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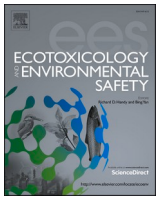

# Urinary polycyclic aromatic hydrocarbons and sex hormones in children and adolescents: Evidence from NHANES

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ABSTRACT

*Background:* Evidences showed that polycyclic aromatic hydrocarbons (PAHs) do harm to human body. However, the association between PAHs and sex hormones in children and adolescents remains unclear.
*Objectives:* The study aims to investigate the associations between PAHs and sex hormones in the general children and adolescent population.
*Methods:* 967 participants aged 6–19 with complete data of PAHs exposure biomarkers, covariates and sex hormones [total testosterone (TT), estradiol (E2) and sex hormone binding globulin (SHBG)] were recruited from National Health and Nutrition Examination Survey (NHANES), 2013–2016. Free androgen index (FAI) was calculated with TT/SHBG. Multivariate linear regression models were performed in six subgroups (male children, male adolescents, male late adolescents, female children, female adolescents and female late adolescents) to estimate the associations between sex hormone alterations and PAHs exposure.
*Results:* In male puberty adolescents, weighted multivariate linear regression indicated that negative trends for 2-Hydroxynaphthalene, 1-Hydroxyphenanthrene, 2&3-Hydroxyphenanthrene and E2 (2-Hydroxynaphthalene: β: −0.104, 95%CI: −0.180, −0.029, $P < 0.01$; 1-Hydroxyphenanthrene: β: −0.112, 95%CI: −0.206, −0.018, $P = 0.019$; 2&3-Hydroxyphenanthrene: β: −0.125, 95%CI: −0.232, −0.018, $P = 0.022$), while exposure to 2-Hydroxynaphthalene was related to TT reduction (β: −0.099, 95%CI: −0.177, −0.020, $P = 0.014$). Same pattern between 2&3-Hydroxyphenanthrene and E2 alteration (2&3-Hydroxyphenanthrene: β: −0.139, 95%CI: −0.236, −0.041, $P < 0.01$) was also observed in male late adolescents. In male children, we determined that 1-Hydroxyphenanthrene was negatively associated with SHBG (β: −0.121, 95%CI: −0.205, −0.037, $P < 0.01$), while the same patterns were observed in male puberty children. We did not observe any significant result in female subgroups. All these results above were determined to have q value < 0.05.
*Conclusion:* PAHs exposure was associated with the alterations of sex hormones in male adolescents and children. Considering the cross-sectional study design, further large-scale epidemiological study is necessary.

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of hazardous substances with two to seven fused aromatic rings and mainly exist in ambient air pollution, soil and food (Kim et al., 2013; Singh et al., 2016). The formations of PAHs are widely acknowledged as a result of combustion of industry or organic materials (Xia et al., 2016). Reports also indicated that there was a wide range of exposure and associations with chronic diseases existed in general population in U.S (Stallings-Smith et al., 2018; Sun et al., 2020). Serious of evidences revealed that PAHs could alter the human health including respiration, reproduction and metabolism with their potential toxicity, mutagenicity and

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carcinogenicity (Burger et al., 2013; Chow and Mahalingaiah, 2016; Mu et al., 2019). Several toxicology studies revealed that PAHs exposure could induce the DNA mutation and chronic inflammation in animals (Attia et al., 2017; Li et al., 2020). The hazard caused by PAHs has become a major issue of public health issue considering the wide exposure pattern and wide-ranged hazardous health effect.

Steroid sex hormones are serious of vital molecules that could be critical to the development and growth of youths, especially adolescents (Herting and Sowell, 2017; Mauras, 2001). According to the previous studies, the total testosterone (TT), sex hormone-binding globulin (SHBG) and estradiol (E2) could play vital roles in the development of bones, muscles and balance the metabolic status (Herbst and Bhasin, 2004; Vandewalle et al., 2015; Wierenga et al., 2018). In the adolescents, they could potentially influence the sex gland maturity and several vital biological processes of reproduction, like spermatogenesis, oogenesis and menstrual periods (Brazert et al., 2019; Wang et al., 2009). Further researches extended the sex hormones' role as risk factors on diseases like polycystic ovary syndrome, infertility and diabetes while they are altered (Goodarzi et al., 2011; Liu and Sun, 2018; Ohlander et al., 2016). Thus, accumulative studies revealed that the sex hormones play a vital role in development and growth in children and adolescents and their alterations could also result in disease burdens in human body (Ruth et al., 2020).

At present, evidences from toxicology studies revealed that exposure to PAHs could further lead to development toxicity and reproductive endocrine failure both in animals and human populations (Drwal et al., 2019; Ni et al., 2019; Scinicariello and Buser, 2014). Several epidemiological studies have shown that a disturbance of sex hormones in children or adolescents will occur when exposure to the several endocrine disrupting chemicals (EDCs) like organophosphate esters or bisphenol A (Luo et al., 2020; Watkins et al., 2017). As one kind of EDCs, PAHs were found to have the potential endocrine disturbance on testosterone synthesis in adults (Wang et al., 2017). However, little is known about associations between PAHs exposure and sex hormone alterations in youths. Given that youths are more vulnerable and sensitive to PAHs exposure, associations between PAHs exposure and sex hormone alterations in youths are considering necessary to be performed (Huang et al., 2019; Poursafa et al., 2017).

Our research selected 967 individuals aged 6–19 years from the National Health and Nutrition Examination Survey (NHANES), 2013–2016. Seven PAHs were included as exposure markers. We performed weighted multivariate linear regression models to identify the associations between PAH exposure and sex hormone alterations in several subgroups. Our research investigated the associations between PAH exposure and sex hormones in children and adolescents and provided a new insight of potential evidence of PAH and sex hormone alterations in youths.

## 2. Materials and methods

### 2.1. Study population

Our study focused on individuals from NHANES, a cross-sectional survey which contains demographic, wide-ranged chemical exposure and biochemical data of general populations in the U.S. 2,2829 individuals from NHANES, 2013–2016 were included in our study (NHANES). We then excluded individuals with missing data of sex hormone outcomes, exposure biomarkers and covariates. Individuals aged beyond 19 were also excluded. Finally, a total of 967 individuals aged 6–19 years were included in our subsequent analysis. The crucial flow chart of our analysis was shown in Fig A. Ethical approval was obtained through the National Center for Health Statistics (NCHS) Research Ethics Review Board (Protocol #2011–17).

### 2.2. Exposure assessment

Seven PAHs were estimated in urine sample of individuals in NHANES, 2013–2016. Urine samples were collected and processed from individuals. An analytical procedure which involved enzymatic hydrolysis of OH-PAH metabolites in urine, extraction by on-line solid phase extraction, and separation and quantification using isotope dilution high performance liquid chromatography-tandem mass spectrometry(online SPE-HPLC-MS/MS) was performed in the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention. Values below the lower limit of detection (LLOD) were calculated as LLOD/$\sqrt{2}$ according to the NHANES laboratory. The details of the laboratory procedure, analysis process and quality control could be downloaded from the website of NHANES (https://wwwn.cdc.gov/nchs/nhanes/default.aspx). Urinary PAHs from participants including 1-Hydroxynaphthalene, 2-Hydroxynaphthalene, 3-Hydroxyfluorene, 2-Hydroxyfluorene, 1-Hydroxyphenanthrene, 2&3-Hydroxyphenanthrene and 1-Hydroxypyrene were initially included in our study as exposure biomarkers. We excluded the 1-Hydroxyprene for its LLOD $<$ 80% (Wang et al., 2018). Finally, six PAHs were included in our analysis. Considering the potential bias caused by kidney condition, the concentrations of each PAH were adjusted by creatinine (Cr) (Suzuki et al., 2010).

### 2.3. Outcomes

Three classic sex hormones including TT, E2 and SHBG were estimated in serum samples of individuals aged 6–19 from NHANES, 2013–2016. Isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) method was used to examine the TT and E2, while SHBG was tested by immuno-antibodies and chemoluminescence measurements (Bai et al., 2020). We also calculated the free androgen index (FAI) using TT/SHBG ratio as an additional outcome to better characterize the potential role of PAHs in sex hormone alterations in youths (Luo et al., 2020).

### 2.4. Covariates

According to the previous reports, potential confounders were considered as age, body measure index (BMI), hispanic origin, six time period, time of blood draw, poverty income ratio (PIR) and educational level as the covariates (Luo et al., 2020; Woodward et al., 2020). These confounders were adjusted into the model as their original format in NHANES. Cigarette smoke exposure status were identified by dividing serum cotinine into two groups according to the limit of detection (Bai et al., 2020). We categorized the smoking status into two levels as $<$ LLOD and $\geq$ LLOD. As to medication use, 11 female individuals had the history of using sex hormone related pills and we adjusted the medication use status in our analysis.

### 2.5. Statistical analysis

All the Cr-adjusted PAHs concentrations and serum sex hormones were nature-log transformed for standardization before our analysis (Woodward et al., 2020). For better understanding the biological meaning of the statistical results, we stratified the population into six groups according to their age and gender. We defined children as individuals aged 6–11 years while adolescents as individuals aged 12–15

years, late adolescents were defined as individuals aged 16–19 years old. To compare differences in demographic, sex hormone and exposure characteristics among genders among these groups, we performed analysis of variance (ANOVA) for continuous variables and chi-square test for categorical variables, respectively.

Due to the great significance of sex hormones existed in puberty status among our individuals, we performed multivariant linear model to four subgroups according to their puberty status. Male children, male puberty adolescents, female children and female puberty adolescents were divided in order to avoid potential biases (Luo et al., 2020). The puberty status was determined by TT $\geq$ 50 ng/dl for males or E2 $\geq$ 20 pg/ml for females. We also determined the female puberty status according to the two questions from the medical condition and reproductive health dataset. Individuals were asked about the questions that "Menopausal periods started yet?" or "Age when menopausal periods occurred?" Female individuals who answered "yes" or with available data on the age when menopausal periods started were considered as puberty (Luo et al., 2020).

The weighted multivariate linear regression model was performed in each group to estimate the coefficient of each PAH on sex hormones. The nature-log transformed concentrations of PAHs were fitted into the multilinear regression model. The associations between PAHs and sex hormones were estimated using the β (95%CI) for per ln unit change, while β was the coefficient of each model. Age, BMI, hispanic origin, six time period, time of blood draw, PIR, educational level and cotinine level were adjusted into each model in order to avoid the potential bias. The subgroup weights were used in the linear model considering the complex design of NHANES (Scinicariello and Buser, 2016).

For sensitive analysis, we calculated false discovery rate (FDR) as q value of significant results to test the robustness of our models. Benjaminiand-Hochberg method was used to adjust the multiple comparison issues. We considered q value $< 0.05$ as robust results (Glickman et al., 2014).

The coefficients estimated were reported as β values and their 95% confidence intervals (CIs). All the analyses were performed using SPSS 26.0 (IBM Company, Armonk, NY, USA) and R version 4.0.1.

## 3. Results

### 3.1. Demographic characteristics

Table 1 revealed the main characteristics involved in our study. Briefly, our analysis included 967 individuals recruited from NHANES (159 male adolescents, 131 male late adolescents, 196 male children, 165 female adolescents, 137 female late adolescents and 179 female children).

Among them, the average age was 12.9 years, and the educational levels of most individuals were below 12th grade. The average BMI of male late adolescents, female late adolescents, male adolescents, female adolescents, male children and female children was 25.1, 25.7, 22.4, 24.0, 19.5 and 19.5 kg/m$^2$, respectively. The mean of PIR was 2.0. About 42.2% of the blood samples were collected in the morning. The non-Hispanic white population was largest and accounting for 26.8% in our study. About 50.1% of the participants was recruited in November 1 through April 30. Male late adolescents had highest cigarette smoke exposure (76.3% of individuals with serum cotinine $\geq$ LLOD) than the

**Table 1**
Main characteristics of study population, NHANES, USA, 2013–2016. Populations were stratified according to their age and gender. Children were defined as 6–11 years old; adolescents were defined as 12–15 years old; late adolescents were defined as 16–19 years old.

| Demographic characteristics[a] | Total | Male | | | Female | | | *P* |
|---|---|---|---|---|---|---|---|---|
| | | Children (N = 196) | Adolescents (N = 159) | Late adolescents (N = 131) | Children (N = 179) | Adolescents (N = 165) | Late adolescents (N = 137) | |
| **Age (Mean, SD)** | 12.9 (3.6) | 9.0 (1.4) | 13.5 (1.1) | 17.4 (1.1) | 9.2 (1.6) | 13.6 (1.2) | 17.3 (1.1) | < 0.01 |
| **PIR (Mean, SD)** | 2.0 (1.5) | 2.1 (1.6) | 2.0 (1.5) | 2.0 (1.4) | 1.9 (1.6) | 2.0 (1.4) | 1.9 (1.4) | 0.722 |
| **BMI (Mean, SD)** | 22.4 (6.1) | 19.5 (4.7) | 22.4 (5.7) | 25.1 (6.0) | 19.5 (4.7) | 24.0 (5.8) | 25.7 (7.1) | < 0.01 |
| **Gender (%)** | | | | | | | | |
| Male | 486 (50.3) | – | – | – | – | – | – | – |
| Female | 481 (49.7) | – | – | – | – | – | – | |
| **Hispanic origin (%)** | | | | | | | | |
| Mexican American | 226 (23.4) | 39 (19.9) | 29 (18.2) | 30 (22.9) | 53 (29.6) | 35 (21.2) | 40 (29.2) | 0.064 |
| Other Hispanic | 125 (12.9) | 25 (12.8) | 15 (9.4) | 16 (12.2) | 24 (13.4) | 25 (15.2) | 20 (14.6) | |
| Non-Hispanic White | 259 (26.8) | 59 (30.1) | 56 (35.2) | 35 (26.7) | 39 (21.8) | 36 (21.8) | 34 (24.8) | |
| Non-Hispanic Black | 197 (20.4) | 38 (19.4) | 41 (25.8) | 27 (20.6) | 37 (20.7) | 31 (18.8) | 23 (16.8) | |
| The Other Race - Including Multi-Racial | 160 (16.5) | 35 (17.9) | 18 (11.3) | 23 (17.6) | 26 (14.5) | 38 (23.0) | 20 (14.6) | |
| **Six months' time period (%)** | | | | | | | | |
| November 1 through April 30 | 484 (50.1) | 95 (48.5) | 93 (58.5) | 71 (54.2) | 83 (46.4) | 77 (46.7) | 65 (47.4) | 0.172 |
| May 1 through October 31 | 483 (49.9) | 101 (51.5) | 66 (41.5) | 60 (45.8) | 96 (53.6) | 88 (53.3) | 72 (52.6) | |
| **Time of blood draw (%)** | | | | | | | | |
| Morning | 408 (42.2) | 71 (36.2) | 73 (45.9) | 61 (46.6) | 67 (37.4) | 73 (44.2) | 63 (46.0) | 0.409 |
| Afternoon | 361 (37.3) | 80 (40.8) | 59 (37.1) | 49 (37.4) | 67 (37.4) | 60 (36.4) | 46 (33.6) | |
| Evening | 198 (20.5) | 45 (23.0) | 27 (17.0) | 21 (16.0) | 45 (25.1) | 32 (19.4) | 28 (20.4) | |
| **Cotinine[b] (%)** | | | | | | | | |
| ≥LLOD | 619 (64.0) | 129 (65.8) | 113 (71.1) | 100 (76.3) | 104 (58.1) | 90 (54.5) | 83 (60.6) | < 0.01 |
| <LLOD | 348 (36.0) | 67 (34.2) | 46 (28.9) | 31 (23.7) | 75 (41.9) | 75 (45.5) | 54 (39.4) | |
| **Educational level[c] (%)** | | | | | | | | |
| below 12th grade | 883 (91.3) | 196 (100.0) | 159 (100.0) | 88 (67.2) | 179 (100.0) | 165 (100.0) | 96 (70.1) | < 0.01 |
| High school graduate | 53 (5.5) | – | – | 26 (19.8) | – | – | 27 (19.7) | |
| GED or equivalent | 1 (0.1) | – | – | – | – | – | 1 (0.7) | |
| More than high school | 30 (3.1) | – | – | 17 (13.0) | – | – | 13 (9.5) | |
| **Sex hormone-related medicine use** | | | | | | | | |
| Yes | 11 (1.1) | – | – | – | – | 5 (3.0) | 6 (4.4) | – |
| No | 956 (98.9) | – | – | – | – | 160 (97.0) | 131 (95.6) | |

[a] : Demographic data were unweighted.
[b] : LLOD was 0.015 ng/ml.
[c] : The educational level was adjusted into the model as its original form in NHANES.

other groups. As expected, we found that there were significant differences in BMI, educational level and serum cotinine among six groups.

Additionally, we listed the demographic characteristics of individuals stratified in puberty and gender in our Supplementary Tables (Table H).

### 3.2. Distribution of PAHs and sex hormone

Table A and Fig B–G displayed the main distributions of PAHs and sex hormones in individuals. All the PAHs included in our analysis have LLOD ≥ 95%. The mean Cr-adjusted concentrations of 1-Hydroxynaphthalene, 2-Hydroxynaphthalene, 3-Hydroxyfluorene, 2-Hydroxyfluorene, 1-Hydroxyphenanthrene, 2&3-Hydroxyphenanthrene were 0.25, 0.80, 0.01, 0.02, 0.01, 0.02 (ng/umol Cr), respectively. Among them, 2-Hydroxynaphthalene had the highest exposure in total participants. The means of TT, E2 and SHBG were 131.65 ng/dl, 35.99 pg/ml, 64.52 nmol/L, respectively. Additionally, the detection frequencies of TT, E2 and SHBG were 99.90%, 72.70%, 100.00%, separately. ANOVA test revealed that there are significant differences of six PAHs and sex hormones among the six subgroups.

### 3.3. Associations between sex hormones and PAHs in adolescents

Multivariate linear regression models were established to assess the associations between sex hormone outcomes and PAHs. Fig. 1 displayed the relationships between PAHs exposure and sex hormones in male children. We determined that 1-Hydroxyphenanthrene was negatively associated with SHBG in male children (β for per ln unit change: −0.121, 95%CI: −0.205, −0.037, $P < 0.01$), while Fig. 2 displayed the same negative pattern of 1-Hydroxyphenanthrene exposure SHBG and in male prepuberty children (β for per ln unit change: −0.118, 95%CI: −0.208, −0.029, $P < 0.01$). All the results above had q value < 0.05.

In male puberty adolescents subpopulation, Fig. 3 indicated that exposure to 2-Hydroxynaphthalene was related to TT reduction (β for per ln unit change: −0.099, 95%CI: −0.177, −0.020, $P = 0.014$). Negative trends for 2-Hydroxynaphthalene, 1-Hydroxyphenanthrene, 2&3-Hydroxyphenanthrene and E2 (2-Hydroxynaphthalene: β for per ln unit change: −0.104, 95%CI: −0.180, −0.029, $P < 0.01$; 1-Hydroxyphenanthrene: β for per ln unit change: −0.112, 95%CI: −0.206, −0.018, $P = 0.019$; 2&3-Hydroxyphenanthrene: β for per ln unit change: −0.125, 95%CI: −0.232, −0.018, $P = 0.022$) were observed. Consistent with the results, in late male adolescent subpopulations, Fig. 4 indicated the negatively patterns of 2&3-Hydroxyphenanthrene and E2 alterations (2&3-Hydroxyphenanthrene: β for per ln unit change: −0.139, 95%CI: −0.236, −0.041, $P < 0.01$). All the above results had q value < 0.05. Additionally, the same trend between E2 reduction and 1-Hydroxyphenanthrene with insignificant q value results was observed (β for per ln unit change: −0.093, 95%CI: −0.180, −0.005, $P = 0.038$). In female subpopulations, no significant association between sex hormone alterations and PAHs exposure were found in our study (Table B–G).

## 4. Discussion

PAHs are sort of hazardous chemicals that widely existed in environment. To our best knowledge, this is the first study assess the associations between PAHs exposure and sex steroid hormones in

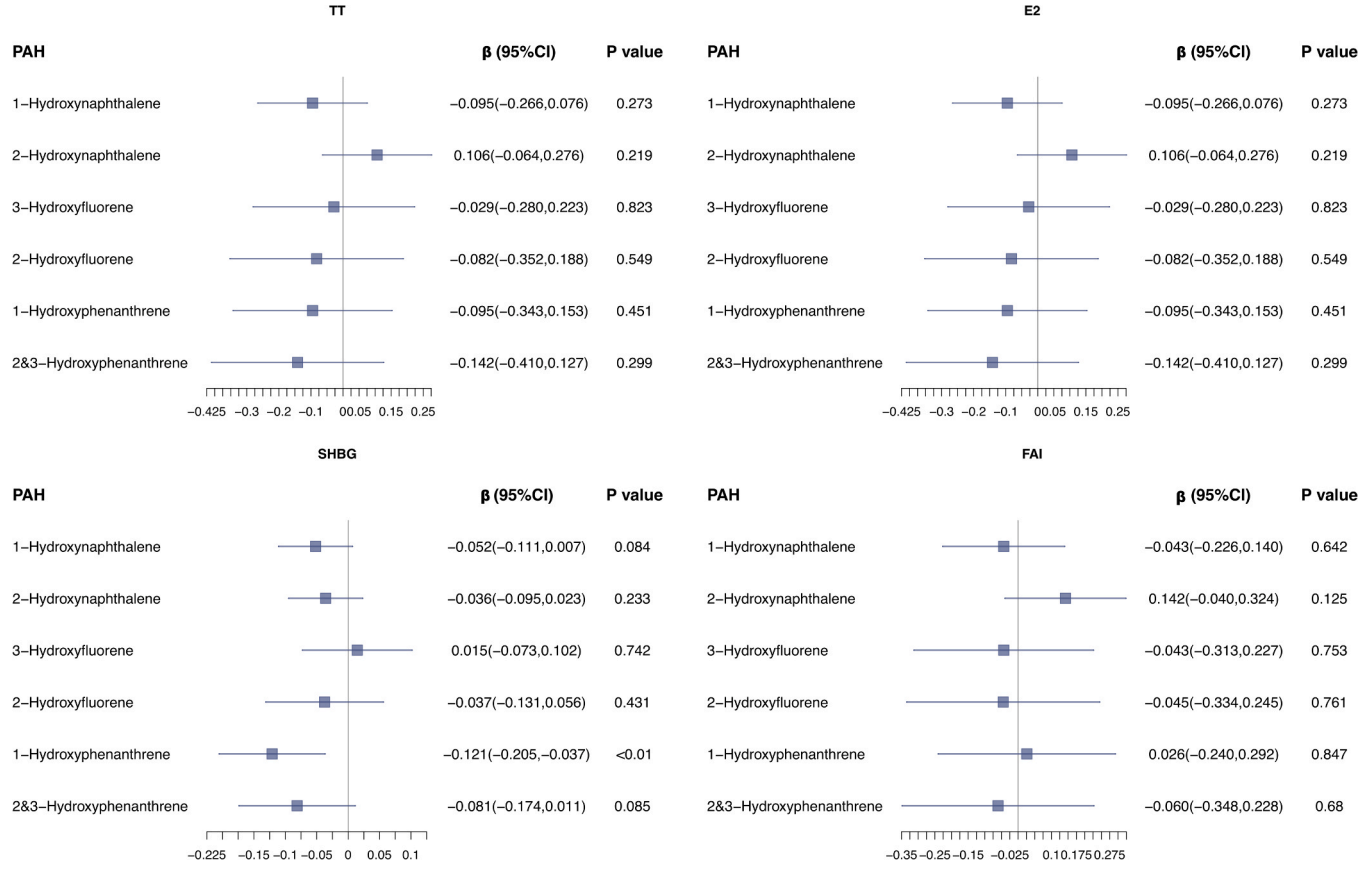


**Fig. 1.** β (95%CI) of associations between PAHs exposure and sex hormones in male children. Model was adjusted by age, body measure index (BMI), Hispanic origin, six months period, time of blood draw, poverty income ratio (PIR), educational level and serum cotinine. q value for association between 1-Hydroxyphenanthrene and SHBG was 0.017.

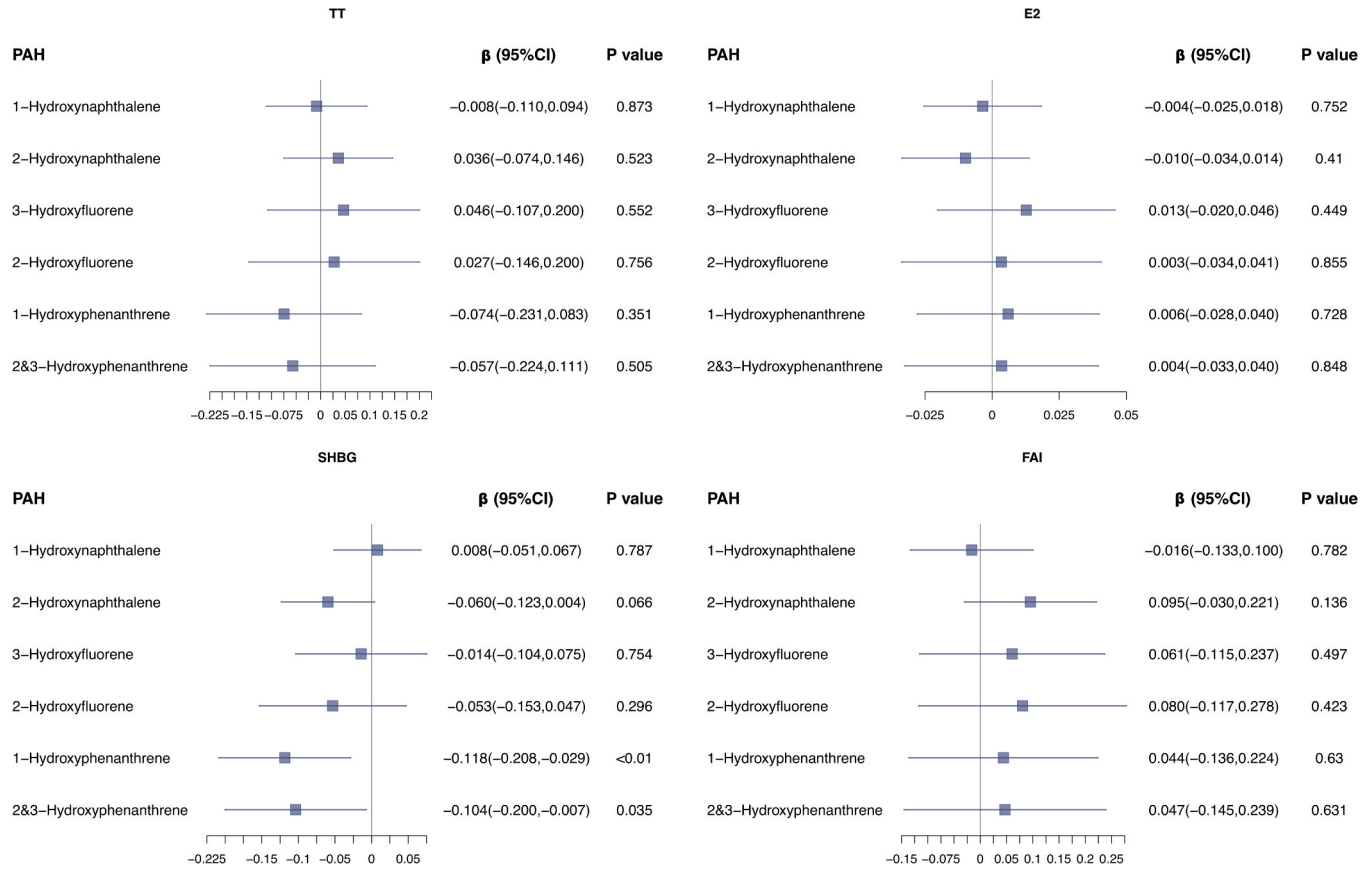


**Fig. 2.** β (95%CI) of associations between PAHs exposure and sex hormones in male prepuberty children. Model was adjusted by age, body measure index (BMI), Hispanic origin, six months period, time of blood draw, poverty income ratio (PIR), educational level and serum cotinine. q values for associations between SHBG and 1-Hydroxyphenanthrene, SHBG and 2&3-Hydroxyphenanthrene were 0.025 and 0.070, respectively.

participants aged 6–19. Through the multivariate linear regression, we determined the negative association between PAHs and SHBG in male children, while negative patterns in TT, E2 were observed in male adolescents. Oppositely, evidences provided from the female individuals were still insufficient. Thus, our analysis revealed the potential risk of PAHs on sex hormone alterations in male children and adolescents.

Epidemiological studies were mounting that exposure to PAHs could alter the serum sex hormones like TT and E2 in male adults. A study recruited male adult individuals from NHANES indicated that urinary 3-hydroxyfluorene and 2-hydroxyfluorene were associated with male TT disturbance (Wang et al., 2017). A cross-sectional study including 208 young male participants in Korea revealed that 1-hydroxypyrene was significantly associated with serum TT alteration (Kim et al., 2005). Another study performed in Chinese hospital was also proved the alteration of TT, E2 and SHBG were associated with PAHs exposure in males (Yang et al., 2017). Thus, these studies showed that PAHs might exist potential reproductive toxicity. However, limited evidence revealed the association between PAHs and sex hormones alterations in children and adolescents.

It is noted that our results indicated a sex-dependent association between PAHs and steroid sex hormone alteration. Negative associations between PAHs exposure and E2, TT in male puberty adolescents were determined in our analysis. These two steroid sex hormones were acknowledged to play vital roles in testis and body development, testicular function homeostasis and spermatogenesis (Ruth et al., 2020; Thomas et al., 1984; Wickman et al., 2001). It is widely acknowledged that PAHs exposure could lead to DNA damage and oxidative stress in testis (Hogstrand and Bohme, 1999; Ma et al., 2019; Yang et al., 2019). In vitro studies proved that PAHs exposure could disrupt testicular gap junctional intercellular communication through MAP kinase-Erk1/2 and PKC pathways targeted on Leydig cells (Kubincová et al., 2019). Another research also revealed that PAHs exposure could disturb the morphology of rat Sertoli cell and F-actin and alpha-tubulin distributions (Raychoudhury and Kubinski, 2003). Additionally, PAHs were also proved to function as estrogen receptor (ER)-binding and anti-estrogen role in breast cancer cell (Arcaro et al., 1999). Thus, the damage or disturbance effect of PAHs on testis could be a potential mechanism explaining our results. However, the evidence that focuses on the mechanisms of specialized PAHs is still limited. Previous studies indicated that exposure to urinary 3-hydroxyfluorene and 2-hydroxyfluorene were positively related in TT in male adults (Wang et al., 2017). Our studies, however, identified negative association between 2-Hydroxynaphthalene and TT in male puberty adolescents. The comparison of our results and the previous epidemiology studies revealed that there may exist a specific endocrine disturbance among PAHs, functional analysis also revealed that PAHs with different structures may have variants of toxicity (Gonzalez et al., 2012). Thus, experimental research is necessary to identify the specific toxicity of PAHs in the future.

Additionally, we also observed the same E2 negative patterns of PAHs exposure in late male adolescents. It is widely acknowledged that this period is crucial for TT synthesis and development of reproductive system (Schulster et al., 2016). While this PAHs-E2 reduction

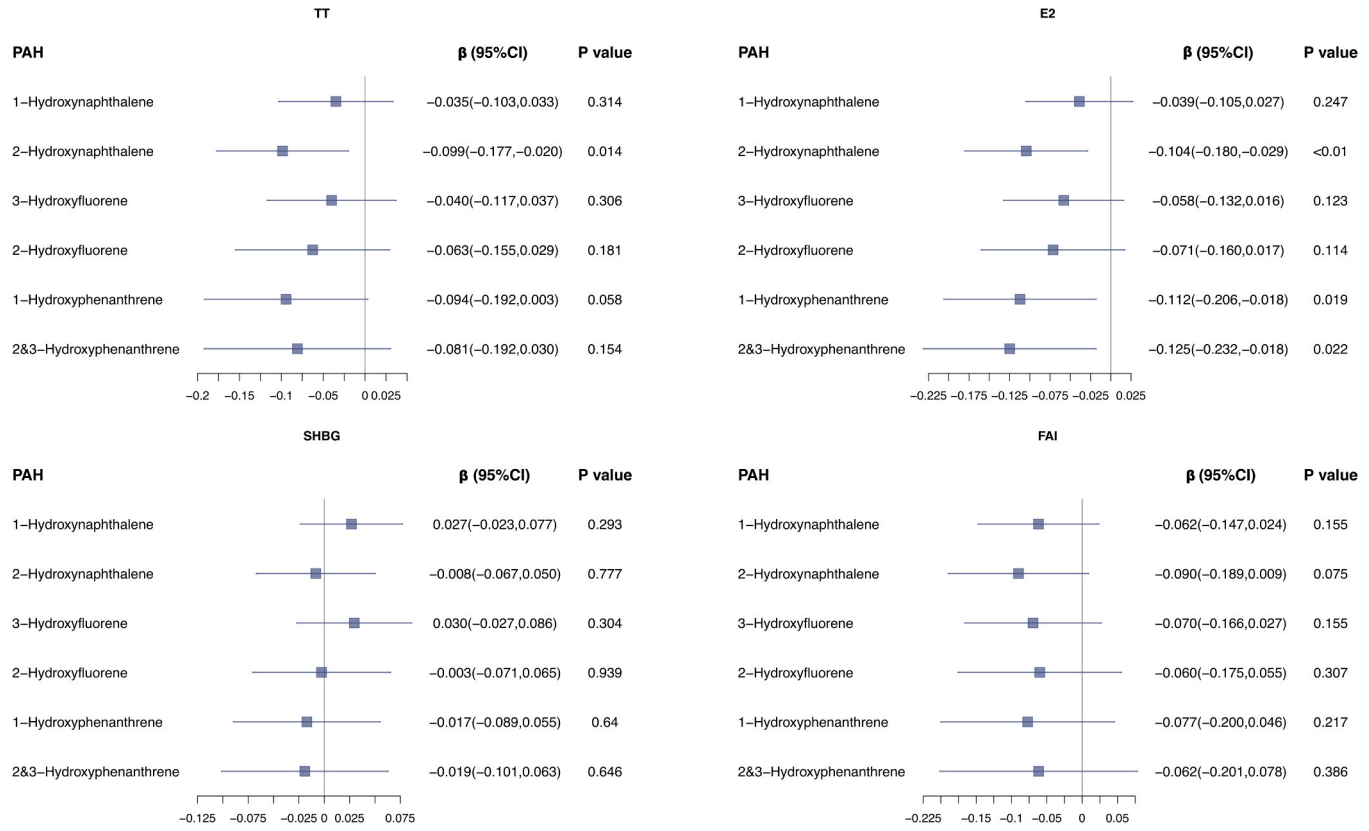


**Fig. 3.** β (95%CI) of associations between PAHs exposure and sex hormones in male puberty adolescents. Model was adjusted by age, body measure index (BMI), Hispanic origin, six months period, time of blood draw, poverty income ratio (PIR), educational level and serum cotinine. q value for associations between TT and 2-Hydroxynaphthalene was 0.028. q values for E2 and 2-Hydroxynaphthalene, 1-Hydroxyphenanthrene, 2&3-Hydroxyphenanthrene were 0.018, 0.041 and 0.044, respectively.

association could be indicated that the potential damage effect on male reproductive system caused by PAHs. Alteration of steroid hormone metabolism will cause several unexpected outcomes as spermatogenesis failure, obesity or male infertility (Vandewalle et al., 2014, 2015). Moreover, we recommended subsequent cohort study to estimate potential associations between PAHs and male adolescents' metabolic alteration.

Our results also indicated that PAHs exposure could be negatively associated with SHBG in male children stratified with age and puberty status. This was the first SHBG negative alteration found in male children. Previously, epidemiological studies included 371 male adults revealed that PAHs exposure was related to serum SHBG in U-shape pattern (Yang et al., 2017). However, negative trend of associations between SHBG and PAHs was observed in our studies. One of the possible mechanisms behind our results could be that PAHs bind to unoccupied receptors and activate a biological response (Yang et al., 2017). Besides, several experimental results revealed that exposure to PAHs could disturb the spatial lipid (including glycerolipids, glycerophospholipids and fatty acid) metabolism and levels in liver in mouse, alter the mRNA level in liver (Li et al., 2020; Pushparajah et al., 2017). These could further influence several metabolic pathways in liver. Thus, considering SHBG is mainly synthesized in liver (Simo et al., 2015), the hepatoxicity and metabolic alteration caused by PAHs exposure may indicate the potential mechanisms behind our results. Additionally, male children are acknowledged as a vital developmental period, while SHBG reduction of this subpopulation could influence the metabolic status (Agirbasli et al., 2009; Dharashivkar et al., 2016). Reports also indicated that SHBG alteration in children could thus lead to obesity, non-alcoholic fatty liver disease or other metabolic syndromes in children (Aydin and Winters, 2016; Park et al., 2020).

Our research had several advances. Firstly, we considered the potential bias caused by time of blood draw, seasons and serum cotinine level, and we also divided individuals into several subgroups according to their age, gender, puberty status and observe the specialized results (Bai et al., 2020; Luo et al., 2020). Second, the estimating methods of urinary PAHs (online SPE-HPLC-MS/MS) performed by NHANES were ascendant and performed strict quality control, which could assure the precision of our data and results (NHANES). Third, our study generated participants from NHANES and could be more representative, which could avoid the potential bias caused by inappropriate selection of study participants.

However, there were still several limitations in our study. First, the urine samples were only measured for one time by NHANES that could lead to potential bias because of the single measurement error. Multiple time points' measurement of PAHs are recommended in future study. Second, the evidence from NHANES may be limited for its cross-sectional design and subsequent large-scale cohort study is necessary to further strengthen our results.

## 5. Conclusion

Therefore, our study revealed the associations between PAHs exposure and sex hormones alterations in male adolescents and children from general population in U.S. Considering the cross-sectional design of NHANES, further large-scale cohort study is necessary.

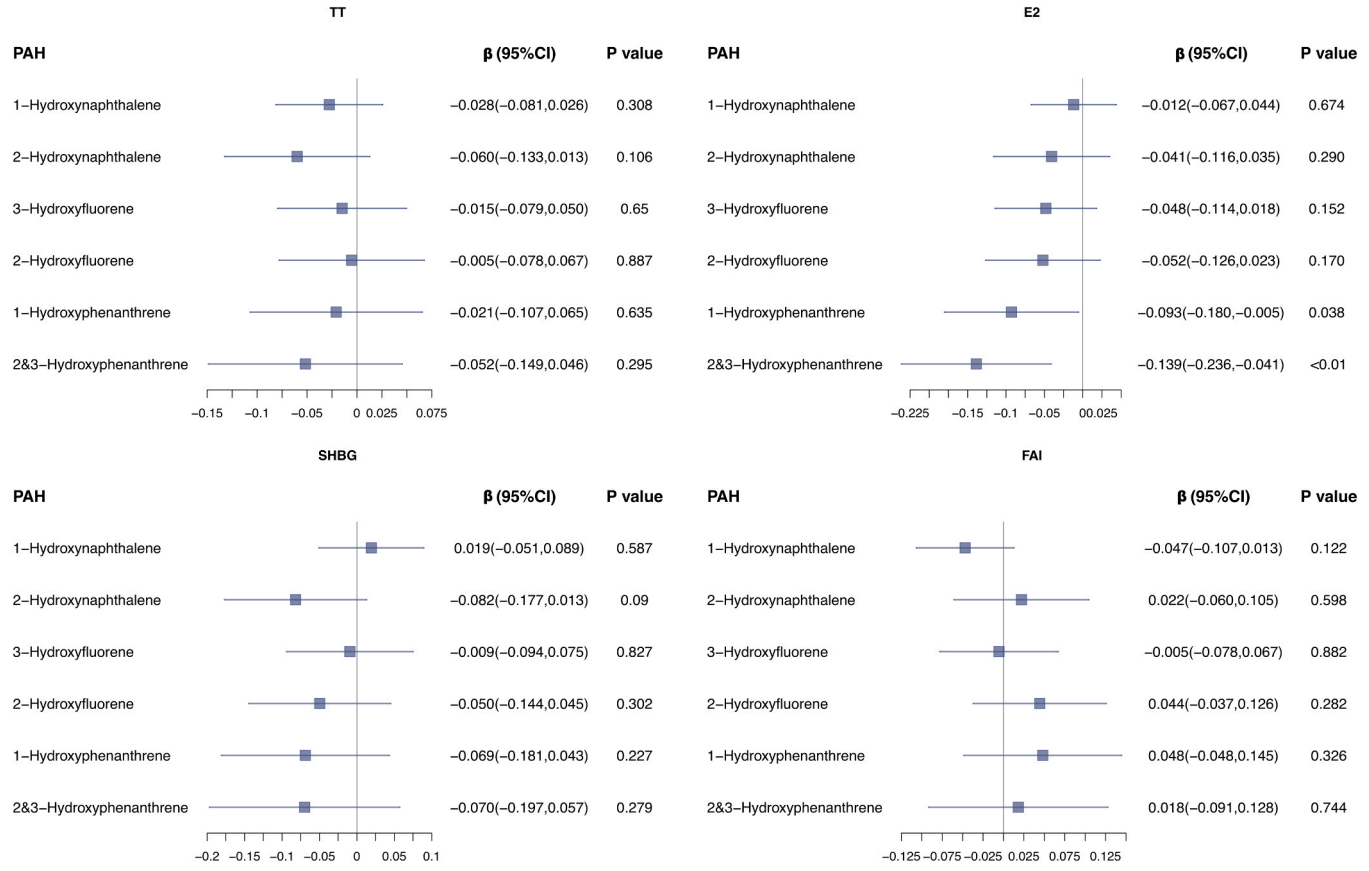


**Fig. 4.** β (95%CI) of associations between PAHs exposure and FAI in male late adolescents. Model was adjusted by age, body measure index (BMI), Hispanic origin, six months period, time of blood draw, poverty income ratio (PIR), educational level and serum cotinine. q values for associations between E2 and 1-Hydroxyphenanthrene, 2&3-Hydroxyphenanthrene were 0.063, 0.016, respectively.

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## CRediT authorship contribution statement

**Chengzhe Tao:** Conceptualization, Methodology, Visualization, Writing - original draft. **Yun Fan:** Conceptualization, Writing - review & editing, Investigation, Software. **Rui Niu:** Conceptualization, Resources, Investigation, Validation. **Zhi Li:** Resources, Investigation. **Hong Qian**: Validation, Data curation. **Hao Yu**: Resources, Investigation. **Qiaoqiao Xu:** validation, Software. **QiuJin Xu:** Supervision, Writing - review & editing, Conceptualization. **Chuncheng Lu**: Conceptualization, Supervision, Writing - review & editing, Funding, acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112215.

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