Association between polycyclic aromatic hydrocarbons and thyroid function among males and females: data from NHANES 2007–2008

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ABSTRACT
The objective of this study was to evaluate the association between thyroid function and exposure to selected polycyclic aromatic hydrocarbons (PAH) among those aged ≥ 20 years. Thyroid variables considered for evaluation were thyroid-stimulating hormone, free and total serum thyroxine (FT4, TT4), free and total triiodothyronine (FT3, TT3), and thyroglobulin. PAH metabolites in urine for which data were analyzed were 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, and 1-hydroxypyrene. Using data from 2007 to 2008 National Health and Nutrition Examination Survey, regression models with logs of thyroid variables as dependent variables and PAH exposure, age, race/ethnicity, iodine sufficiency, smoking status, and others as independent variables were fitted. For females, increased levels of 2-hydroxynaphthalene, 2-hydroxyphenanthrene, and 1-hydroxypyrene were associated with elevated levels of TT3. For males, increased levels of 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, and 9-hydroxypyrene were associated with decreased levels of FT4.

INTRODUCTION
Thyroid gland is one of the largest endocrine glands in the human body. Principal hormones produced by thyroid are triiodothyronine (T3) and thyroxine (T4). Thyroid hormones play an important role in normal development of brain (Bernal 2005, 2007), lung (Bizzarro and Gross 2004), heart (Danzi et al. 2005; Grover et al. 2005), and other organs in human body. Thyroid hormones regulate the rate of metabolism (Oliva et al. 2013; Villanueva et al. 2013). Iodine is a key component of thyroid hormones necessary to synthesize T3 and T4 (Steinmaus et al. 2013). Thus, inadequate iodine uptake may perturb thyroid homeostasis which is maintained by a multi-loop feedback system called the hypothalamic–pituitary–thyroid axis. In a review article, Miller et al. (2009) identified eight classes of chemicals that can disrupt thyroid homeostasis. These include iodine transport chemicals like perchlorate, nitrates, and thiocyanates; synthesis inhibitors like propylthioureia, methimazole, and soy isoflavones; transport disruptors like hydroxy-polychlorinated biphenyls and pentachlorophenol; chemicals which enhance hepatic catabolism like acetochlor, phenobarbital, and polychlorinated biphenyls; enhanced cellular...
transporters like rifampicin, oltipraz, and 1,4-Bis[2-(3,5-dichloropyridyloxyl] benzene; sulfotransferases like triclosan and hydroxyl-polychlorinated biphenyls; deiodinases like FD&C red dye no. 3 and octylmethoxycinnamate; and finally thyroid receptor agonists and antagonists like tetrabromo-bisphenol A and bisphenol A. Miller et al. (2009) provides comprehensive references to the mechanism of how these chemicals affect thyroid homeostasis.

However, there may be chemicals that may affect thyroid homeostasis that may be from a different class of chemicals other than those listed in Miller et al. (2009). In addition, certain chemicals may not yet have been determined to be in one of these classes of chemicals. For example, in a study by Ciarrocca et al. (2012), values of the levels of thyroid-stimulating hormones (TSH), free thyroxine (FT4), free triiodothyronine (FT3), and thyroglobulin (TGN) were compared between traffic policemen in urban areas who were exposed to arsenic in the amount of 2.9 μg/m³ and roadmen in rural areas who were exposed to arsenic in the amount of 0.1 μg/m³. Traffic policemen were found to have statistically significantly higher levels of TSH and TGN than roadmen and statistically significantly lower levels of both FT3 and FT4 than roadmen. Thus, a positive correlation between arsenic levels and TSH and TGN, and a negative correlation between arsenic levels and FT3 and FT4 was shown to exist. In a study of 400 Chinese males, Zhu et al. (2009) found positive correlations between the levels of creatinine adjusted 2-hydroxyfluorene and serum TSH levels. However, no association was found between urinary polycyclic aromatic hydrocarbons (PAH) levels and serum FT3, FT4, and TT4 levels. Rappaport et al. (2004) have also shown PAH metabolites to have endocrine-disrupting properties. Sun et al. (2008) have shown PAH metabolites to interfere THR-mediated transcription. Some of the animal studies that have evaluated the association between one or more PAH metabolites and thyroid functions are due to Goldstein and Taurog (1968), Newman and Moon (1967), Newman et al. (1971), and He et al. (2012). These studies suggest enhanced biliary excretion of FT4 in the presence of exposure to PAH in males. It is possible that the mechanism of how PAHs affect thyroid function might be considered under hepatic catabolism, one of the classifications suggested by Miller et al. (2009).

It should be noted that structurally, PAHs can be composed of two or more aromatic rings. PAHs that contain two aromatic rings include naphthalene and those containing three aromatic rings include phenanthrene and anthracene. PAHs may also contain 4–7 rings with those having five or six rings being the most common. Those having four rings include chrysene, triphenylene, and pyrene. Those having five rings include pentacene and benzo[a]pyrene, and those having six rings include corannulene and benzo[ghi]perylenes. PAHs are lipophilic and most are not soluble in water (Choi et al. 2010). Aqueous solubility decreases as molecular mass increases (Johnsen et al. 2005). Two-ring PAHs, and to a lesser extent three-ring PAHs, dissolve in water, making them more available for biological uptake and degradation. Further, two- to four-ring PAHs volatilize sufficiently to appear in the atmosphere predominantly in gaseous form, although the physical state of four-ring PAHs can depend on temperature. In contrast, compounds with five or more rings have low solubility in water and low volatility; they are therefore predominantly in solid state, bound to particulate air pollution, soils, or sediments. In solid state, these compounds are less accessible for biological uptake or degradation, increasing their persistence in the environment. Several four-ring monohydroxilated PAHs, for example, 3-, 4-, and 10-hydroxybenz[a]anthracenes as well as 2-hydroxychrysene, have been shown to strongly exhibit estrogenic activities (Hayakawa et al. 2007). In addition, certain four-ring monohydroxilated PAHs, for example, 2- and 3-hydroxybenzo[c]phenanthrenes as well as 2-hydroxybenz[a]anthracene and 3-hydroxychrysene, have exhibited strong antiestrogenic activity (Hayakawa et al. 2007).

To the best of our knowledge, no study has been done to assess the impact of PAH on thyroid function in general population. Thus, the objective of this study is to evaluate the association between selected PAHs and six thyroid variables, namely TT3, FT3, TT4, FT4, TSH, and TGN, separately in males and females using data from National Health and Nutrition Examination Survey (NHANES) for the years 2007–2008. Based on the results of a preliminary investigation (data not shown), it is hypothesized that for females, elevated levels of TT3 are associated with increasing levels of selected PAHs. Based on the results of some of the animal studies, it is hypothesized that males will have decreased levels of FT4 and possibly TT4 associated with increasing levels of selected PAH metabolites.
Material and methods

The focus of this study was to evaluate the association between selected PAHs and six thyroid variables, namely TT3, FT3, TT4, FT4, TSH, and TGN, separately in males and females. NHANES conducted by the United States Centers for Disease Control and Prevention (www.cdc.gov/nchs/nhanes.htm) has publically released data on PAH exposure since 1999–2000 for a representative sample of non-institutionalized US population. Starting with years 2007–2008, comprehensive data on the thyroid hormones have been made available. Thus, this study evaluated the relationship between PAH exposure and thyroid function by analyzing data from NHANES for the years 2007–2008 for participants aged 20 years and over. While data were available for all those aged 12 years and over, the sample size for those aged 12–19 years was too small to have stable statistical estimates and as such, they were excluded from the study. At the time of this study, data for thyroid variables were available for 2009–2010 also, but the data for PAH for the years 2009–2010 were not available. A preliminary analysis based on un-weighted NHANES 2007–2008 data (Supplementary Table S1) indicated that most PAH metabolites do affect the levels of thyroid variables. In addition, while this study was not intended to directly compare the levels of thyroid variables between males and females when exposed to PAH, there were gender differences in the levels of thyroid variables when exposed to PAH (Table S1). Instead, this study was intended to evaluate how exposure to PAH affects the levels of thyroid variables separately for males and females.

Data source

Starting with 2007–2008 cycle (www.cdc.gov/nchs/nhamnes/nhanes2007-2008/nhanes07_08.htm) of NHANES, comprehensive data on thyroid profile have become available. Specifically, the variables available on thyroid profile are thyroglobulin antibodies (TgAb), FT3, FT4, TGN, TSH, thyroid peroxidase antibodies (TPOAb), TT3, and TT4. Data were available for NHANES participants aged 12 years and older. Description of laboratory methodology used to detect and measure various thyroid profile variables is given elsewhere (www.cdc.gov/nchs/nhanes/nhanes2007-2008/thyrod_e.htm#Description_of_Laboratory_Methodology). For PAH, data for nine mono-hydroxylated metabolites were available in urine samples, namely 1-hydroxynaphthalene (NAP1), 2-hydroxynaphthalene (NAP2), 2-hydroxyfluorene (FLU2), 3-hydroxyfluorene (FLU3), 1-hydroxyphenanthrene (PHE1), 2-hydroxyphenanthrene (PHE2), 3-hydroxyphenanthrene (PHE3), 1-hydroxypyrene (PYE1), and 9-hydroxyfluorene (FLU9). Percent weighted observations at or above the limit of detection (LOD) for all of these nine PAHs were above 80 % and as such, all of them were selected for further analysis. All observations below the LOD were set at LOD/√2. Laboratory methods used to detect and measure various PAH variables are available at http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/PAH_E.htm#Description_of_Laboratory_Methodology. For the purpose of this study, a variable that represented the sum of all nine PAH metabolites, ΣPAH, as listed above was also created. ΣPAH = NAP1 + NAP2 + FLU2 + FLU3 + FLU9 + PHE1 + PHE2 + PHE3 + PYE1.

Data from demographic, thyroid profile, PAH variables, C-reactive protein (CRP), body measures, serum cotinine, urine iodine, and fasting data files for 2007–2008 were downloaded and match merged. It should be noted that fasting data files provide data on NHANES participants’ fasting status prior to blood being drawn in the Mobile Examination Center for blood chemistries. For example, how long it has been since the participant consumed alcohol. The fasting time used for this study was the time in hours since the participant ate or drank anything other than water at the time of venipuncture. The sampling plan for NHANES is a complex, stratified, multistage, probability cluster designed to be representative of the civilian, non-institutionalized US population. Sampling weights were created in NHANES to account for the complex survey design, including oversampling, survey non-response, and post-stratification.
Sample selection

First, in concurrence with the objectives of this study, data for only those who were aged ≥ 20 years were selected. Next, since pregnancy may affect thyroid function, those females who were pregnant \((N = 20)\) at the time of participation in NHANES were also removed from the study database. Then, those who reported having current thyroid problems \((N = 113)\) and/or reported using one or more thyroid treatment drugs \((N = 11)\) were also removed from the study database. Since the presence of high levels of thyroid antibodies may indicate thyroid damage, those with TPOAb ≥ 35 IU/ml and/or TgAb ≥ 20 IU/ml \((N = 94)\) were excluded from the study. These cut-offs have been recommended in Greenspan’s Basic and Clinical Endocrinology (Greenspan 2011) as quoted at http://emedicine.medscape.com/article/2086819-overview. Final database available for data analysis had 1355 participants. In addition, since there were gender differences in the levels of thyroid variables when exposed to PAH (Table S1), separate databases for males \((N = 740)\) and females \((N = 615)\) were generated. Sample selection process is detailed in Figure 1. Detailed sample sizes are also given in Table 1.

![Sample selection process diagram](image)

**Table 1.** Un-weighted sample sizes by age, race/ethnicity, smoking status, and iodine sufficiency status for participants aged ≥ 20 years.

|                          | Females | Males |
|--------------------------|---------|-------|
| **Total**                | 615     | 740   |
| **Age**                  |         |       |
| 20–64 years old          | 485     | 542   |
| 65+ years old            | 130     | 198   |
| **Race/ethnicity**       |         |       |
| Non-Hispanic white       | 274     | 392   |
| Non-Hispanic black       | 145     | 138   |
| Mexican American         | 196     | 210   |
| **Smoking status**       |         |       |
| Non-smoker               | 472     | 518   |
| Smoker                   | 143     | 222   |
| **Iodine sufficiency status** |       |       |
| Iodine deficient         | 204     | 173   |
| Iodine replete           | 411     | 567   |

Note: Data from National Health and Nutrition Examination Survey 2007–2008.
**Outcome variables**

Outcome variables for this study were log10 transformed values of FT3, FT4, TGN, TSH, TT3, and TT4.

**Exposure variables**

Exposure variables for this study were NAP1, NAP2, FLU2, FLU3, PHE1, PHE2, PHE3, PYE1, FLU9, and ∑PAH.

**Covariates**

Based on the results of previous research and our own preliminary analysis, the independent variables/covariates considered for regression modeling were age, race (non-Hispanic whites (NHW), non-Hispanic blacks (NHB), Hispanics (HISP)), smoking status (non-smoker, smoker), iodine sufficiency status (iodine deficient, iodine replete), CRP, body mass index (BMI), fasting time (FTIME) before the blood was drawn for analysis, and 10 PAH metabolites in urine as previously mentioned. Race/ethnicity and age differences for TSH and TT4 have been documented by Hollowell et al. (2002). For this reason, they were included as covariates in the regression models. CRP was used as a biomarker of inflammatory conditions that may alter thyroid function (Blount et al. 2006). Kelly (2000), in a review article, found aging and fasting to be factors that may affect the levels of TT3 and as such fasting time was also included in the regression models. Estrogen use and menopausal status were also used as independent variables for females as they may affect thyroid function (Blount et al. 2006). Also, as has been considered among others by Blount et al. (2006) and Turyk et al. (2007), a consideration was given to the possibility of the use of drugs other than thyroid treatment drugs having association with thyroid variables. In order to evaluate that, all those who were using one or more prescription drugs like non-steroidal anti-inflammatory drugs, beta blockers, blood glucose regulators, and others like interferon, lithium, phenytoin, etc. were identified. An indicator variable, use or non-use of these drugs was created and used in all models as an independent variable. Since menopausal status and use of estrogen may affect the levels of thyroid variables (Blount et al. 2006) and since these variables are only applicable to females, the data were analyzed separately for males and females. In addition, since iodine is an essential component needed to produce thyroid hormones, iodine sufficiency status has the potential to affect thyroid profile, urine iodine was categorized as those who were iodine deficient, meaning those with urinary iodine levels were < 100 ng/ml, and those who were iodine replete defined as those for whom urinary iodine levels were ≥ 100 ng/ml. This scheme based on urinary iodine concentration levels to classify those who are iodine deficient has also been used among others by Blount et al. (2006). WHO (2007) also defines iodine deficiencies as those who had urinary iodine levels below 100 ng/ml. It should be noted that criterion to define iodine sufficiency status among females is different than non-pregnant females because of the increased need for iodine during pregnancy to fulfill the iodine needs for the developing fetus (http://www.endocrine.niddk.nih.gov/pubs/pregnancy/#eating). A variable for smoking status was created by splitting serum cotinine data in two different levels. Those whose serum cotinine levels were < 10 ng/ml were defined as non-smokers and those whose serum cotinine levels were ≥ 10 ng/ml were defined as smokers. Those with serum cotinine levels of ≥ 10 ng/ml have been defined as smokers among others by Jain and Wang (2011) and Jain (2013a). Because of positively skewed distributions, all thyroid as well as PAH variables were log10 transformed before analysis.

**Statistical analysis**

All analyses incorporated information on sampling design variables. All variables on thyroid profile were multiplied by 1000 before analysis so as to avoid working with very small values and as such cause unstable estimations of statistical parameters. This change in unit should be carefully considered in interpreting the magnitude of regression slopes. It should be noted that tests of significance are not
affected if variable values are rescaled (for example, multiplied) before regression, only the magnitude of regression slopes are affected.

In a preliminary analysis, first order interactions between age, race/ethnicity, and smoking status were considered but were found to be statistically insignificant at $\alpha = 0.05$ and as such interactions terms were not entered into the regression models. Only one PAH variable at a time was considered for analysis for each model. Since, there were a total of six thyroid variables, namely, FT3, FT4, TT3, TT4, TSH, and TGN, and ten PAH variables including $\Sigma$PAH; a total of 60 regression models each for males and females were fitted.

All analyses were done using SAS version 9.2 (www.sas.com, SAS, Cary, North Carolina, USA) and SUDAAN version 11.0 (www.rti.org/SUDAAN, Research Triangle Institute International, Research Triangle Park, North Carolina, USA). All analyses used appropriate weights as provided in the data files. Specifically, the variable WTSB2YR provided weight for all data.

Results

Tremendous amount of data were generated in this study. There were a total of 60 regression models fitted to evaluate the linear relationship of ten PAH variables with each of the six thyroid variables for each of the two databases, namely, for females and males. This meant generation of 60 sets of regression coefficients and adjusted geometric means for males and females separately. Reproducing all these data in this manuscript will be a waste of journal space and in fact, confusing and counterproductive. Consequently, only the regression coefficients generated when evaluating linear association between only one PAH variable and six thyroid variables are presented. In addition, for each thyroid variable for each of the two databases used in this study, adjusted geometric means are presented for only one PAH variable. The differences between the regression coefficients and adjusted geometric means presented here and not presented in the manuscript were minimal, if any.

Table 2. Actual sample sizes ($N$) and $R^2$ for the various regression models fitted with thyroid variables* as dependent and polycyclic aromatic hydrocarbons (PAH) as one of the independent variables by database and gender used for the study for participants aged ≥ 20 years.

| Dependent variable | Log10(TSH) | Log10(TT3) | Log10(FT3) | Log10(TT4) | Log10(FT4) | Log10(TGN) |
|--------------------|------------|------------|------------|------------|------------|------------|
| PAH variable       | $N$ | $R^2$ | $N$ | $R^2$ | $N$ | $R^2$ | $N$ | $R^2$ | $N$ | $R^2$ | $N$ | $R^2$ |
| Database           |   |   |   |   |   |   |   |   |   |   |   |   |
| Females            |   |   |   |   |   |   |   |   |   |   |   |   |
| 1-Hydroxynaphthalene | 539 | 11.8 | 539 | 15.1 | 539 | 16.8 | 539 | 12.2 | 539 | 4.0 | 539 | 10.5 |
| 2-Hydroxynaphthalene | 552 | 11.5 | 552 | 17.3 | 552 | 16.8 | 552 | 13.8 | 552 | 3.4 | 552 | 10.9 |
| 3-Hydroxyflurene   | 563 | 11.8 | 563 | 15.4 | 563 | 15.6 | 563 | 12.7 | 563 | 3.5 | 563 | 11.5 |
| 2-Hydroxyfluorene  | 563 | 12.0 | 563 | 15.2 | 563 | 15.6 | 563 | 13.0 | 563 | 3.6 | 563 | 11.1 |
| 3-Hydroxyphenanthrene | 566 | 12.0 | 566 | 14.3 | 566 | 14.9 | 566 | 12.7 | 564 | 3.6 | 566 | 11.1 |
| 1-Hydroxyphenanthrene | 569 | 11.5 | 569 | 14.7 | 569 | 14.9 | 569 | 12.9 | 569 | 3.2 | 569 | 11.1 |
| 2-Hydroxyphenanthrene | 563 | 11.6 | 563 | 16.6 | 563 | 14.5 | 563 | 13.5 | 563 | 3.5 | 563 | 11.1 |
| 1-Hydroxypyrene    | 561 | 11.2 | 561 | 16.0 | 561 | 15.5 | 561 | 13.3 | 561 | 3.5 | 561 | 10.7 |
| 9-Hydroxyfluorene  | 569 | 11.5 | 569 | 14.8 | 569 | 15.0 | 569 | 13.3 | 569 | 3.3 | 569 | 11.1 |
| $\Sigma$PAH        | 539 | 9.3  | 539 | 12.6 | 539 | 3.1  | 539 | 14.6 | 539 | 10.1 | 539 | 7.4  |
| Males              |   |   |   |   |   |   |   |   |   |   |   |   |
| 1-Hydroxynaphthalene | 698 | 6.9  | 698 | 14.5 | 698 | 19.9 | 698 | 2.8  | 698 | 3.6  | 698 | 7.1  |
| 2-Hydroxynaphthalene | 709 | 6.4  | 709 | 14.4 | 709 | 19.7 | 709 | 3.8  | 709 | 3.9  | 709 | 6.6  |
| 3-Hydroxyflurene   | 718 | 6.9  | 718 | 13.9 | 718 | 19.6 | 718 | 2.9  | 718 | 3.7  | 718 | 7.5  |
| 2-Hydroxyfluorene  | 721 | 7.5  | 721 | 14.3 | 721 | 19.7 | 721 | 2.8  | 721 | 3.8  | 721 | 6.9  |
| 3-Hydroxyphenanthrene | 721 | 6.8  | 721 | 14.7 | 721 | 19.7 | 721 | 3.5  | 721 | 3.9  | 721 | 7.1  |
| 1-Hydroxyphenanthrene | 725 | 6.7  | 725 | 14.8 | 725 | 19.4 | 725 | 4.4  | 725 | 4.0  | 725 | 7.6  |
| 2-Hydroxyphenanthrene | 714 | 6.4  | 714 | 14.9 | 714 | 19.3 | 714 | 3.5  | 714 | 4.3  | 714 | 7.3  |
| 1-Hydroxypyrene    | 713 | 6.6  | 713 | 14.6 | 713 | 19.9 | 713 | 3.3  | 713 | 2.9  | 713 | 7.0  |
| 9-Hydroxyfluorene  | 725 | 6.5  | 725 | 14.5 | 725 | 19.8 | 725 | 3.5  | 725 | 4.2  | 725 | 7.2  |
| $\Sigma$PAH        | 698 | 6.2  | 698 | 20.5 | 698 | 3.6  | 698 | 16.1 | 698 | 3.0  | 698 | 7.6  |

Notes: *TSH = Thyroid-Stimulating Hormone, TT3 = Total triiodothyronine, FT3 = Free triiodothyronine, TT4 = Total thyroxine, FT4 = Free thyroxine, TGN = Thyroglobulin.
For females, actual sample sizes used in the analyses varied from 539 to 569. $R^2$ for various models for females varied from 3.2 to 17.3 %. For males, actual sample sizes used in the analyses varied from 698 to 725. $R^2$ for various models for males varied from 2.8 to 19.9 % (Table 2).

**Association between PAH and thyroid variables**

**Females**

For females, levels of FT3 increased with increase in the levels of NAP1 ($\beta = 0.0083, p = 0.015$, Table 3) and PYE1 ($\beta = 0.0128, p = 0.033$) and just about with NAP2 ($\beta = 0.015, p = 0.051$). The levels of TSH decreased with increase in the levels of FLU2 ($\beta = -0.0876, p = 0.043$). The levels of FT4 increased with the levels of NAP1 ($\beta = 0.0089, p = 0.049$). However, most significantly, the levels of TT3 increased with the levels of NAP2 ($\beta = 0.046, p = 0.032$), PHE2 ($\beta = 0.0521, p = 0.025$), and PYE1 ($\beta = 0.0438, p = 0.01$).

Based on models which used logs of PAH metabolites as dependent variables and gender and logs of PAH metabolites as independent variables, an interquartile range (IQR) change in PAH metabolites was associated with ≥0.2 μIU/ml change in TSH for FLU2, FLU3, and PHE3; and <0.2 μIU/ml change in TSH for other six metabolites (Table S2). For an IQR change in the levels of PAH metabolites, levels of FT3 decreased by ≥0.08 pg/ml for NAP2 and PYE1, between 0.05 and 0.08 for FLU2, FLU3, and PHE2, and <0.05 for other metabolites (Table S2). Change in FT4 was insignificant and <0.01 for every PAH metabolite (Table S2). An IQR change in NAP2, FLU2, FLU3, PHE2, and PYE1 was associated with decrease in TT3 levels by more than 2 ng/dl; and ≤1 ng/dl for PHE3, PHE1, and FLU9 (Table S2). An IQR change in NAP1 was associated with increase in the level of TT3 by 0.37 ng/dl. An IQR change in the levels of PHE3, PHE1, and FLU9 was associated with increase in TT4 levels by >0.1 μg/dl; between 0.05 and 0.1 μg/dl for NAP1, FLU3, and PYE1, and <0.05 for FLU2 and PHE2. However, IQR change in the levels of NAP2 was associated with decrease in TT4 level by 0.08 μg/dl. For an IQR change in NAP1, NAP2, FLU2, FLU3, PHE3, and FLU9, the levels of TGN decreased by >1 ng/ml, and by <1 ng/ml for PHE1, PHE2, and PYE1 (Table S2).

**Males**

For ≥20 year old males, there was a statistically significant decrease in the levels of FT4 with increase in the levels of PHE1 ($\beta = -0.0163, p = 0.02$, Table 3), PHE2 ($\beta = -0.0207, p = 0.003$), and FLU9 ($\beta = -0.0169, p = 0.003$). There was also a negative association between the levels of TT3 and PHE1 ($\beta = -0.0283, p = 0.041$, Table 3). There was a positive association between the levels of TT4 and NAP2 ($\beta = 0.0226, p = 0.007$, Table 3). However, levels of TT4 decreased with increase in the levels of PHE1 ($\beta = -0.0353, p = 0.001$), PHE2 ($\beta = -0.0244, p = 0.035$), PHE3 ($\beta = -0.0219, p = 0.052$), and FLU9 ($\beta = -0.0214, p = 0.014$). There was a negative association between TGN and PHE1 ($\beta = -0.1168, p = 0.009$, Table 3).

Based on models which used logs of PAH metabolites as dependent variables and gender and logs of PAH metabolites as independent variables, an IQR change in PAH metabolites was associated with increase in TSH levels by >0.2 μIU/ml for FLU2, FLU3, PHE3, and PYE1, and by <0.1 but <0.2 μIU/ml for NAP2, PHE1, PHE2, and FLU9 (Table S2). FT3 levels decreased by <0.1 pg/ml for IQR change in PAH metabolite levels and FT4 levels increased by <0.01 ng/dl for all PAH metabolites except NAP2 for which there was a decrease by 0.002 ng/dl (Table S2). For an IQR change in NAP2 and PYE1, the levels of TT3 decreased by more than 3 ng/dl; by ≥2 but <3 ng/dl for FLU2 and FLU3; by 1.95 ng/dl for PHE2, and by <1 ng/dl for PHE3, PHE1, and FLU9. However, for NAP1, TT3 levels increased by 0.32 ng/dl. TT4 levels increased for every PAH metabolite except NAP2 for which a decrease was observed (Table S2). TGN levels decreased for NAP1, FLU2, and FLU3 by >1.0 ng/ml, and by <1.0 ng/ml for all other metabolites.

**Association between thyroid variables and demographic and other factors**

**Females**

For females, BMI was positively associated with the levels of TT4 ($\beta = 0.0016, p = 0.013$, Table 4). CRP was positively associated with TT4 ($\beta = 0.0312, p = 0.003$, Table 4). Urine creatinine was not found...
Table 3. Regression slopes with p-values for selected polycyclic aromatic hydrocarbons (PAH) with various thyroid variables for participants aged ≥ 20 years.

| Data-set | Log10 of | NAP1 | NAP2 | FLU3 | FLU2 | PHE3 | PHE1 | PHE2 | PYE1 | FLU9 | ΣPAH |
|----------|----------|------|------|------|------|------|------|------|------|------|------|
| Females  | TSH      | -0.0037 (0.9) | -0.0108 (0.77) | -0.046 (0.25) | -0.0876 (0.04) | -0.0643 (0.30) | -0.032 (0.64) | -0.0552 (0.33) | -0.0185 (0.73) | -0.0295 (0.55) | -0.04412 (0.45) |
|          | FT3      | 0.0083 (0.02) | 0.015 (0.051) | 0.0142 (0.06) | 0.0139 (0.09) | 0.0035 (0.61) | 0.0061 (0.38) | 0.0108 (0.2) | 0.0128 (0.03) | 0.0082 (0.24) | 0.01586 (0.05) |
|          | FT4      | 0.0089 (0.049) | 0.0109 (0.40) | 0.0122 (0.23) | 0.0142 (0.14) | 0.0123 (0.32) | 0.0041 (0.74) | 0.0025 (0.85) | 0.0052 (0.66) | -0.0066 (0.54) | 0.01183 (0.34) |
|          | TT3      | -0.0043 (0.49) | 0.046 (0.03) | 0.0265 (0.20) | 0.035 (0.14) | 0.0044 (0.79) | 0.0201 (0.28) | 0.0521 (0.03) | 0.0438 (0.01) | 0.0205 (0.40) | 0.02685 (0.07) |
|          | TT4      | -0.0001 (0.98) | 0.0232 (0.27) | 0.0025 (0.83) | 0.0136 (0.28) | 0.0058 (0.65) | -0.0014 (0.92) | 0.0211 (0.14) | 0.0032 (0.77) | -0.0163 (0.10) | 0.00267 (0.85) |
|          | TGN      | 0.0148 (0.71) | 0.0152 (0.81) | 0.0291 (0.60) | 0.0467 (0.43) | -0.0062 (0.91) | 0.0267 (0.66) | -0.0456 (0.42) | 0.0138 (0.82) | -0.0328 (0.67) | -0.01455 (0.82) |
| Males    | TSH      | -0.0061 (0.76) | -0.0082 (0.78) | -0.0544 (0.17) | -0.0726 (0.16) | -0.0478 (0.30) | -0.0419 (0.25) | -0.0519 (0.21) | -0.0289 (0.33) | -0.0093 (0.82) | -0.02444 (0.43) |
|          | FT3      | 0.0029 (0.47) | -0.0012 (0.86) | -0.0014 (0.82) | -0.0007 (0.92) | -0.0032 (0.51) | -0.0069 (0.19) | -0.0013 (0.80) | 0.0041 (0.34) | -0.0106 (0.11) | -0.00199 (0.7) |
|          | FT4      | 0.0004 (0.95) | -0.0015 (0.87) | -0.0062 (0.49) | -0.0121 (0.20) | -0.0122 (0.09) | -0.0163 (0.02) | -0.0207 (0.03) | 0.0035 (0.62) | -0.0169 (0.03) | -0.00302 (0.71) |
|          | TT3      | 0.0007 (0.93) | 0.0033 (0.81) | -0.0084 (0.47) | -0.0119 (0.29) | -0.0251 (0.06) | -0.0283 (0.04) | -0.0251 (0.06) | 0.0018 (0.90) | -0.0211 (0.09) | -0.00006 (1) |
|          | TT4      | 0.0015 (0.83) | 0.0226 (0.01) | -0.0034 (0.71) | -0.0031 (0.79) | -0.0219 (0.05) | -0.0353 (0.01) | -0.0244 (0.04) | -0.0172 (0.17) | -0.0214 (0.01) | 0.00732 (0.47) |
|          | TGN      | -0.0302 (0.27) | 0.0219 (0.58) | -0.0133 (0.76) | -0.0507 (0.31) | -0.0772 (0.16) | -0.1168 (0.01) | -0.0884 (0.07) | -0.0455 (0.36) | -0.0686 (0.33) | -0.02991 (0.38) |

Notes: NAP1 = 1-hydroxynapthalene, NAP2 = 2-hydroxynaphthalene, FLU3 = 3-hydroxyfluorene, FLU2 = 2-hydroxyfluorene, PHE3 = 3-hydroxyphenanthrene, PHE2 = 2-hydroxyphenanthrene, PHE1 = 1-hydroxyphenanthrene, PYE1 = 1-hydroxypyrene, FLU9 = 9-hydroxyfluorene.

Data from National Health and Nutrition Examination Survey 2007–2008.
to be associated with any of the six thyroid variables. Fasting time also, did not affect the levels of any thyroid variable. In addition, use of prescription drugs other than the thyroid treatment drugs also did not affect the levels of any thyroid variable. Use of estrogen drugs was associated with increased levels of TT3 ($\beta = 0.0509$, $p = 0.014$). Being in menopause was associated with increased levels of TSH ($\beta = 0.0906$, $p = 0.003$) and $\Sigma$PAH ($\beta = 0.09202$, $p = 0.03$) and decreased levels of FT3 ($\beta = -0.0106$, $p = 0.006$) and TT3 ($\beta = -0.0295$, $p = 0.022$).

### Males

BMI was positively associated with FT3 ($\beta = 0.0008$, $p = 0.023$, Table 4) and TGN ($\beta = 0.0066$, $p = 0.009$, Table 4) for males. Use of prescription drugs other than thyroid treatment drugs was associated with decreased levels of FT3 ($\beta = -0.0201$, $p < 0.001$) and TT3 ($\beta = -0.0273$, $p = 0.004$).

### Effect of age, race/ethnicity, smoking, and iodine sufficiency status on the levels of thyroid variables

#### Females

Those females who were aged 20–64 year had statistically significantly lower levels of TSH (1.48 μIU/ml) as compared of those who were 65+ years old (1.81 μIU/ml, Table 5). NHB had statistically significantly lower levels of TSH than HISP (1.34 μIU/ml vs. 1.60 μIU/ml, Table 5). Neither smoking nor iodine sufficiency status affected the levels of TSH.

Those who were 20–64 years old had statistically significantly higher levels of FT3 (3.09 pg/ml) as compared of those who were 65+ years old (2.98 pg/ml, Table 5). Also, among 20 years and older, HISP had statistically significantly higher levels of FT3 than both NHW and NHB (3.16 pg/ml vs.

### Table 4. Regression slopes with $p$-values for continuous variables for the models for various thyroid variables for participants aged ≥ 20 years.

| Data-set | TSH | FT3 | FT4 | TT3 | TT4 | TGN | $\Sigma$PAH |
|----------|-----|-----|-----|-----|-----|-----|-------------|
|          | $\beta$ (p-value) | $\beta$ (p-value) | $\beta$ (p-value) | $\beta$ (p-value) | $\beta$ (p-value) | $\beta$ (p-value) | $\beta$ (p-value) |
| Females  | C-reactive protein | $0.0255$ (0.331) | $0.0023$ (0.721) | $0.0036$ (0.767) | $0.0188$ (0.888) | $0.0312$ (0.003) | $-0.0224$ (0.607) | $0.04675$ (0.15) |
|          | Body mass index | $-0.0006$ (0.841) | $0.0013$ (0.051) | $0.0007$ (0.29) | $0.0016$ (0.096) | $0.0016$ (0.013) | $0.0063$ (0.157) | $-0.00012$ (0.97) |
|          | Urine creatinine | $-0.0003$ (0.674) | $0 (0.472)$ (0.142) | $-0.0001$ (0.798) | $0 (0.605)$ (0.468) | $-0.0003$ (0.157) | $-0.00051$ (0.45) |
|          | Fasting time | $0.0053$ (0.116) | $0.0007$ (0.081) | $0 (0.972)$ (0.96) | $-0.0001$ (0.874) | $0.0002$ (0.864) | $-0.0009$ (0.18) | $0.00522$ (0.45) |
|          | Use of other prescription drugs | $-0.0416$ (0.333) | $-0.0119$ (0.052) | $0.0097$ (0.328) | $-0.0076$ (0.606) | $0.0191$ (0.222) | $0.0219$ (0.723) | $-0.06411$ (0.2) |
|          | Estrogen | $0.0695$ (0.411) | $0.0152$ (0.113) | $-0.0001$ (0.993) | $0.0509$ (0.014) | $0.0286$ (0.097) | $-0.1517$ (0.072) | $0.07057$ (0.46) |
|          | Menopause | $0.0906$ (0.003) | $-0.0106$ (0.006) | $-0.0007$ (0.914) | $-0.0295$ (0.022) | $-0.0055$ (0.342) | $0.0754$ (0.075) | $0.09202$ (0.03) |
| Males    | C-reactive protein | $-0.0007$ (0.982) | $0.0037$ (0.426) | $0.0119$ (0.133) | $0.0007$ (0.951) | $0.0143$ (0.169) | $-0.0003$ (0.992) | $-0.0109$ (0.7) |
|          | Body mass index | $0.0032$ (0.305) | $0.008$ (0.023) | $-0.0009$ (0.176) | $0.0011$ (0.703) | $-0.0002$ (0.097) | $0.0006$ (0.099) | $0.00172$ (0.59) |
|          | Urine creatinine | $0.0002$ (0.408) | $0 (0.109)$ (0.611) | $0.0947$ (0.641) | $0 (0.447)$ (0.76) | $-0.0002$ (0.611) | $-0.00006$ (0.0033) |
|          | Fasting time | $-0.0011$ (0.52) | $0.0007$ (0.116) | $0.0006$ (0.352) | $-0.0007$ (0.362) | $-0.0007$ (0.424) | $0.0003$ (0.913) | $-0.0033$ (0.85) |
|          | Use of other prescription drugs | $0.0023$ (0.958) | $-0.0201$ (0) | $0.015$ (0.058) | $-0.0273$ (0.004) | $0.012$ (0.434) | $0.0963$ (0.06) | $0.00492$ (0.92) |

Note: Data from National Health and Nutrition Examination Survey 2007–2008.
Table 5. Adjusted geometric means with 95 % confidence intervals for thyroid variables for participants aged ≥ 20 years
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| Data-set | TSH | FT3 | FT4 | TT3 | TT4 | TGN |
|----------|-----|-----|-----|-----|-----|-----|
| Females  |     |     |     |     |     |     |
| 20–64 years | 1.48 | 3.09 | 0.75 | 109.99 | 7.67 | 11.64 (10.25–13.23) |
| old (A20–64) | (1.36–1.61)* | (3.05–3.14)* | (0.73–0.78) | (106.07–114.05) | (7.46–7.88) | (9.6–12.85)^ |
| 65+ years old | 1.81 | 2.98 | 0.76 | 107.4 | 7.54 | 9.54 |
| (A65+) | (1.52–2.14)* | (2.93–3.03)* | (0.74–0.79) | (102.69–112.32) | (7.29–7.81) | (7.12–12.8) |
| Non-Hispanic | 1.56 | 3.07 (3.03–3.11)^ | 0.75 | 109.71 | 7.59 (7.36–8.81) | 11.11 |
| white (NHW) | (1.39–1.75) | (0.72–0.77) | (105.75–113.81) | (7.83)^ | (9.6–12.85)^ |
| Non-Hispanic | 1.34 | 3.02 | 0.76 | 105.13 | 7.55 | 15.4 (13.24–17.92)^ |
| black (NHB) | (1.17–1.52)^ | (2.95–3.1)^ | (0.7–0.83) | (99.67–110.9)^ | (7.16–7.97)^ | (117.37)^ |
| Hispanic (HISP) | 1.61 (1.45–1.76)^ | 3.16 (3.1–3.22)^ | 0.78 | 113.18 | 8.06 (7.73–8.77) | 7.77 (7.32–8.37) |
| | (1.39–1.75)^ | (0.75–0.81) | (109.14–117.3^) | (8.41)^ | (10.51)^ |
| Non-smokers | 1.56 | 3.08 | 0.75 | 110.54 | 7.66 | 10.23 |
| | (1.42–1.71) | (3.04–3.12) | (0.73–0.78) | (107.09–114.1) | (7.48–7.84) | (9.11–11.5)^ |
| Smokers | 1.46 | 3.06 | 0.76 | 106.4 | 7.6 | 15.19 (12.12–19.04)^ |
| | (1.12–1.9) | (2.93–3.2) | (0.73–0.79) | (94.19–120.19) | (7.14–8.09) | (9.21–13.44) |
| Iodine deficient | 1.38 | 3.1 | 0.74 | 112.93 | 7.64 | 11.12 |
| | (1.19–1.6) | (3.05–3.15) | (0.72–0.77) | (108.66–117.37) | (7.38–7.91) | (9.21–13.44) |
| Iodine replete | 1.62 | 3.06 | 0.76 | 107.88 | 7.65 | 11.31 |
| | (1.46–1.79) | (3.02–3.1) | (0.73–0.79) | (103.56–112.37) | (7.42–7.88) | (9.46–13.51) |
| Males |     |     |     |     |     |     |
| 20–64 years | 1.64 | 3.3 (3.25–3.35)^ | 0.76 | 114.18 | 7.32 | 9.46 |
| old (A20–64) | (1.53–1.75) | (0.74–0.78) | (114.1–117.01)^ | (7.21–7.45) | (8.81–10.56) | (7.9) |
| 65+ years old | 1.69 | 3.05 | 0.77 | 99.73 | 7.28 | 7.9 |
| (A65+) | (1.52–1.88) | (2.98–3.12)* | (0.73–0.81) | (94.78–104.94)^ | (6.78–7.83) | (6.48–9.63) |
| Non-Hispanic | 1.73 | 3.24 | 0.77 | 111.09 | 7.25 | 9.44 (8.63–10.34)^ |
| white (NHW) | (1.62–1.85)^ | (3.19–3.29) | (0.75–0.79)* | (108.01–114.26)^ | (7.09–7.42)^ | (10.34)^ |
| Non-Hispanic | 1.26 | 3.18 (3.1–3.26)^ | 0.74 | 108.26 | 7.38 | 13.45 (10.99–16.45)^ |
| black (NHB) | (1.17–1.36)^ | (0.71–0.76)^ | (103.36–113.39)^ | (7.08–7.69) | (16.45)^ |
| Hispanic (HISP) | 1.51 | 3.42 (3.34–3.5)^ | 0.77 | 118.25 | 7.62 | 6.94 (6.12–7.87)^ |
| | (1.37–1.67)^ | (0.74–0.8) | (114.36–122.27)^ | (7.37–7.87) | (7.87)^ |
| Non-smokers | 1.69 | 3.23 | 0.76 | 109.71 | 7.28 | 8.52 |
| | (1.54–1.86) | (3.18–3.29) | (0.73–0.78) | (106.78–112.71)^ | (7.08–7.48) | (7.73–9.38)^ |
| Smokers | 1.54 | 3.32 | 0.78 | 116.61 | 7.41 | 11.47 |
| | (1.35–1.76) | (3.19–3.46) | (0.74–0.81) | (112.1–121.3)^ | (7.14–7.69) | (9.51–13.83)^ |
| Iodine deficient | 1.62 | 3.27 | 0.77 | 111.13 | 7.43 | 9.49 |
| | (1.44–1.82) | (3.2–3.34) | (0.74–0.81) | (104.41–118.29) | (7.16–7.72) | (8.28–10.86) |
| Iodine replete | 1.65 | 3.26 | 0.76 | 112.05 | 7.28 | 9.31 |
| | (1.55–1.76) | (3.21–3.3) | (0.74–0.78) | (109.98–114.16) | (7.15–7.42) | (8.37–10.36) |

*Pairs with the same group with same symbol, *, ^, ^^, or ** are statistically significantly different from each other.

Note: Data from National Health and Nutrition Examination Survey 2007–2008.

3.07 and 3.02 pg/ml). Age did not affect the levels of TT3 but among those aged 20 years and older, HISP had statistically significantly higher levels than NHB (113.18 ng/dl vs. 105.13 ng/dl). Neither smoking nor iodine sufficiency status affected the levels of FT3 or TT3.

Levels of FT4 were not affected by age, race/ethnicity, smoking, or iodine sufficiency status. Age, smoking, or iodine sufficiency status did not affect the levels of TT4. However, among those aged...
For 20 years and older males, the order of TSH levels by race/ethnicity was in the order: NHW (1.73 μIU/l) > HISP (1.51 μIU/l) > NHB (1.26 μIU/l) and all pairwise differences were statistically significantly different. Those who were 20–64 years old had statistically significantly higher FT3 (3.30 pg/ml vs. 3.05 pg/ml) and TT3 (114.18 ng/dl vs. 99.73 ng/dl) levels than those who were 65+ years old. Both NHW and NHB had statistically significantly lower levels of FT3 than HISP (3.24 and 3.18 pg/ml vs. 3.42 pg/ml). The same was true for TT3 (111.09 and 108.26 ng/dl vs. 118.25 ng/dl). NHW had statistically significantly higher levels of FT4 than NHB (0.77 ng/dl vs. 0.74 ng/dl). NHW had statistically significantly lower levels of TT4 than HISP (7.25 μg/dl vs. 7.62 μg/dl). The order of TGN levels by race/ethnicity was in the order: NHB (13.45 ng/ml) > NHW (9.44 ng/ml) > HISP (6.94 ng/ml) and all pairwise differences were statistically significant. Smokers had statistically significantly higher levels than non-smokers for both TT3 (116.61 ng/dl vs. 109.71 ng/dl) and TGN (11.47 ng/ml vs. 8.52 ng/ml).

Intercorrelations between individual PAH metabolites

Spearmen correlations between all nine PAH metabolites were found to be positive (supplementary Table S3). The magnitude of correlations varied from a relatively low of 0.53 for females and 0.56 for males between NAP1 and PYE1 to a high of 0.95 for females and 0.96 for males between FLU2 and FLU3. The positive correlations among PAH metabolites are reflected in all positive associations that PAH had with FT3 and FT4 for females (Table 3) and all negative associations that PAH had with TSH for females. However, when pairwise correlation between two PAHs was relatively low, their association with thyroid variables were found to be in opposite directions even though statistical significance may be missing in one or both circumstances. For example, the correlation between NAP1 and NAP2 for females was 0.61 and the association of NAP2 with TT3 was positive (β = 0.046) but the association of NAP1 with TT3 was negative (β = −0.0043). In general, lower the correlation between a pair of PAH metabolites, greater is the possibility that they will have associations with one or the other thyroid variable in opposite directions.

Discussion

The main focus of this communication was to determine the association between thyroid variables and PAH exposure adjusted for the differential contribution of age, race/ethnicity, smoking status, and other variables like CRP, BMI, and fasting times. In this process, geometric means adjusted for other variables in the model for different thyroid variables were also generated. These adjusted means have been generated previously also by quite a few authors, for example, by Jain (2013b) and Jain (2014). Results generated here are quite similar to the results generated in these publications in spite of the differential focus of these previous publications. Briefly, the order in which various thyroid variables were observed by race/ethnicity among those aged 20 years and over was: TSH: NHW > HISP > NHB; FT4: HISP or NHW > NHB; FT3: HISP > NHW or NHB; TT4: HISP > NHW or NHB; TT3: HISP > NHW > NHB; TGN: NHB > NHW > HISP; and the results are almost the same as in Jain (2013b). By age, the order in which FT3 and TT3 levels was observed was A20–64 > A65+. These results are also the same as observed by Jain (2013b). These differences based on age and race/ethnicity can be explained by the life style differences and how thyroid hormones are metabolized by various age and race/ethnic groups. It should be noted that there are racial/ethnic differences in the levels of PAH metabolites also (Suwan-ampai et al. 2009). It should be noted that here that the racial/
ethnic differences among thyroid variables observed here are also affected by the racial/ethnic differences in exposure to PAHs.

The order in which TSH and TT4 levels were observed by smoking status was non-smoker > smoker, and the order in which FT3, TT3, and TGN levels by smoking status were observed was smoker > non-smoker. These results are exactly the same as reported by Jain (2013b). It is possible that smoking may inhibit (or induce) certain enzymes that may decelerate (or accelerate) clearance of different thyroid hormones. Constituents such as PAH in smoking can induce CYP1A2 by binding to AhR (Jain & Wang 2011). As suggested by Petersen et al. (2006), smoking can induce CYP1A2 which can result in enhanced elimination of certain chemicals. Jain and Wang (2011) showed that smoking was associated with lower serum concentration of polychlorinated dibenzo-p-dioxins and furans possibly because of enhanced elimination induced by CYP1A2. Thus, it may be possible that induction of CYP1A2 by constituents in tobacco smoke may be possible in lower levels associated with smoking for TSH and TT4. Then, why the reverse was observed for FT3, TT3, and TGN will require additional explanations.

Higher levels of almost all PAH metabolites have been associated with smoking (Suwan-ampai et al. 2009). Since PAH was used as an independent variable in this study, there may be some kind of interactive, possibly contradicting and interacting effect of smoking on the clearance of PAH and thyroid hormones which may explain the opposite effect of smoking on TSH and TT4 vs. FT3, TT3, and TGN.

According to the results reported by Jain (2013b), those who were iodine deficient had statistically significantly higher levels of TSH, TT3, TT4, and TGN. These results could not be replicated in this study. This may be due to the differences in two study designs. Jain (2013b) analyzed data for males and females as well as all age groups together. In this study, data were analyzed separately for males, females, and for only those who were aged 20 years and older.

In this study, for 20+ years old females, relatively higher levels of NAP2, PHE2, and PYE1 (Table 3) were found to be associated with elevated levels of TT3. FT3 levels were also found to be elevated with increased levels of NAP1 and PYE1. TSH levels were also not found to be elevated with increased levels of any PAHs studied here. In fact, levels of TSH were found to be negatively associated with increased levels of PAHs though statistical significance was reached for FLU2 only ($p = 0.043$, Table 3). It is entirely possible that selected PAHs may be selectively affecting TT3 levels in females via a mechanism not yet known. It may be beneficial to study the effect of PAHs on TT3 and other thyroid hormones in laboratory using animal models among females.

While no association was found between the levels of PAHs and FT4 for females; for males 20 years and older, the levels of FT4 were found to statistically significantly decrease with increase in the levels of PHE1, PHE2, and FLU9 (Table 3). TT4 levels were also found to be statistically significantly reduced with increased levels of NAP2, PHE1, PHE2, PHE3, and FLU9 (Table 3). These results seem to be in accordance with enhanced biliary elimination of FT4 in the presence of exposure to PAHs as has been suggested in animal models (Newman & Moon 1967; Newman et al. 1971; Bastomsky and Papapetrou 1973). In general, low levels of FT4 should be of concern since the levels of FT4 below the lower limit of the normal range or below 0.7 ng/dl if observed in combination with elevated levels of TSH above the upper limit of the normal range or above 6.0 μU/ml are indicative of hypothyroidism.

Hayakawa et al. (2007) showed three-ring FLU2 and PHE2 to have some though not strong estrogenic activity. The statistically significant association of TSH with FLU2 and of TT3 with PHE2 for females as observed in this study seems to follow the results provided by Hayakawa et al. (2007). The same is true of the statistically significant association of PHE2 with FT4 and TT4 for males as observed for this study. Hayakawa et al. (2007) also showed four-ring PYE1 to have some though not strong estrogenic activity. Statistically significant association of PYE1 with FT3 and TT3 for females as seen in this study seems to be in support of the observations made by Hayakawa et al. (2007). It should, however, be realized that the levels of PAHs observed in this study may not have been high enough to exhibit strong estrogenic activity.

Exposure to PAHs for most people occur on a regular basis through air, water, soil, and food sources and routes of exposure include ingestion, inhalation, and dermal contact in both occupational and non-occupational setting (www.atsdr.cdc.gov/csem/csem.asp?csem=13&po=6). The
background levels of 17 priority PAHs in ambient air are reported to be 0.02–1.2 ng/m³ in rural areas and 0.15–19.3 ng/m³ in urban areas (www.atsdr.cdc.gov/csem/csem.asp?csem=13&po=6) and both environmental and side-stream smoke contain a variety of PAHs. Soil contains measurable amounts of PAHs primarily from airborne fallout and PAHs can leak from soil to water. About 70 % of PAH exposure for non-smokers can be associated with diet (Skupinska et al. 2004). However, people are always exposed to a mixture of PAHs, rather than single PAH (www.toxipedia.prg/display/toxiepdia/Polycyclic+Aromatic+Hydrocarbons). Selected metabolites of naphthalene, fluorene, phenanthrene, and pyrene were found to affect selected thyroid function variables in this study. Using data from NHANES 2001–2002, Shin et al. (2013) suggested (i) indoor exposure as the primary route of exposure to naphthalene, fluorene, and phenanthrene, (ii) benzo[al]pyrene exposure to be mainly from food ingestion, and (iii) multiple routes of exposure for pyrene.

Levels of exposure to PAHs, particularly in occupational settings, may differ between industrial settings and between countries as has been shown by Olsson et al. (2010) in a multicenter study conducted in seven European countries. It should be expected that the observed levels of PAHs may affect the magnitude of association between thyroid function variables and PAH. Consequently, the results presented in Tables 3–5 in this study may be affected if this study was to be conducted data obtained from countries other than USA and mix of industrial and other settings in which participants work.

While more work needs to be done to determine the mechanism by which PAHs may influence thyroid function; it does seem to be evident that elevated levels of PAHs are associated with the levels of thyroid hormones in males and females in different manners. Among females, TT3 levels seem to be primarily affected and among males, FT4 and TT4 levels seem to be primarily affected.

As hypothesized, for females, elevated levels of TT3 were associated with increasing levels of NAP2, PHE2, and PYE1 (Table 3). Also, for males, as hypothesized, the levels of FT4 decreased with increasing levels of PHE1, PHE2, and FLU9 (Table 3) and the levels of TT4 also decreased with increasing levels of PHE1, PHE2, PHE3, and FLU9.

In summary, (i) for females aged 20 years and over, elevated levels of TT3 are associated with increase in the levels of selected PAH metabolites, namely, 2-hydroxynaphthalene, 3-hydroxyphenanthrene, and 1-hydroxyphenanthrene without corresponding changes, if any, in the levels of FT4 and TT4, and (ii) for males aged 20 years and older, the levels of FT4 decreased with increasing levels of 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, and 9-hydroxyfluorene. Thus, in general, PAH exposure is associated with increased levels of TT3 among adult females and decreased levels of FT4 and TT4 among adult males.

Cross-sectional nature of the data used in this study limits the confidence that can be placed in the results obtained from this analysis. Neither the magnitude nor the source of exposure to PAH was available. The timing of the exposure was also not available. A relatively long-term follow-up study that can keep track of the ongoing PAH exposure and observed urinary PAH metabolite levels may better be able to evaluate association between PAH exposure and thyroid function. Thus, the limitations of the conclusions drawn based on a cross-sectional study design are obvious. Without these conclusions being verified by a longitudinal study, all judgments must be reserved. However, a longitudinal study of the size and scale of NHANES will be prohibitively expensive and may not even be possible. The stability or instability of statistical estimates is reflected in the standard errors associated with the estimates. If standard errors are not provided in the results, width of the confidence intervals can be looked at since the standard errors are used to compute confidence intervals. Larger the widths of the confidence intervals, less stable the statistical estimates are considered to be. Large confidence intervals may preclude reaching the appropriate conclusions, for example, when comparing geometric means for two groups.

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All data used in this research are available free of charge from www.cdc.gov/nchs/nhanes.htm.
Disclosure statement

No potential conflict of interest was reported by the author.

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