percentile versus when they were fixed at the other percentile. We then explored the univariate exposure–response function when a single chemical is at the 75th *versus* the 25th percentile, while all other chemicals are fixed at their median values. Finally, potential interactions within mixtures mediated by the change in THs were assessed by comparing each individual chemical concentration at the 25th to 75th percentiles, when setting the remaining exposures at their 25th, 50th, or 75th percentile levels (Bobb). We also calculated the conditional posterior inclusion probability (PIP) to identify the relative importance of individual exposure variables to the overall mixture effect (Coker et al. 2018). All BKMR models were fitted 10,000 iterations using the Markov Chain Monte Carlo sampler.

Quantile-based g-computation (QGC)

The QGC model was introduced in this study to assess the overall association of mixed chemical exposures and to identify the most toxic agents. QGC reflects mixed exposures to multiple contaminants by constructing a weighted index, with the weight of each contaminant representing the corresponding relative importance. The method combines the inferential simplicity of weighted quantile regression with the flexibility of g-calculation, relaxes the assumption of directional homogeneity, allows individual components of the mixture to contribute positive or negative weights to the mixture index, and also allows for some consideration of nonlinear relationships between mixed exposures and single contaminants and outcomes (Keil et al. 2020). In the QGC model, a threshold of weight $\geq 1/q$ (q represents the number of exposure variables) was set to identify significant toxicants, and confidence bounds for the mixture slope and overall model were derived from 10,000 bootstrap iterations.

Sensitivity analyses

Participants with high serum levels of TPOAb (>9 IU/mL) or TgAb (>4 IU/mL) were excluded to reanalyze the association between mixed exposure to 16 EDCs and THs to

avoid the effect of pre-existing immunological disturbances in thyroid tissue.

Results

Participant characteristics

Table 1 presented the characteristics of the study population by age and sex. A total of 704 adolescents and 2911 adults were included in the study. The mean age of adolescents was 15.68 years, and 51.98% of them were boys. Compared to girls, boys had higher level waist circumference (WC) and a higher frequency of active smoking but a lower prevalence of TPOAb positive rate. The mean age of adults was 46.11 years, and approximately 53% were male. There were statistically significant differences between males and females in the distribution of most of the variables; for example, males had a higher percentage of below high school and higher ratese of overweight but lower rates of TPOAb and TgAb positivity.

Distribution of EDCs and serum thyroid function measures

Table 2 summarized the GM and the median of the 16 EDCs and thyroid function indictors. In adolescents, the 16 EDCs had the highest and lowest GM values for MEP and BPA of 62.92 μg/L and 2.04 μg/L, respectively. Boys generally had lower levels of chemical exposure than girls, but boys had higher levels of TSH, T3, and FT3. Among adults, males had much higher concentrations of all phthalates and BPA than females but lower median concentrations of TCS, BP3, PPB, and MPB. Compared to adults, adolescents had lower concentrations of MEP, TCS, and PPB in urine and higher levels of the other 13 metabolites.

Spearman correlation analysis

Spearman's correlation analysis was performed to evaluate the correlation between 16 EDCs, and the results were presented in Fig. 2. Among these 16 EDCs, there existed strong positive correlation among MEHP, MiBP, and MEOHP for boys ($r = 0.95$–0.99, Fig. 2A); MEP, MEHHP, and MEHP for girls ($r = 0.93$–0.99, Fig. 2B); MCNP, MECPP, and MnBP for adult males ($r = 0.94$–0.98, Fig. 2C); and TCS, BP3, and PPB for females ($r = 0.93$–0.98, Fig. 2D). Furthermore, mild-to-moderate positive correlations were also found between other metabolites, with coefficients

ranging from 0.12 to 0.90. The details are presented in Fig. 2 ($p<0.05$).

Association between 16 EDCs and THs

Generalized linear regression was performed to evaluate the association between different EDCs and thyroid profiles. For adolescents, after adjusting confounding factors including age, sex, race, education, family poverty level, BMI, serum cotinine, urine iodine (ln-transformed), and urinary creatinine (ln-transformed), elevated T3 levels were positively associated with MCOP, MECPP, MnBP, MBzP, and BPA (Fig. 3), and elevated FT3 levels were positively associated with MECCP, MiBP, and MEOHP (Fig. S1). MCPP was negatively correlated with T4 in boys (Fig. S2), and MECCP, MnBP, MEHHP, MEHP, and MEOHP were negatively correlated with FT4 in girls (Fig. S3), and MiBP was negative associated with TSH in girls (Fig. S4). Besides, there was a positive association of MCOP, MCNP, and MCPP with Tg levels in girls (Fig. S5) and a negative association of BPA, TCS, and MPB with TPOAb levels in boys (Fig. S6). For adults, after adjusting age, sex, race, education, family poverty level, BMI, serum cotinine, urine iodine (ln-transformed), urinary creatinine (ln-transformed), marital status, hypertension, and diabetes, the study found that T3 was negatively correlated with the concentrations of MECPP, MEHHP, and MEOHP in males (Fig. 3); T4 was negatively correlated with MECPP, MEHHP, and MEOHP in both males and females (Fig. S2); and FT4 was negatively correlated with MECPP, MEHHP, MEOHP, TCS, PPB, and MPB (Fig. S3). MCOP was positively correlated with FT4 both in males and females, and MiBP was positively correlated with male's TSH levels and female's TPOAb levels (Fig. S4, Fig. S6).

In addition, each kind of EDCs was divided into 4 quartiles. Then, generalized linear regression analyses were performed to assess the independent association between EDCs and the thyroid function after adjusting confounders. As presented in Fig. 4, serum T3 levels in boys were 9%, 11%, 8%, 10%, and 10% higher when comparing extreme quartiles of MCOP, MnBP, MEHHP, MEOHP, and BPA, respectively, with statistically significant linear P-trend values. And there was also an agreement between other outcomes and the use of EDCs as the continuous variables in adolescents, including T3 (Fig. 4), FT3 (Fig. S8), T4 (Fig. S9), FT4 (Fig. S10), TSH (Fig. S11), and Tg (Fig. S12). In adults, using the lowest quartile as the reference group, there was a 5% decrease in T3 levels in the highest quartile of MECCP in males, with a clear dose–response relationship ($p<0.01$). And there were negative associations of MnBP, MBzP, and BPA with T3 concentrations in men in the highest quartiles compared to the lowest

Table 1 Weighted study population characteristics for adolescents and adults, NHANES (2007–2012)

Variable	Adolescent (ages 12–19)				Adults (ages ≥20)			
	Total (n=704)	Boy (n = 366)	Girl (n=338)	P	Total (n = 2911)	Male (n = 1542)	Female (n = 1369)	P
Age, mean (SE)	15.68 (0.09)	15.69 (0.13)	15.67 (0.14)	0.67	46.11 (0.53)	46.03 (0.67)	46.18 (0.61)	0.89
Race, n (%)								
Non-Hispanic White	409 (58.14)	212 (58.09)	197 (58.20)	0.52	1972 (67.75)	1067 (69.22)	906 (66.17)	0.06
Non-Hispanic Black	106 (15.07)	54 (14.69)	52 (15.49)		325 (11.17)	151 (9.81)	173 (12.65)	
Mexican American	96 (13.71)	56 (15.14)	41 (12.13)		184 (6.31)	137 (8.86)	106 (7.72)	
Other	93 (13.08)	44 (12.08)	48 (14.18)		371 (12.76)	187 (12.11)	184 (13.46)	
Education, n (%)								
Below high school	625 (88.76)	315 (0.8618)	310 (91.54)	0.16	525 (18.23)	300 (19.44)	231 (16.91)	0.01
High school graduate/GED	11 (1.63)	4 (1.18)	7 (2.12)		646 (22.2)	371 (24.04)	279 (20.36)	
Post-high school	68 (9.61)	46 (12.63)	21 (6.34)		1732 (59.50)	872 (56.52)	872 (63.73)	
Marital status, n (%)								
Married/living with partner	---	---	---		1868 (64.16)	1037 (67.26)	832 (60.80)	<0.001
Widowed/divorced/separated	---	---	---		475 (16.31)	171 (11.10)	301 (21.97)	
Never married	---	---	---		569 (19.53)	329 (21.64)	236 (17.23)	
Family income–to-poverty ratio								
<1.30	219 (31.16)	114 (31.09)	106 (31.24)	0.92	593 (20.38)	274 (17.78)	317 (23.19)	<0.01
1.30–3.49	242 (34.34)	123 (33.62)	119 (35.13)		931 (31.97)	517 (33.56)	414 (30.24)	
≥3.50	243 (34.50)	129 (35.09)	113 (35.13)		1387 (47.66)	750 (48.66)	638 (46.57)	
BMI (kg/m^2)								
Underweight (<18.5)	63 (8.89)	42 (11.37)	21 (6.14)	0.10	45 (1.53)	6 (0.40)	38 (2.76)	<0.001
Normal weight (18.5–24.9)	388 (55.18)	184 (50.37)	204 (60.48)		917 (31.49)	422 (27.37)	492 (35.96)	
Overweight (25–29.9)	125 (17.75)	74 (20.06)	51 (14.97)		957 (32.86)	573 (37.19)	386 (28.17)	
Obese (30–34.9)	128 (18.19)	66 (17.99)	62 (18.41)		993 (34.12)	540 (35.04)	453 (33.11)	
WC (cm), mean (SE)	82.19 (0.99)	83.17 (0.95)	81.07 (1.58)	0.01	98.35 (0.47)	101.72 (0.71)	94.96 (0.49)	<0.001
Cotinine (ng/mL), n (%)								
No exposure (<1.0)	530 (75.36)	265 (72.50)	265 (78.52)	0.02	2058 (70.71)	1017 (65.93)	1039 (75.90)	<0.001
Environmental exposure (1.0–9.9)	68 (9.72)	31 (8.57)	37 (11.00)		118 (4.06)	78 (5.05)	41 (2.99)	
Active smoking (≥10)	105 (14.92)	69 (18.93)	36 (11.48)		734 (25.23)	447 (29.02)	289 (21.11)	
Hypertension, n (%)	9 (1.27)	7 (1.78)	2 (0.70)	0.13	1129 (38.79)	651 (42.19)	470 (34.33)	<0.01
Diabetes mellitus, n (%)	4 (0.62)	3 (0.94)	1 (0.26)	0.18	368 (12.63)	225 (14.62)	143 (10.47)	0.02
TPOAb positive (≥ 9 IU/mL)	50 (7.07)	7 (2.03)	43 (12.64)	<0.001	264 (9.06)	106 (6.87)	157 (11.45)	<0.01
TgAb positive (≥4 IU/mL)	40 (5.63)	16 (4.33)	24 (7.07)	0.20	174 (5.98)	73 (4.75)	101 (7.32)	0.04

SE standard error; *BMI* body mass index; *WC* waist circumference. The weighted *t*-test and χ^2 test were between the males and females

Table 2 Weighted distribution of exposure biomarkers for adolescents and adults, NHANES (2007–2012)

Variables	Total		Boy/male		Girl/female		P
	GM	M (IQR)	GM	M (IQR)	GM	M (IQR)	
Adolescent (ages 12–19, N=704)							
MCOP	14.71	13.80 (5.70, 34.60)	12.63	11.30 (5.20, 27.20)	17.40	16.70 (6.50, 49.30)	0.13
MCNP	2.83	2.60 (0.50, 5.30)	2.78	2.50 (1.50, 4.90)	2.87	2.80 (1.60, 6.60)	0.78
MECPP	26.13	22.90 (11.50, 54.10)	25.17	20.60 (10.70, 51.60)	27.22	25.30 (12.00, 58.80)	0.47
MnBP	16.49	18.50 (8.40, 40.70)	14.30	15.00 (7.40, 35.60)	19.31	22.60 (9.90, 45.20)	<0.001
MCPP	3.70	3.50 (1.70, 8.30)	3.45	3.20 (1.50, 7.60)	4.00	4.00 (1.90, 9.70)	0.54
MEP	62.92	55.11 (24.00, 162.69)	50.08	42.37 (21.30, 106.06)	80.97	93.20 (27.70, 189.55)	<0.001
MEHHP	16.71	13.80 (6.30, 36.90)	15.39	12.80 (6.00, 36.00)	18.31	17.40 (7.00, 42.40)	0.56
MEHP	2.27	2.10 (0.78, 4.80)	2.03	1.80 (0.78, 4.40)	2.57	2.50 (0.78, 5.00)	0.02
MiBP	9.23	9.50 (4.60, 21.30)	8.40	8.50 (4.50, 20.00)	10.23	10.80 (4.70, 24.20)	<0.01
MEOHP	10.27	9.40 (4.20, 21.20)	9.24	8.70 (3.50, 19.70)	11.52	10.15 (4.9, 23.5)	0.26
MBzP	9.08	10.59 (3.74, 21.96)	8.25	9.43 (3.40, 20.09)	10.09	11.95 (4.80, 24.77)	0.06
BPA	2.04	2.10 (1.00, 3.90)	2.04	2.00 (1.10, 3.50)	2.04	2.20 (1.00, 3.90)	0.36
TCS	13.75	9.20 (3.30, 40.40)	11.92	8.40 (2.90, 32.10)	16.09	9.40 (3.50, 49.00)	0.36
BP3	24.49	48.30 (6.20, 78.70)	14.40	11.60 (4.10, 33.80)	44.06	31.40 (11.40, 164.00)	<0.001
PPB	5.95	5.10 (1.10, 29.80)	2.36	1.70 (0.50, 6.90)	16.51	19.10 (4.20, 72.70)	<0.001
MPB	42.04	41.20 (9.20, 187.00)	22.39	15.70 (6.40, 75.90)	84.30	91.90 (23.90, 350.00)	<0.001
TSH	1.45	1.48 (1.00, 2.12)	1.52	1.54 (1.10, 2.17)	1.38	1.43 (0.97, 1.98)	<0.01
T3	131.44	129.00 (117.00, 146.00)	135.14	134.00 (120.00, 152.00)	127.47	125.00 (113.00, 142.00)	<0.001
FT3	3.58	3.60 (3.3,0 3.86)	3.72	3.69 (3.50, 4.00)	3.43	3.40 (3.20, 3.70)	<0.001
T4	7.53	7.40 (6.70, 8.40)	7.30	7.27 (6.65, 8.17)	7.79	7.60 (6.85, 8.85)	<0.001
FT4	0.81	0.8 (0.70, 0.90)	0.80	0.80 (0.70,0.90)	0.80	0.80 (0.71, 0.90)	0.24
Tg	7.97	8.80 (5.74, 14.36)	7.70	8.09 (5.47, 12.83)	8.29	9.56 (6.39, 15.19)	0.06
Adults (ages ≥20, N=2911)							
MCOP	11.35	10.00 (4.20, 29.10)	12.87	11.30 (4.50, 33.90)	9.90	8.50 (3.90, 24.40)	<0.01
MCNP	2.38	2.30 (1.20, 4.60)	2.74	2.60 (1.40, 5.40)	2.04	2.00 (0.90, 4.10)	<0.001
MECPP	19.35	18.50 (9.60, 39.90)	20.95	19.90 (10.00, 39.90)	17.75	16.30 (8.00, 39.20)	0.03
MnBP	11.06	13.10 (5.60, 26.30)	11.41	13.20 (6.00, 26.30)	10.70	13.00 (5.20, 26.00)	0.54
MCPP	2.70	2.50 (1.20, 5.60)	3.12	2.70 (1.50, 5.90)	2.31	2.30 (1.00, 5.20)	<0.001
MEP	63.18	56.60 (19.50, 178.20)	66.11	58.60 (19.90, 185.00)	60.15	57.30 (19.27, 173.50)	0.06
MEHHP	12.48	12.10 (5.40, 26.00)	13.78	13.10 (6.20, 26.90)	11.21	10.80 (4.70, 23.60)	<0.01
MEHP	1.88	1.70 (0.78, 3.90)	2.07	1.90 (0.78, 4.20)	1.69	1.40 (0.78, 3.40)	<0.001
MiBP	6.15	6.70 (3.20,13.30)	6.65	7.20 (3.60, 13.60)	5.66	6.20 (2.70, 12.90)	0.02
MEOHP	7.34	7.10 (3.30, 14.80)	7.94	7.60 (3.70, 14.80)	6.74	6.50 (3.00, 14.40)	0.06
MBzP	5.02	5.40 (2.20, 12.53)	5.28	5.62 (2.30, 12.24)	4.74	5.11 (2.00, 12.46)	0.05
BPA	1.72	1.70 (0.90, 3.40)	1.85	1.80 (1.00,3.60)	1.60	1.60 (0.70, 3.20)	<0.01
TCS	14.59	9.80 (2.50, 61.40)	14.35	9.40 (2.50, 60.60)	14.84	10.10 (2.80, 62.20)	0.13
BP3	20.18	17.10 (4.40, 73.80)	14.08	12.20 (3.50, 44.80)	29.83	25.60 (6.10, 129.00)	<0.001
PPB	6.83	7.00 (1.10, 41.60)	2.84	2.10 (0.60,12.00)	17.69	22.10 (4.70, 77.50)	<0.001
MPB	53.28	55.90 (14.10, 200.00)	29.64	26.10 (8.10, 92.90)	100.66	113.00 (35.40, 315)	<0.001
TSH	1.58	1.59 (1.09, 2.300)	1.60	1.59 (1.11, 2.34)	1.55	1.57 (1.09, 2.20)	0.91
T3	111.43	112.00 (99.00, 126.00)	112.56	114.00 (100.00, 127.00)	110.21	111.00 (97.00, 123.00)	<0.01
FT3	3.16	3.17 (2.91, 3.40)	3.25	3.26 (3.01, 3.50)	3.07	3.08 (2.89, 3.30)	<0.001
T4	7.57	7.60 (6.70, 8.56)	7.43	7.43 (6.55, 8.40)	7.72	7.70 (6.90, 8.68)	<0.001
FT4	0.79	0.80 (0.70, 0.89)	0.79	0.80 (0.70, 0.89)	0.78	0.80 (0.70, 0.89)	0.02
Tg	9.80	10.47 (6.08, 17.89)	8.97	9.67 (5.74, 15.21)	10.80	11.49 (6.39, 20.46)	<0.001

GM geometric mean, *M* median, *IQR* interquartile range. *P* were used the Wilcoxon rank sum test
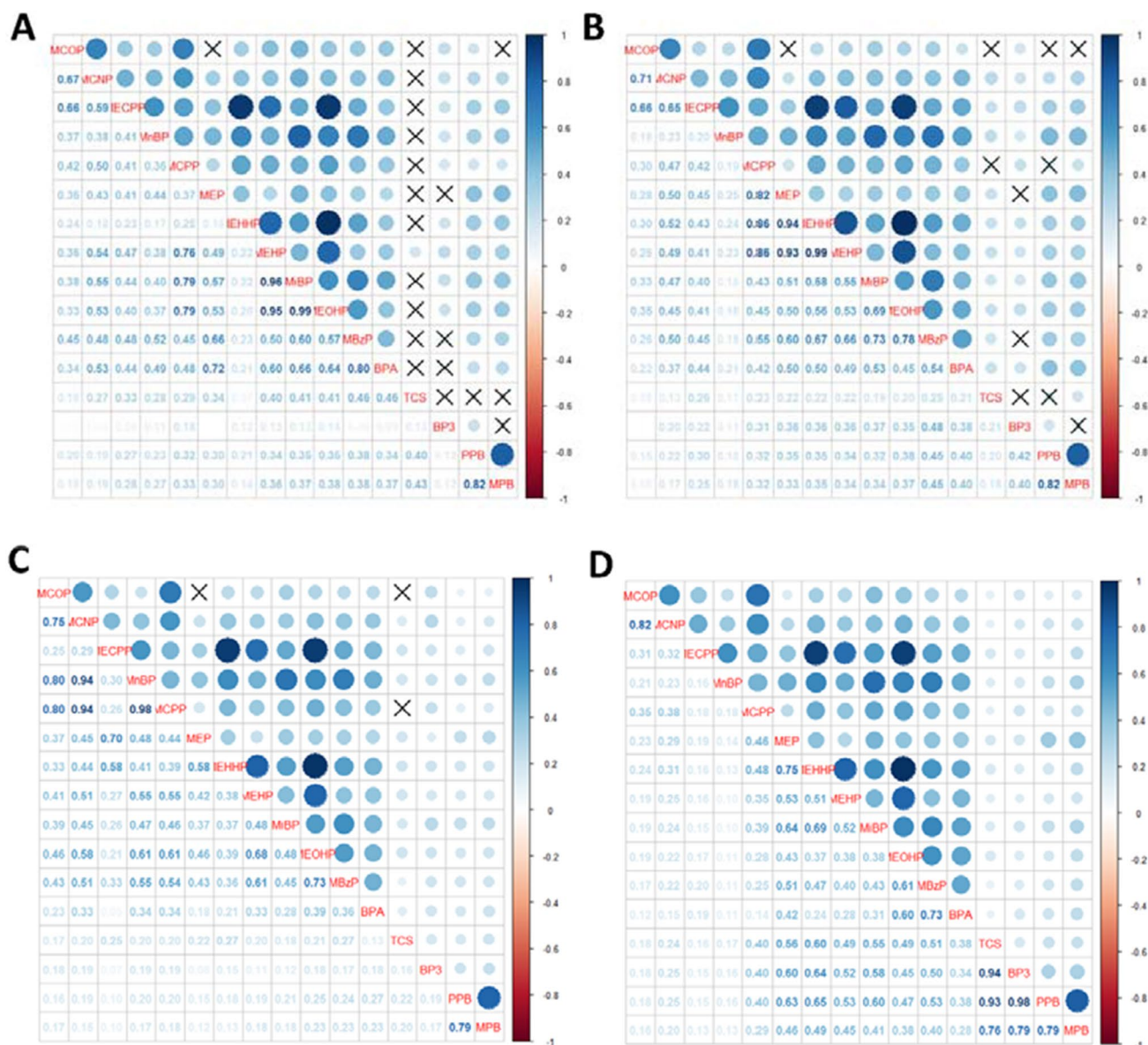
Fig. 2 Spearman's correlation among 16 ln-transformed urine phenols, parabens, and phthalate metabolites in the study. **A** In adolescent boys; **B** in adolescent girls; **C** in adult males; **D** in adult females. ✗ P value > 0.0001

quartiles (Fig. S13). And the use of EDCs as categorical variables in FT3 (Fig. S14), T4 (Fig. S15), FT4 (Fig. S16), TSH (Fig. S17), and Tg (Fig. S18) was consistent with the results previously obtained as continuous variables.

Bayesian kernel machine regression (BKMR) models

The associations between the mixed chemical exposures and thyroid function in adolescents, analyzed by BKMR models, were presented in Fig. 5. We found that mixed exposures to 16 chemicals were positively associated T3 levels and inversely associated T4 levels, and a bell-shaped curve was identified between the mixed exposures and risk

of TPOAb and TgAb positivity. The PIP in the BMKR model reflects the importance of each compound in the overall association, with the largest effect on T3 being MCOP (PIP = 0.02, Fig. S19A), the largest effect on T4 being BPA (PIP = 0.67, Fig. S19C), and almost all mixtures playing a potentially important role in the risk of TPOAb and TgAb positivity in girls (Fig. S19G and H). When we set the other 15 chemicals at the 25th, 50th, and 75th percentiles, this study found that as the IQR of MCOP increased (Fig. S20A), T3 for boys increased by 0.03, 0.03, and 0.03, respectively. As the IQR of BPA increased, T4 for girls decreased by −0.03, −0.03, and −0.03, respectively (Fig. S20B), and the risk of TPOAb positivity for

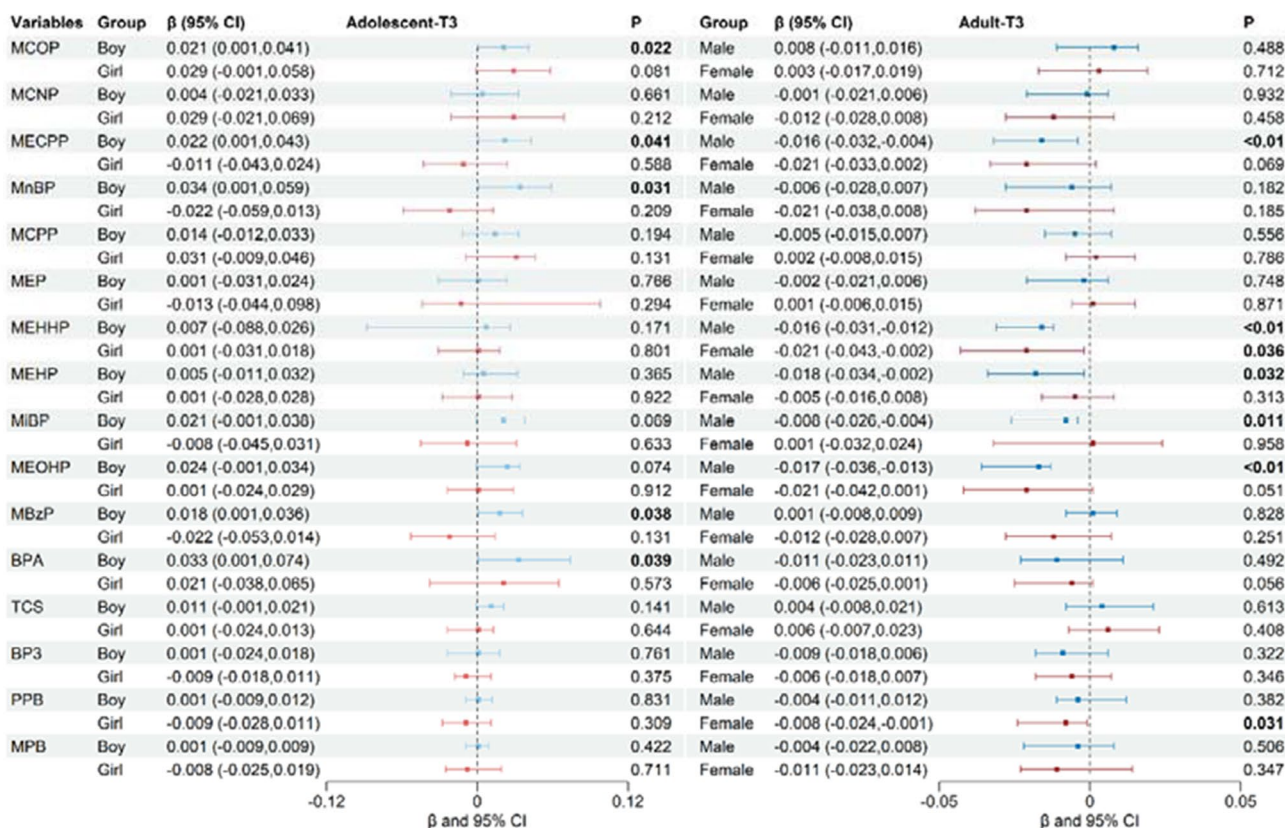| Variables	Group	β (95% CI)	Adolescent-T3	P	Group	β (95% CI)	Adult-T3	P
MCOP	Boy	0.021 (0.001,0.041)		**0.022**	Male	0.008 (-0.011,0.016)		0.488
	Girl	0.029 (-0.001,0.058)		0.081	Female	0.003 (-0.017,0.019)		0.712
MCNP	Boy	0.004 (-0.021,0.033)		0.661	Male	-0.001 (-0.021,0.006)		0.932
	Girl	0.029 (-0.021,0.069)		0.212	Female	-0.012 (-0.028,0.008)		0.458
MECPP	Boy	0.022 (0.001,0.043)		**0.041**	Male	-0.016 (-0.032,-0.004)		**<0.01**
	Girl	-0.011 (-0.043,0.024)		0.588	Female	-0.021 (-0.033,0.002)		0.069
MnBP	Boy	0.034 (0.001,0.059)		**0.031**	Male	-0.006 (-0.028,0.007)		0.182
	Girl	-0.022 (-0.059,0.013)		0.209	Female	-0.021 (-0.038,0.008)		0.185
MCPP	Boy	0.014 (-0.012,0.033)		0.194	Male	-0.005 (-0.015,0.007)		0.556
	Girl	0.031 (-0.009,0.046)		0.131	Female	0.002 (-0.008,0.015)		0.786
MEP	Boy	0.001 (-0.031,0.024)		0.766	Male	-0.002 (-0.021,0.006)		0.748
	Girl	-0.013 (-0.044,0.098)		0.294	Female	0.001 (-0.006,0.015)		0.871
MEHHP	Boy	0.007 (-0.088,0.026)		0.171	Male	-0.016 (-0.031,-0.012)		**<0.01**
	Girl	0.001 (-0.031,0.018)		0.801	Female	-0.021 (-0.043,-0.002)		**0.036**
MEHP	Boy	0.005 (-0.011,0.032)		0.365	Male	-0.018 (-0.034,-0.002)		**0.032**
	Girl	0.001 (-0.028,0.028)		0.922	Female	-0.005 (-0.016,0.008)		0.313
MiBP	Boy	0.021 (-0.001,0.038)		0.069	Male	-0.008 (-0.026,-0.004)		**0.011**
	Girl	-0.008 (-0.045,0.031)		0.633	Female	0.001 (-0.032,0.024)		0.958
MEOHP	Boy	0.024 (-0.001,0.034)		0.074	Male	-0.017 (-0.036,-0.013)		**<0.01**
	Girl	0.001 (-0.024,0.029)		0.912	Female	-0.021 (-0.042,0.001)		0.051
MBzP	Boy	0.018 (0.001,0.036)		**0.038**	Male	0.001 (-0.008,0.009)		0.828
	Girl	-0.022 (-0.053,0.014)		0.131	Female	-0.012 (-0.028,0.007)		0.251
BPA	Boy	0.033 (0.001,0.074)		**0.039**	Male	-0.011 (-0.023,0.011)		0.492
	Girl	0.021 (-0.038,0.065)		0.573	Female	-0.006 (-0.025,0.001)		0.056
TCS	Boy	0.011 (-0.001,0.021)		0.141	Male	0.004 (-0.008,0.021)		0.613
	Girl	0.001 (-0.024,0.013)		0.644	Female	0.006 (-0.007,0.023)		0.408
BP3	Boy	0.001 (-0.024,0.018)		0.761	Male	-0.009 (-0.018,0.006)		0.322
	Girl	-0.009 (-0.018,0.011)		0.375	Female	-0.006 (-0.018,0.007)		0.346
PPB	Boy	0.001 (-0.009,0.012)		0.831	Male	-0.004 (-0.011,0.012)		0.382
	Girl	-0.009 (-0.028,0.011)		0.309	Female	-0.008 (-0.024,-0.001)		**0.031**
MPB	Boy	0.001 (-0.009,0.009)		0.422	Male	-0.004 (-0.022,0.008)		0.506
	Girl	-0.008 (-0.025,0.019)		0.711	Female	-0.011 (-0.023,0.014)		0.347

Fig. 3 Associations of 16 ln-transformed urine chemicals with ln-transformed T3 in adolescents and adults. *CI* confidence interval. For adolescents, adjusted β coefficient were adjusted for age, sex, race, education, family poverty level, BMI, serum cotinine, urine iodine (ln-transformed), and urinary creatinine (ln-transformed). And for adults, additional adjustments were needed for marital status, hypertension, and diabetes

girls decreased by 0.02, 0.02, and 0.02, respectively (Fig. S20C). And the risk of TgAb positivity in girls decreased progressively with increasing IQR for TCS, MnBP, and MCOP, respectively (Fig. S20D). Mixed exposure to these 16 EDCs was not associated with serum levels of FT3 (Fig. 5B), FT4 (Fig. 5D), TSH (Fig. 5E), and Tg (Fig. 5F) in adolescents.

Fig. 6 summarized the total effects of mixed exposure on thyroid function in adults. T4 levels decreased approximately linearly with increasing percentile of mixed exposure, with MEHHP having the highest PIPs on T4 levels for both males and females (Fig. S21C). When the exposure percentiles for the other 15 chemicals were set at the 25th, 50th, and 75th percentiles, MEHHP was negatively associated with T4, whereas MEOHP was positively associated with T4, with the sum of the negative contributions being greater than the sum of the positive contributions, and the overall effect remaining negative. In addition, in the female subgroup, as the mixed exposure percentile increased, FT4 levels gradually decreased and were no longer statistically significant beyond the 50th percentile; also the increase in the risk of TPOAb and TgAb positivity was progressively

slower and no longer significant beyond the 75th percentile. The chemicals with the highest effects on female THs were as follows: MCOP, MEHHP, and MEOHP had the highest PIPs for FT4 levels (Fig. S21D), MEOHP and TCS had the highest PIPs for TPOAb (Fig. S21H), and TCS had the highest PIPs for TgAb (Fig. S21I). Statistically significant association of any single chemical with the risk of TPOAb or TgAb positivity was not observed when other chemical concentrations were fixed at the 25th, 50th, or 75th percentiles (Fig. S20H and Fig. S20I).

Quantile-based g-computation (QGC) models

Analyses based on the QGC model further determined that the overall effect of the chemical mixture was associated with an increase in adolescent T3 and a decrease in adolescent and adult T4. As shown in Table 3, there was a significant positive association between mixed exposure and increased T3 levels in boys (β = 0.05, P = 0.01), with MECCP contributing the most in the positive direction (Fig. S22A, weight = 0.32). Mixture exposure also proved to be significantly and inversely associated with

Fig. 4 Odds ratios (95% confidence interval) for ln-transformed serum T3 among quartile subgroup of 16 urine chemicals by sex. *CI* confidence interval, *OR* odd ratio. Adjusted *OR* were adjusted for age, sex, race, education, family poverty level, BMI, serum cotinine, urine iodine (ln-transformed), and urinary creatinine (ln-transformed)

T4 levels in girls ($\beta = -0.05$, $P = 0.04$), with MnBP contributing the most in the negative direction (Fig. S22B, weight = 0.24). In adults, increased concentrations of mixture chemicals were also shown to be negatively correlated with T4 levels (for males, $\beta = -0.03$, $P<0.01$; for females, $\beta = -0.04$, $P < 0.01$), with MEHHP having the greatest relative importance in the negative direction both in males and in females (Fig. S22C and D). Finally, mixed exposure was found to be negatively associated with FT4 levels only in female subgroup, with MEHHP having the largest negative contribution of a value of 0.23 (Fig. S22E).

Sensitivity analysis

Previous studies confirmed that thyroid autoantibody status should be considered in the association study of thyroid-damaging chemicals (Choi et al. 2020). To avoid the impact of pre-existing immunologic disturbance of the thyroid tissue, we excluded participants with high serum TPOAb levels (>9 IU/mL) or TgAb levels (>4 IU/mL) and conducted a sensitivity analysis using BKMR and QGC models. As shown in Table S3, Fig. S23, and Fig. S24, the correlation between mixed exposure and adolescent T4 was no longer significant, but it hardly affects the trend of association with adult THs. The association between chemicals and THs seems to be affected by the state of autoantibody.

Discussion

This study was among the latest studies to estimate the associations of individual and mixtures of EDC chemicals with thyroid hormones among the US adolescents and adults. We found that exposure to mixtures of phenol, parabens, and phthalates, either individually or as a mixture, was generally associated with T3, T4, FT4, and their antibodies. These associations were stronger among adolescents than adults. In our analyses, the 3 phthalate metabolites including MCOP, MECCP, and MEHHP were the most highly weighted chemicals in both BKMR and QGC models. In addition, there was a strong age-specific difference in the effect of mixed exposure on T3 level, as well as an age–sex difference in the risk of TPOAb and TgAb positivity.
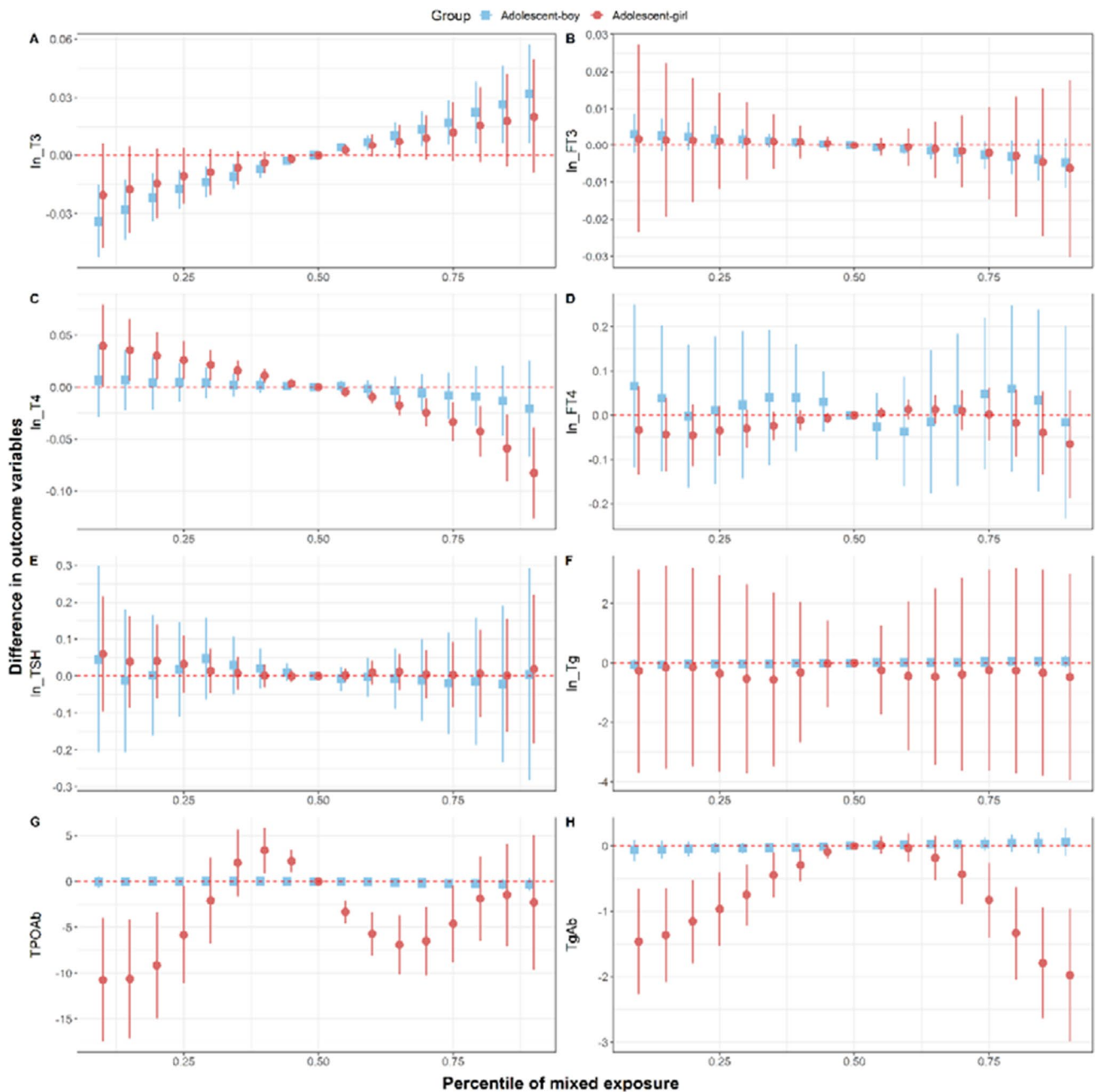
Fig. 5 Overall association estimates with 95% *CI* for 16 the mixture exposure of 16 urine chemicals and thyroid function in the BKMR models and stratified by the sex in adolescents. All the chemicals at particular percentiles from 0.10 to 0.90 increment by 0.05 were compared to all the chemicals at their 50th percentile. Overall association estimates were adjusted for age, sex, race, education, family poverty level, BMI, serum cotinine, urine iodine (ln-transformed), and urinary creatinine (ln-transformed). **A** Association of mixed exposure with ln-transformed T3; **B** association of mixed exposure with ln-transformed FT3; **C** association of mixed exposure with ln-transformed T4; **D** association of mixed exposure with ln-transformed FT4; **E** association of mixed exposure with ln-transformed TSH; **F** association of mixed exposure with ln-transformed Tg; **G** association of mixed exposure with the risk of positive TPOAb; **H** association of mixed exposure with the risk of positive TgAb

Effect of mixed exposure to 16 EDCs on serum levels of T3 and T4

We confirmed that MCOP, MECPP, MnBP, MiBP, and MBzP were positively associated with ln-transformed

T3 in boys. Our results were partly supported by Meeker et al., the authors who used the data from NHANES found a positive correlation between MEHP, MEHHP, MEOHP, and MECPP and serum T3 in adolescents (Meeker and Ferguson 2011). Recently, Koeppe et al. reported a
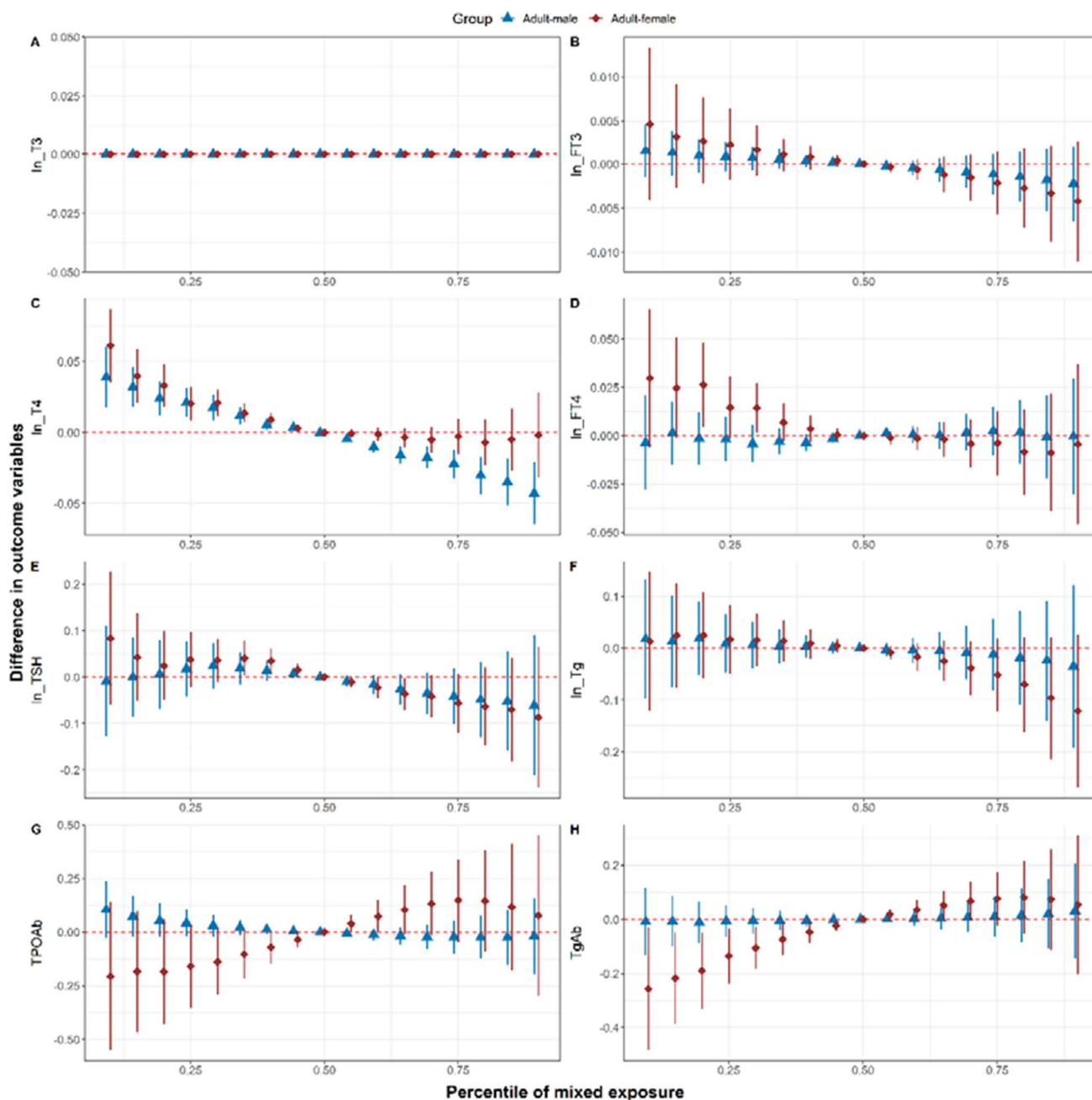
Fig. 6 Overall association estimates with 95% *CI* for 16 mixture exposure and thyroid function in the BKMR models and stratified by the sex in adults. All the chemicals at percentiles from 0.10 to 0.90 increments by 0.05 were compared to all the chemicals at their 50th percentile. Overall association estimates were adjusted for age, sex, race, education, family poverty level, BMI, serum cotinine, urine iodine (ln-transformed), urinary creatinine (ln-transformed), marital status, hypertension, and diabetes. **A** Association of mixed expo-sure with ln-transformed T3; **B** association of mixed exposure with ln-transformed FT3; **C** association of mixed exposure with ln-trans-formed T4; **D** association of mixed exposure with ln-transformed FT4; **E** association of mixed exposure with ln-transformed TSH; **F** association of mixed exposure with ln-transformed Tg; **G** association of mixed exposure with the risk of positive TPOAb; **H** association of mixed exposure with the risk of positive TgAb

positive association between TCS and T3 in adolescents (Koeppe et al. 2013). In this study, we explored for the first time the total effect of mixed exposure to 16 compounds and confirmed a statistical correlation between increased

levels of MCOP and T3 only when immobilizing the other 15 chemicals by BMKR and QGC modeling. For serum T4, the present study identified that MECCP, MEHHP, MEOHP, and PPB were negatively associated with T4

Table 3 The total effect of exposure mixture on serum thyroid hormones stratified by age and sex based on quantile-based g-computation model

	Boy/male			Girl/female		
	Coefficient	95%CI	P	Coefficient	95%CI	P
Adolescent						
T3[a]	0.048	(0.008, 0.091)	0.011	0.009	(−0.038, 0.059)	0.671
FT3 [a]	0.031	(0.001, 0.048)	0.051	0.008	(−0.021, 0.042)	0.705
T4 [a]	−0.022	(−0.056, 0.031)	0.405	−0.046	(−0.101, −0.009)	0.038
FT4 [a]	−0.001	(−0.051, 0.028)	0.708	−0.022	(−0.058, 0.021)	0.391
TSH [a]	−0.031	(−0.171, 0.102)	0.612	−0.081	(−0.225, 0.057)	0.274
Tg [a]	0.028	(−0.189, 0.246)	0.778	0.188	(−0.101, 0.468)	0.213
TPOAb[b]	0.078	(−7.181, 7.332)	0.982	0.305	(−1.116, 1.738)	0.672
TgAb[b]	−1.351	(−4.712, 2.001)	0.431	−0.381	(−1.687, 0.925)	0.567
Adult						
T3[a]	0.011	(−0.019, 0.028)	0.611	−0.011	(−0.028, 0.019)	0.685
FT3 [a]	−0.012	(−0.021, 0.013)	0.625	−0.008	(−0.021, 0.012)	0.461
T4 [a]	−0.028	(−0.052, −0.011)	<0.01	−0.038	(−0.064, −0.013)	<0.01
FT4 [a]	−0.009	(−0.021, 0.008)	0.591	−0.021	(−0.052, −0.012)	0.048
TSH [a]	−0.017	(−0.089, 0.046)	0.652	−0.062	(−0.145, 0.028)	0.211
Tg [a]	−0.035	(−0.158, 0.078)	0.518	−0.054	(−0.201, 0.104)	0.503
TPOAb[b]	−0.358	(−0.805, 0.094)	0.121	0.155	(−0.278, 0.605)	0.455
TgAb[b]	0.076	(−0.501, 0.667)	0.792	0.231	(−0.315, 0.778)	0.412

CI confidence interval, *a* T3, FT3, T4, FT4, TSH, and Tg were included as continuous variables with coefficient representing β value; *b* TPOAb and TgAb were included as categorical variable with coefficient representing odd ratio (*OR*). For adolescents, the adjusted covariates were as follows: age, sex, race, education, family poverty level, BMI, serum cotinine, urine iodine (ln-transformed), and urinary creatinine (ln-transformed). And for adults, additional adjustments were needed for marital status, hypertension, and diabetes. All 16 urine chemicals and 6 continuous thyroid hormones were ln-transformed before analysis

levels in males and females; MnBP and MCPP were also negatively associated with T4 levels in boys and girls, respectively. And the total effect of mixed exposures was shown to be positively correlated of decrease in T4 levels both in adults and adolescents. These above results were similar to the conclusions of most previous studies. For example, Huang et al. conducted a population-based cross-sectional study of 279 Taiwanese adults (≥18 years old) and demonstrated that serum T4 levels were negatively associated with phthalate and the sum of phthalate metabolites (Huang et al. 2017). Koeppe et al. collected 1831 subjects (age ≥12 years) from the 2007–2008 NHANES and evaluated the relationship between urinary triclosan and parabens and serum thyroid markers, and they found that evidence of some inverse associations between parabens and circulating THs levels in adults (Koeppe et al. 2013). Park et al. collected data from nationally representative Korean adults (*n* = 6003) recruited for the second round of the Korean National Environmental Health Survey (KoNEHS), 2012–2014, and confirmed that urinary phthalate metabolites were generally associated with lowered total T4 or T3. When grouped by sex, urinary MEHHP levels were inversely associated with T4 only in males, and MnBP levels were inversely associated with T3 in females (Park et al. 2017). Findings with adolescents were similar; Zhao et al. involved 347 Chinese adolescents which showed a negative correlation between phthalate metabolites in the urine of adolescents and serum T4 levels (Zhao et al. 2022). Another study based on 166 adolescents in Taiwan, China, also found that MMP was associated with decreased T4 (Huang et al. 2020). And a recent meta-analysis confirmed a significant negative correlation between adult DEHP metabolites and serum T4 (Kim et al. 2019).

Associations of urinary phthalate metabolites with T3 and T4 may be in part explained by increasing thyroxine-binding globulin (TBG) and enhanced deiodinase (DIO) activity in peripheral tissues. TBG is the major thyroid hormone-binding protein in humans and might play an important role in phthalate-related thyroid disruption. Increasing TBG can lead to increase of total form of thyroid hormones while leaving lesser thyroid hormones in free forms. Experiments based on rats and zebrafish have found that exposure to phthalates could reduce T4 concentrations by interfering with the effects of hormone synthesis-related proteins, deiodinase, transthyretin, and thyroid hormone receptors (Liu et al. 2015; Zhai et al. 2014). Cell culture experiments have also confirmed that phthalate exposure was associated with the antagonistic activity of TH action (Shen et al. 2009). Dietrich et al. provided clinical evidence that DIO activity

is among possible mechanisms of thyroid-disrupting chemicals. An experimental study involving a long-term exposure to low-dose DEHP in rat found that long exposure to low-dose DEHP in rats showed a tendency to upregulate dio1 expression; however, short-term exposure to DEHP significantly downregulated dio1 mRNA expression or reduce DIO activity in the liver (Dong et al. 2017). Choi et al. employed a subpopulation of adults ($n = 1254$) who participated in the KoNEHS 2015–2017 and suggested that change of DIO activity might play an important role in thyroid disruption by phthalates (Choi et al. 2020). However, the effects of phthalate on binding proteins in humans are equivocal, and there is no clear evidence to support an association between phthalate exposure and thyroxine-binding globulin (Huang et al. 2017).

In contrast, BPA has been shown to affect thyroid function and is age-specific. Fernandez et al. found that BPA exposure in adult rats could increase T4 levels, whereas neonatal exposure decreased T4 levels and increased TSH levels (Fernandez et al. 2018). And Lee et al. demonstrated that exposure to BPA in zebrafish larvae would increase T3 levels (Lee et al. 2019). BPA could inhibit the synthesis of THs by reducing the uptake of thyroid iodide and TPO activity (Silva et al. 2018), and it could also destroy thyroid function through thyroid hormone receptor antagonism (Heimeier et al. 2009). For parabens, it was found to significantly decrease T4 levels and enhance thyroid peroxidase mRNA expression in female Wistar rats (Gogoi and Kalita 2020). All these experiments indicate that EDCs such as phenols, parabens, and phthalates can affect thyroid function, but the effects might vary according to the route, dose, duration, or age at exposure.

Thyroid disruption by thyroid autoantibody status

Thyroid antibody positivity, which reflects thyroid autoimmunity, is an important risk factor for thyroid dysfunction. This study found that the effect of mixed exposure on thyroid antibodies was sex-specific, and the bell–curve association between mixed exposure and risk of TPOAb and TgAb was significant only in girls, and the linear positive correlations between mixed exposure and the risk of TPOAb or TgAb were significant only in females. Mixed exposure was not associated with antibody positivity either in boys or in adult males.

Thyroid autoimmunity is an important cause of thyroid dysfunction; if chemicals can induce thyroid autoimmunity, it can also reduce thyroid hormone production. In a human crossover–crossback study, it was demonstrated that treatment with DBP-coated drugs could lower thyroid autoantibody levels in humans (Nassan et al. 2019). But this observation cannot be directly applied to the general population because this study was based on small number of males ($n = 70$) with inflammatory bowel disease, and the level of exposure was expected to be much greater than those occurring in the general population. In previous animal studies, DBP treatment to rats increased the production of Tg antibody and chronic lymphocytic thyroiditis induced by Tg (Duan et al. 2018).

Our observation in TCS is comparable to the report of a populational based study involving 317 women enrolled in the Environment and Reproductive Health Study (Skarha et al. 2019). They reported that each tenfold increase in TCS was inversely associated with TPOAb (−10%; 95% CI, −19, −0.4) and TgAb (−12%; 95% CI, −23, 0.9) concentrations. While a randomized controlled clinical trial based on 132 subjects comparing the use of 0.3% triclosan toothpaste with a placebo toothpaste did not find any effect of TCS on THs during a 4-year-long follow-up (Cullinan et al. 2012). Animal studies identified that TCS decreases the serum levels of T4, T3, and TSH in rats, affecting the thyroid homeostasis and histology and leading to symptoms of hypothyroidism. The same was not found in larvae where the T4 levels increased, and there were no significant changes in the other hormones; thus, further studies are needed in this area as the effects of TCS on the thyroid function in animals are not yet fully defined.

Recently, several human population studies have been conducted on the link between BPA and thyroid autoantibodies, but the results were inconsistent. A study based on the Thai population over 15 years old showed that there was a significant increasing trend for subjects with TgAb and TPOAb positivity as BPA quartiles increased (Chailurkit et al. 2016), and another research based on Chinese females also confirmed that higher urinary BPA concentrations were associated with increased risk of thyroid nodules in those with positive thyroid autoantibodies (Li et al. 2019). Besides, Kim et al. based on NHANES 2007–2008 data identified that urinary DEHP metabolites were not associated with thyroid autoimmunity in healthy adult females (Kim et al. 2017); however, a study from Beijing, China, showed a negative and positive correlation between total metabolite levels of phthalates in urine and serum TPOAb levels in normal-weight women and underweight women, respectively (Yang et al. 2022). Previous studies have identified BPA could interfere with TH action at multiple points of thyroid hormone synthesis, secretion, and metabolism. In rats, BPA treatment decreased thyroid iodide uptake and TPO activity (Silva et al. 2018). Dong and Wade reported that BPA can inhibit thyroid hormone uptake via the thyroid hormone transporter monocarboxylate transporter 8 (MCT8) in the brain (Dong and Wade 2017). In the blood, thyroid hormone is transported in conjunction with proteins such as TBG and transthyretin (TTR). BPA can bind TTR (Cao et al. 2011). The structure of BPA and its analogs resembles that of T3, BPA can bind

TR, particularly the beta isoform of TR, and acts as an antagonist(Moriyama et al. 2002), which may be the main mechanism through which it disrupts thyroid function.

To avoid the impact of pre-existing immunologic disturbance of the thyroid tissue, this study excluded participants with higher serum TPOAb levels (>9 IU/mL) or TgAb levels (>4 IU/mL) and performed sensitivity analyses using BKMR and QGC models. The results found that the correlation between mixed exposure and serum T4 level in adolescent was no longer significant, but it hardly affects the trend of association with adult THs. Such modifying effects of the thyroid autoantibody status have rarely been assessed in a human population, especially for exposure to a mixture of phenols, parabens, and phthalates. Our observations of different association by the presence of thyroid autoantibodies suggest potential modifying effects of thyroid autoantibody status. Such modifying effects of the thyroid autoantibody status have rarely been assessed in a human population. For perfluoroalkyl substances (PFAS), different directions of association by the thyroid autoantibodies were reported (Itoh et al. 2019). In the adult population of the US NHANES 2007–2008, the association between PFAS and thyroid hormones were modified when both TPO antibodies were positive and iodine status was low (Webster et al. 2016). Therefore, further epidemiological and experimental studies are needed to elucidate the association between these metabolites and thyroid autoimmunity.

Strengths and limitations

There are three unique strengths to our study. First, NHANES is a large study that collects data utilizing a standardized study protocol, employs extensive quality control measures, and utilizes technicians who are trained and certified in data collection procedures. NHANES is nationally representative, which allows our results to be generalizable to the noninstitutionalized US civilian population. Second was that use of the novel and sophisticated methods to estimate the joint effects of 16 chemicals mixtures on THs. BKMR and QGC are two models recently developed to analyze the effects of chemical mixtures on health, which can be taken into account a series of chemicals with high correlations. Finally, different associations between mixtures and THs were observed in different sex and age subgroups, and stratified analysis broadened our understanding of mixed exposure to thyroid dysfunction.

However, the study has some limitations. First, there may be some concern regarding exposure misclassification because of the episodic exposure of these nonpersistent chemicals and their short biological half-lives. However, prior research in NHANES populations has demonstrated that BPA, parabens, and phthalate concentrations were correlated with many lifestyles and building characteristics

(Zota et al. 2016). The relative stability of these characteristics may result in persistent exposure to shorter-lived chemicals (Tanner et al. 2020). Second, we performed numerous association tests between a large number of outcome measures and 16 chemicals; multiple comparisons might result in potential spurious statistically significant results. For this reason, we have focused on the total effect of mixed exposure on the thyroid function because they are the most consistent ones across outcomes and modeling approaches. Finally, NHANES survey weights were not employed in BKMR programs (Luo et al. 2020), since the weighting technique was not accessible in the BKMR models. However, when using the QGC for effect estimation of mixtures, we compared weighted and unweighted analyses and found that the presence or absence of weighting had little effect on the total effect value. We estimated that the results of the three statistical models are not affected by the weighting factor.

Conclusion

In conclusion, the effects of mixed exposure to phenol, parabens, and phthalates on serum T4 were consistent across sex and age with negative correlations in both adolescents and adults. In contrast, the effect of mixed exposure on thyroid autoantibodies varied by sex and age, with a curvilinear correlation in girls, a positive linear correlation in women, and no statistically significant effect in boys and men. More detailed studies of populations at different life stages are needed to reconcile the specific results of the limited human and animal studies conducted to date in this area, to determine the temporal relationships between exposure markers and effects, to elucidate the biological mechanisms involved, and to determine the potential clinical and public health implications of these associations.

Data availability The data used in this study can be downloaded for free in NHANES (website: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Demographics).

Declarations

Ethical approval The study protocols were approved by the National Center for Health Statistic's Research Ethics Review Board, Centers for Disease Control and Prevention (NHANES, 2022).

Consent to participate Informed consent was provided by all NHANES survey participants before the health examination.

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

References

Barr DB et al (2005) Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 113:192–200

Bobb JF (2017) bkmr: Bayesian Kernel Machine Regression. R package version 0.2.0. https://github.com/jenfb/bkmr. Accessed 6 Apr 2022

Bobb JF et al (2018) Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. Environ Health 17:67

Cao J et al (2011) In vitro fluorescence displacement investigation of thyroxine transport disruption by bisphenol A. J Environ Sci (China) 23:315–321

Chailurkit LO et al (2016) The association of serum bisphenol A with thyroid autoimmunity. Int J Environ Res Public Health 13:1153

Choi S et al (2020) Thyroxine-binding globulin, peripheral deiodinase activity, and thyroid autoantibody status in association of phthalates and phenolic compounds with thyroid hormones in adult population. Environ Int 140:105783

Coker E et al (2018) Association between prenatal exposure to multiple insecticides and child body weight and body composition in the VHEMBE South African birth cohort. Environ Int 113:122–132

Cullinan MP et al (2012) Long term use of triclosan toothpaste and thyroid function. Sci Total Environ 416:75–79

Derakhshan A et al (2021) Association of phthalate exposure with thyroid function during pregnancy. Environ Int 157:106795

Dong H, Wade MG (2017) Application of a nonradioactive assay for high throughput screening for inhibition of thyroid hormone uptake via the transmembrane transporter MCT8. Toxicol In Vitro 40:234–242

Dong X et al (2017) Effects of long-term in vivo exposure to di-2-ethylhexylphthalate on thyroid hormones and the TSH/TSHR signaling pathways in Wistar rats. Int J Environ Res Public Health 14:44

Drakvik E et al (2020) Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. Environ Int 134:105267

Duan J et al (2018) Exposure to DBP and high iodine aggravates autoimmune thyroid disease through increasing the levels of IL-17 and thyroid-binding globulin in Wistar rats. Toxicol Sci 163:196–205

Fernandez MO et al (2018) Neonatal exposure to bisphenol A alters the hypothalamic-pituitary-thyroid axis in female rats. Toxicol Lett 285:81–86

Gogoi P, Kalita JC (2020) Effects of butylparaben exposure on thyroid peroxidase (TPO) and type 1 iodothyronine deiodinase (D1) in female Wistar rats. Toxicology 443:152562

Gore AC et al (2015) EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev 36:E1–e150

Heimeier RA et al (2009) The xenoestrogen bisphenol A inhibits postembryonic vertebrate development by antagonizing gene regulation by thyroid hormone. Endocrinology 150:2964–2973

Huang HB et al (2017) Does exposure to phthalates influence thyroid function and growth hormone homeostasis? The Taiwan Environmental Survey for Toxicants (TEST) 2013. Environ Res 153:63–72

Huang PC et al (2020) Characterization of phthalate exposure in relation to serum thyroid and growth hormones, and estimated daily intake levels in children exposed to phthalate-tainted products: a longitudinal cohort study. Environ Pollut 264:114648

Itoh S et al (2019) Association between perfluoroalkyl substance exposure and thyroid hormone/thyroid antibody levels in maternal and cord blood: the Hokkaido study. Environ Int 133:105139

Kahn LG et al (2020) Endocrine-disrupting chemicals: implications for human health. Lancet Diabetes Endocrinol 8:703–718

Keil AP et al (2020) A quantile-based g-computation approach to addressing the effects of exposure mixtures. Environ Health Perspect 128:47004

Kim K et al (2022) Associations of exposure to metal and metal mixtures with thyroid hormones: results from the NHANES 2007-2012. Environ Res 212:113413

Kim MJ et al (2019) Association between diethylhexyl phthalate exposure and thyroid function: a meta-analysis. Thyroid 29:183–192

Kim S et al (2017) Considering common sources of exposure in association studies - urinary benzophenone-3 and DEHP metabolites are associated with altered thyroid hormone balance in the NHANES 2007-2008. Environ Int 107:25–32

Koeppe ES et al (2013) Relationship between urinary triclosan and paraben concentrations and serum thyroid measures in NHANES 2007-2008. Sci Total Environ 445-446:299–305

Kortenkamp A, Faust M (2018) Regulate to reduce chemical mixture risk. Science 361:224–226

Lang IA et al (2008) Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. Jama 300:1303–1310

Lee S et al (2019) Comparison of thyroid hormone disruption potentials by bisphenols A, S, F, and Z in embryo-larval zebrafish. Chemosphere 221:115–123

Li L et al (2019) Bisphenol A exposure and risk of thyroid nodules in Chinese women: a case-control study. Environ Int 126:321–328

Liu C et al (2015) DEHP reduces thyroid hormones via interacting with hormone synthesis-related proteins, deiodinases, transthyretin, receptors, and hepatic enzymes in rats. Environ Sci Pollut Res Int 22:12711–12719

Luo K et al (2020) Associations between organophosphate esters and sex hormones among 6-19-year old children and adolescents in NHANES 2013-2014. Environ Int 136:105461

Martínez MA et al (2018) Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: a Catalonia (Spain) case study. Environ Res 166:25–34

Meeker JD, Ferguson KK (2011) Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007-2008. Environ Health Perspect 119:1396–1402

Miller MD et al (2009) Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. Environ Health Perspect 117:1033–1041

Morgenstern R et al (2017) Phthalates and thyroid function in preschool age children: sex specific associations. Environ Int 106:11–18

Moriyama K et al (2002) Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metab 87:5185–5190

Muncke J et al (2020) Impacts of food contact chemicals on human health: a consensus statement. Environ Health 19:25

Murphy E et al (2010) Thyroid function within the upper normal range is associated with reduced bone mineral density and an increased risk of nonvertebral fractures in healthy euthyroid postmenopausal women. J Clin Endocrinol Metab 95:3173–3181

Nassan FL et al (2019) Dibutyl-phthalate exposure from mesalamine medications and serum thyroid hormones in men. Int J Hyg Environ Health 222:101–110

Ouidir M et al (2020) Association of maternal exposure to persistent organic pollutants in early pregnancy with fetal growth. JAMA Pediatr 174:149–161

Park C et al (2017) Associations between urinary phthalate metabolites and bisphenol A levels, and serum thyroid hormones among the Korean adult population - Korean National Environmental Health Survey (KoNEHS) 2012-2014. Sci Total Environ 584-585:950–957

Philippat C et al (2015) Exposure to select phthalates and phenols through use of personal care products among Californian adults and their children. Environ Res 140:369–376

Pirkle JL et al (1996) Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. Jama 275:1233–1240

Przybyla J et al (2018) A cross sectional study of urinary phthalates, phenols and perchlorate on thyroid hormones in US adults using structural equation models (NHANES 2007-2008). Environ Res 163:26–35

Shen O et al (2009) Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol Lett 191:9–14

Silva MMD et al (2018) Bisphenol A increases hydrogen peroxide generation by thyrocytes both in vivo and in vitro. Endocr Connect 7:1196–1207

Skarha J et al (2019) Cross-sectional associations between urinary triclosan and serum thyroid function biomarker concentrations in women. Environ Int 122:256–262

Sun Y et al (2021) Relationship between blood trihalomethane concentrations and serum thyroid function measures in U.S. adults. Environ Sci Technol 55:14087–14094

Tanner EM et al (2020) Early prenatal exposure to suspected endocrine disruptor mixtures is associated with lower IQ at age seven. Environ Int 134:105185

Taylor PN et al (2018) Global epidemiology of hyperthyroidism and hypothyroidism. Nat Rev Endocrinol 14:301–316

Webster GM et al (2016) Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. adults: variation according to TPOAb and iodine status (NHANES 2007-2008). Environ Health Perspect 124:935–942

Xing W et al (2023) Sex-specific effect of urinary metabolites of polycyclic aromatic hydrocarbons on thyroid profiles: results from NHANES 2011-2012. Environ Sci Pollut Res Int 30:47168–47181

Yang TC et al (2023) Interventions to reduce exposure to synthetic phenols and phthalates from dietary intake and personal care products: a scoping review. Curr Environ Health Rep:1–31

Yang Z et al (2022) Associations between phthalate exposure and thyroid function in pregnant women during the first trimester. Ecotoxicol Environ Saf 242:113884

Yehuda M et al (2017) Parity and risk of thyroid autoimmunity based on the NHANES (2001-2002, 2007-2008, 2009-2010, and 2011-2012). J Clin Endocrinol Metab 102:3437–3442

Yorita Christensen KL (2013) Metals in blood and urine, and thyroid function among adults in the United States 2007-2008. Int J Hyg Environ Health 216:624–632

Zhai W et al (2014) Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP). PLoS One. 9:e92465

Zhao Y et al (2022) The associations of urinary DEHP metabolite levels, serum thyroid hormones, and thyroid-related genes among the adolescent students from China: a cross-sectional study. Environ Sci Pollut Res Int 29:19081–19097

Zota AR et al (2016) Recent fast food consumption and bisphenol A and phthalates exposures among the U.S. population in NHANES, 2003-2010. Environ Health Perspect 124:1521–1528