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# Polycyclic aromatic hydrocarbon biomarkers and serum markers of inflammation. A positive association that is more evident in men

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## ABSTRACT

**Background:** Polycyclic aromatic hydrocarbons (PAHs) are potent atmospheric pollutants, occurring from anthropogenic and natural sources. Several animal studies have reported a positive association of PAHs with inflammation. However, it is not clear if lower background exposure to PAHs is associated with inflammation in humans, independent of smoking, a major source of PAHs.

**Methods:** We examined participants from the National Health and Nutrition Examination Survey 2001–2002, 2003–2004, and 2005–2006. Our exposures of interest were eight urinary monohydroxy polycyclic aromatic hydrocarbon biomarkers. Our outcomes were serum markers of inflammation; C-reactive protein (CRP) ( $\leq 10$  mg/L) and total white blood cell (WBC) count (4000–12,000 cells/ $\mu$ L).

**Results:** Compared to participants with summed biomarkers of low-molecular weight (LMW) PAHs in the lowest quartile, the multivariable odds ratios (95% confidence interval) of high serum CRP ( $\geq 3$  mg/L) and high total WBC count (defined as at or above the 95 percentile of total WBC distribution) among participants in the highest exposure quartile were 1.77 (1.13, 2.76) and 1.34 (1.12, 1.60) respectively. Urinary 1-hydroxypyrene, the biomarker of the higher molecular weight pyrene, was positively associated with total WBC count, and to lesser extent with serum CRP. In subsequent analyses, the positive association between LMW PAHs and serum CRP and total WBC count was found to be present within the stratified subgroups, independent of smoking and other potential confounders. The positive association was more evident among adult males when compared to females.

**Conclusions:** Urinary PAH biomarkers were found to be positively associated with serum CRP and total WBC count independent of smoking and other potential confounders. The association was more evident in men.

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## 1. Introduction

Systemic inflammation is considered a key risk factor for atherosclerosis and subsequent development of cardiovascular disease (CVD) (Tracy, 1998). Several studies have reported a positive association between baseline elevations of C-reactive protein (CRP), a serum inflammatory marker, and future risk of CVD (Ridker et al., 1997; Ridker et al., 1998). Clinical and public health groups have recommended serum CRP levels to be used as a CVD risk stratifying tool (Yeboah, 2012). In addition, elevations in total white blood cells (WBC) count within the normal range

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(4000–12,000 cells/ $\mu$ L) were found to be independently associated with increased risk of CVD and have been proposed as an alternate serum inflammatory marker (Kannel et al., 1992).

Polycyclic aromatic hydrocarbons (PAHs) are potent atmospheric pollutants composed of fused aromatic rings (Talaska et al., 1996; Angerer et al., 1997; Warshawsky, 1999). PAHs may occur in oil, coal, and tar deposits, and are produced as byproducts of indoor and outdoor fuel burning (Liu et al., 2008; Achten and Hofmann, 2009). PAHs can be also found in contaminated water and in food as a result of food processing, preparation, and cooking (Ramesh et al., 2004; Šimko, 2005). Further, exposure to PAHs is markedly increased by cigarette smoking. Several in-vitro and animal studies have reported a positive association between exposure to PAHs and systemic inflammation (Albert et al., 1977; Penn et al., 1981; Curfs et al., 2005; Jeng et al., 2011). However, it is not clear if the lower background exposure to PAHs is associated with inflammatory effects in humans in the general population.

In this context, we examined the association of eight urinary biomarkers of PAHs, the monohydroxy-PAHs (OH-PAH), with serum CRP and total WBC count in a nationally representative sample of United States (US) adults. Since exposure to active smoking and second hand cigarettes smoke are major sources of PAHs, we sought to determine if this relationship was independent of serum cotinine, self-reported cigarettes smoking status, and other potential confounders.

Due to the multiple sources of PAHs in the environment, exposure to a single PAH compound is implausible. Metabolism, and consequently health effects of exposure to multiple PAHs were found to be different from that of exposure to an individual PAH compound (Olatubi, 2005). Enzyme competition was evident in the metabolism of PAH mixtures, changing significantly the metabolism patterns from that of individual PAHs (Olatubi, 2005). Therefore in the current study, and similar to analytical strategies employed by previous authors (Xia et al., 2009), we created a summed variable as a measure of cumulative exposure to multiple low molecular weight PAHs simultaneously.

## 2. Methods

### 2.1. Study population

The present study is based on merged data from the 2001–2002, 2003–2004 and 2005–2006 National Health and Nutrition Examination Survey (NHANES). Detailed description of NHANES study design and methods are available elsewhere (Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS) 2001). NHANES included a stratified multistage probability sample representative of the civilian non-institutionalized US population. Selection was based on counties, blocks, households and individuals within households, and included oversampling of non-Hispanic Blacks and Mexican Americans in order to provide stable estimates of these groups. Out of 31,509 participants in NHANES 2001–2006, there were 11,512 who were 20–65 years of age. Urinary PAH biomarkers were only measured in a subsample of individuals. The subsample is nationally representative, but with a smaller analytic sample size.

We excluded participants with missing information on serum CRP or with CRP levels > 10 mg/L, indicating potential underlying non-cardiovascular causes of inflammation (Pearson et al., 2003). We further excluded participants with missing information on serum cotinine level, or other covariates included in the final CRP model. Similarly, to minimize the confounding effect of infection, only subjects with a WBC count within the normal range (4000–12,000 cells/ $\mu$ L) were included in the final WBC analysis. We also excluded participants with missing information on total WBC count, or other covariates included in the final model.

### 2.2. Main outcome of interest: serum inflammatory markers

#### 2.2.1. High sensitivity serum C-reactive protein

Serum CRP was measured using latex-enhanced nephelometry. Details of the laboratory collection, processing, and analysis are available in the laboratory procedures manual (Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS) 2001). High CRP level was defined as values  $\geq 3$  mg/dL, consistent with American Heart Association/Centers for Disease Control & Prevention (AHA/CDC) guidelines for identifying subjects with high risk of CVD (Pearson et al., 2003).

#### 2.2.2. Total white blood cell count within normal values

The methods used to derive WBC count are based on the Beckman Coulter method of counting. High WBC count was defined as values at or above the 95th percentile of the total WBC count distribution, consistent with previous studies examining the association between total WBC count within normal ranges and CVD risk (Kannel, 1992; Twig et al., 2012).

### 2.3. Main exposure: urinary levels of monohydroxy-PAH

Urine specimens collected during the clinical exam portion of the survey were processed, stored, and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for analysis. The specific analytes measured in this study were monohydroxy-PAH (OH-PAH). By evaluating these analytes in urine, a measurement of the body burden from PAH exposure is obtained (Castano-Vinyals et al., 2004). The procedure involves enzymatic hydrolysis of urine, extraction,

derivatization and analysis using capillary gas chromatography combined with high resolution mass spectrometry (GC-HRMS). Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM).

Seven LMW PAH urinary biomarkers, naphthalene biomarkers; 1-hydroxy-naphthalene, 2-hydroxynaphthalene, fluorene biomarkers; 2-hydroxyfluorene, 3-hydroxyfluorene, phenanthrene biomarkers; 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene and 1-hydroxypyrene, the biomarker of the higher molecular weight PAH pyrene, were consistently available in NHANES 2001–2006. All analytes were measured in the same unit; ng/L. Urinary OH-PAH were corrected for urinary creatinine concentration, a urinary marker of kidney function to adjust for urinary dilution (Barr et al., 2005). Urinary levels of OH-PAH (ng/L) were divided by urinary creatinine level (mg/dL) multiplied by 0.01; [(ng/L)/(mg/dL  $\times$  0.01)] and expressed as nanogram per gram of creatinine (ng/g creatinine).

### 2.4. Exposure measurements

Information on age, gender, race/ethnicity, alcohol intake, income, diabetes and cigarette smoking were obtained from a standardized questionnaire during a home interview. Alcohol consumption was categorized into none and alcohol drinker. Income-poverty ratio (income/poverty guideline) was used as a measure of the socioeconomic status. The Department of Health and Human Services' poverty guidelines were used as the poverty measure to calculate this ratio. Cigarettes smoking status was categorized into never smokers (smoked < 100 cigarettes during their lifetime), former smokers (smoked  $\geq 100$  cigarettes during their lifetime and currently not smoking), current smokers (smoked  $\geq 100$  cigarettes during their lifetime and currently smoking). Information on anthropometric, physical and laboratory components were obtained during the medical examination center examination. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Seated blood pressure was measured using a mercury sphygmomanometer according to the American Heart Association and recommendations (Chaturvedi, 2004). Up to 3 measurements were averaged for blood pressure.

### 2.5. Statistical analysis

Urinary levels of OH-PAH were analyzed both as continuous as well as categorical variables. For analysis as continuous variables, urinary OH-PAH levels were log-transformed as a result of their skewed distribution. Weighted Pearson correlation coefficients between individual OH-PAH were calculated to evaluate the correlations between pairwise combinations of all eight urinary metabolites. We created a summed LMW PAH biomarkers variable by summing urinary levels of metabolites of the low molecular weight PAHs (naphthalene, fluorene and phenanthrene).

We ran linear regression models to calculate the multivariable change and 95% confidence interval (CI) in serum CRP and total WBC count with increasing individual and additive urinary OH-PAH levels. In addition, we ran logistic regression models to calculate the multivariable odds ratio (OR) and 95% CI of high serum CRP ( $\geq 3$  mg/L) and total WBC count in the 95th percentile, for each higher urinary OH-PAH quartile by using the lowest quartile as the referent. Variables were included in the model if they satisfied a plausible association with the main exposure/outcome. In addition, we used stepwise adjustment to identify the variables that could predict changes in serum CRP as well as total WBC count. Inclusion and retention of variables were allowed at a 10% change of odds ratio after adjusting for the potential confounder. Accordingly, final models were adjusted for age (years), sex (men, women), ethnicity (non-Hispanic White, non-Hispanic Black, all others), poverty-income ratio (%), alcohol drinking (yes/no), diabetes (absent/present), BMI (normal, overweight, obese), total cholesterol (mg/dL), serum cotinine (ng/mL) and systolic blood pressure (mm Hg).

To further ensure that the association is parallel for subgroups, we performed subgroup analyses by gender, race/ethnicity, BMI and self-reported cigarettes smoking categories. Sample weights that account for the unequal probabilities of selection, oversampling, and nonresponse in the NHANES survey were applied for all analyses. Analyses were conducted using SAS (version 9.3, SAS Institute, Cary, NC) software. Standard errors were estimated using the Taylor series linearization method.

## 3. Results

Table 1 presents the baseline characteristics of the study population with CRP levels < 10 mg/L. The study population was primarily non-Hispanic white (72.9%). Approximately one-half (50.5%) were never smokers, and the remainders were former smokers (21.7%) and current cigarettes smokers (27.8%). The arithmetic mean of serum cotinine level was 74.2 ng/mL.

Tables 2 and 3 present the weighted percentiles and means of individual and the summed LMW urinary OH-PAH biomarkers used in the final analysis with CRP and total WBC count, respectively. Correlations between pairwise combinations of all eight urinary biomarkers (results not shown in a table), were statistically significant, with Pearson correlation coefficients that ranged from 0.41 to 0.93.

Table 4 presents the results of the linear regression analyses measuring the association between urinary OH-PAH levels, serum CRP and total WBC count. Urinary levels of OH-PAH were positively associated with serum CRP levels independent of potential confounders. All the associations were statistically significant except for 1-hydroxynaphthalene, 3-hydroxyphenanthrene and 1-hydroxypyrene. Similarly urinary levels of OH-PAH were positively associated with total WBC count independent of potential confounders. The results of our stepwise linear regression (not presented in a table) showed a positive association between serum cotinine and total WBC count [unadjusted odds ratio (95% C.I) 2.99

(1.48, 4.50)]. Serum cotinine showed less strong association with total WBC count [unadjusted odds ratio (95% C.I) 0.002(–0.001, 0.005)].

Table 5 presents the odds ratio of the association between urinary levels of OH-PAH and high CRP, defined as CRP levels  $\geq 3$  mg/L. Overall, urinary levels of OH-PAH were positively associated with high CRP in the multivariable adjusted models. Using urinary OH-PAH levels as continuous variables, the observed associations were still positive. Although positive, the associations between urinary 3-hydroxyphenanthrene and serum CRP were not statistically significant.

Table 6 presents the odds ratios of the association between urinary levels of OH-PAH and high WBC count, defined as total WBC count in the 95th percentile of the study population. Similar to the results for CRP, urinary levels of OH-PAH were positively associated with high total WBC count in the multivariable adjusted models. Using urinary OH-PAH levels as continuous variables, the observed associations were still positive. Although positive, the associations between urinary 1-hydroxyphenanthrene and 3-hydroxyphenanthrene and total WBC were not statistically significant.

Table 7 investigates several types of potential confounding. Overall, consistent with the findings for the whole cohort, we found that higher urinary levels of summed LMW PAH biomarkers were positively associated with high CRP and high total WBC count within these stratified subgroups. Compared to females, urinary biomarkers of LMW PAH showed stronger association with serum CRP and high total WBC count among males. Compared to non-smokers, summed urinary biomarkers of LMW PAH showed stronger association with high total WBC count among current cigarettes smokers. Although in the positive direction, some of the odds ratios failed to reach the conventional levels of statistical significance. P-interaction values for cross-product terms between urinary OH-PAH levels and stratifying variables were all  $> 0.15$  except for gender ( $P=0.001$ ) in the CRP analysis and ( $P=0.003$ ) in the WBC analysis.

#### 4. Discussion

In a multiethnic sample of US adults, we found that higher levels of urinary PAH biomarkers were positively associated with high serum CRP levels and total WBC count, independent of surveyed cigarettes smoking status, serum cotinine and other potential confounders. The association was stronger for the low

**Table 1**  
Baseline characteristics of the study population with measured urinary levels of OH-PAH and CRP  $\leq 10$  mg/L.

Characteristics	Mean values (Std error of mean) or sample size (weighted percentages)
Women (%)	1136 (47.7)
Age (years)	40.9 (0.34)
Race/Ethnicity (%)	
Non-Hispanic Whites	1200 (72.9)
Non-Hispanic Blacks	463 (10.0)
Others	691 (17.1)
Education categories (%)	
Less than high school	547 (14.2%)
High school	541 (24.2)
Above high school	1266 (61.6)
Below poverty level (%)	392 (12.1)
Smoking categories (%)	
Never	1229 (50.5)
Former smokers	501 (21.7)
Current smokers	624 (27.8)
Alcohol drinking (%)	
Yes	1731 (77.2)
Body mass index (%)	
Normal weight ( $< 25.0$ kg/m <sup>2</sup> )	784 (36.5)
Overweight (25.0–29.9 kg/m <sup>2</sup> )	827 (32.9)
Obese ( $\geq 30.0$ kg/m <sup>2</sup> )	743 (30.6)
Serum cotinine (ng/mL)	74.2 (4.25)
Total cholesterol (mg/dL)	201.6 (1.43)
Systolic blood pressure (mmHg)	119.1 (0.47)

**Table 2**  
Weighted percentiles of OH-PAH (ng/g creatinine) among participants included in the final analysis with serum CRP levels  $\leq 10$  mg/L.

Chemicals	Selected percentiles						
	Sample size	Mean	Minimum	25th	50th	75th	Maximum
1-Hydroxynaphthalene	2480	44,914	50.8	843.7	2009.3	7116.1	39,226,536
2-Hydroxynaphthalene	2488	6235.5	93.5	1341.8	2820.7	7947.9	404,573
2-Hydroxyfluorene	2465	654.4	2.2	143.3	247.8	751.2	29,914
3-Hydroxyfluorene	2450	339.0	1.2	52.5	97.6	407.4	19,084
1-Hydroxyphenanthrene	2469	215.31	0.7	89.3	139.7	232.7	8341.9
2-Hydroxyphenanthrene	2448	96.6	0.5	35.1	56.9	99.9	5069.4
3-Hydroxyphenanthrene	2444	191.5	0.7	55.4	91.8	176.2	20,248
1-Hydroxypyrene	2461	136.5	0.9	35.5	66.1	136.6	10,014
Summed LMW PAH biomarkers	2369	54,230	620.0	3156.6	6140.5	18,777	39,235,478

**Table 3**Weighted percentiles of OH-PAH (ng/g creatinine) among participants included in the final analysis with total WBC count between 4000 and 12,000 cells/ $\mu$ L.

Chemicals and blood markers	Selected percentiles						
	Sample size	Mean	Minimum	25th	50th	75th	Maximum
1-Hydroxynaphthalene	2620	43,340	50.8	825.2	1963.4	6878.0	39,226,536
2-Hydroxynaphthalene	2628	6156.1	9.2	1357.5	2825.1	7829.2	404,573
2-Hydroxyfluorene	2604	632.8	2.2	143.4	244.4	735.1	29,914
3-Hydroxyfluorene	2588	325.5	1.2	52.3	95.7	377.7	19,084
1-Hydroxyphenanthrene	2610	213.2	0.7	89.7	140.4	229.3	8341.9
2-Hydroxyphenanthrene	2587	96.3	0.5	35.7	58.3	101.2	5069.4
3-Hydroxyphenanthrene	2585	189.1	0.7	54.9	90.0	170.6	20,248
1-Hydroxypyrene	2600	133.9	0.9	35.4	65.4	132.0	10,014
Summed LMW PAH biomarkers	2505	52,480	508.7	6085.4	18,255	18,255	39,235,478

**Table 4**Linear regression: association between urinary OH-PAH levels (ng/g creatinine) and serum CRP (mg/L) and total WBC count (cells/ $\mu$ L).

Chemicals	Multivariable change in CRP*		Multivariable change in total WBC count*	
	Sample size	Log OH-PAH	Sample size	Log OH-PAH
1-Hydroxynaphthalene	2480	0.07(−0.01, 0.14)	2620	89.7(8.9, 170.5)
2-Hydroxynaphthalene	2488	0.14(0.04, 0.25)	2628	229.2(103.2, 355.3)
2-Hydroxyfluorene	2465	0.16(0.06, 0.26)	2604	310.1(190.1, 430.0)
3-Hydroxyfluorene	2450	0.10(0.02, 0.18)	2588	235.9(135.9, 335.9)
1-Hydroxyphenanthrene	2469	0.21(0.10, 0.33)	2610	132.4(19.5, 245.3)
2-Hydroxyphenanthrene	2448	0.19(0.01, 0.36)	2587	213.6(117.3, 309.9)
3-Hydroxyphenanthrene	2444	0.05(−0.06, 0.17)	2585	199.8(81.8, 317.7)
1-Hydroxypyrene	2461	0.11(−0.004, 0.21)	2600	144.5(53.8, 235.2)
Summed LMW PAH biomarkers	2369	0.15(0.04, 0.25)	2505	186.0(73.0, 299.0)

\* Adjusted for age (years), sex (male/female), BMI (normal weight/overweight/obese), race (non-Hispanic White/non-Hispanic Black/all others), alcohol drinking (yes/no), poverty-income ratio, total cholesterol (mg/dL), serum cotinine (ng/mL), diabetes mellitus (absent/present) and systolic blood pressure (mm Hg).

**Table 5**Logistic regression: association between urinary OH-PAH levels (ng/g creatinine) and CRP  $\geq$ 3 mg/L.

Chemicals	Sample size	Quartile 1				Log-PAH
		Multivariable odds ratio (95% confidence interval)*				
1-Hydroxynaphthalene	2480	1 (Referent)	0.94(0.69, 1.28)	1.07(0.75, 1.52)	1.53(1.01, 2.32)	1.08(0.98, 1.20)
2-Hydroxynaphthalene	2488	1 (Referent)	1.10(0.80, 1.51)	1.22(0.81, 1.82)	1.66(1.10, 2.48)	1.15(1.01, 1.32)
2-Hydroxyfluorene	2465	1 (Referent)	1.09(0.79, 1.50)	1.34(0.95, 1.89)	1.63(1.14, 2.33)	1.22(1.09, 1.37)
3-Hydroxyfluorene	2450	1 (Referent)	0.97(0.72, 1.31)	1.17(0.87, 1.59)	1.58(1.15, 2.16)	1.14(1.03, 1.25)
1-Hydroxyphenanthrene	2469	1 (Referent)	1.45(1.00, 2.12)	1.84(1.38, 2.46)	1.67(1.25, 2.22)	1.23(1.09, 1.40)
2-Hydroxyphenanthrene	2448	1 (Referent)	1.23(0.74, 2.05)	1.58(1.00, 2.51)	1.88(1.26, 2.80)	1.25(1.01, 1.54)
3-Hydroxyphenanthrene	2444	1 (Referent)	1.01(0.71, 1.43)	1.03(0.76, 1.40)	1.21(0.90, 1.64)	1.09(0.96, 1.24)
1-Hydroxypyrene	2461	1 (Referent)	1.24(0.86, 1.78)	1.41(1.09, 1.81)	1.38(0.92, 1.95)	1.14(1.01, 1.29)
Summed LMW PAH biomarkers	2369	1 (Referent)	1.09(0.73, 1.61)	1.18(0.80, 1.75)	1.77(1.13, 2.76)	1.16(1.03, 1.31)

\* Adjusted for age (years), sex (male/female), BMI (Normal weight/overweight/obese), race (non-Hispanic White/non-Hispanic Black/all others), alcohol drinking (yes/no), poverty-income ratio, total cholesterol (mg/dL), serum cotinine (ng/mL), diabetes mellitus (absent/present) and systolic blood pressure (mm Hg).

molecular weight PAH biomarkers, compared to 1-hydroxypyrene, a urinary metabolite of the higher molecular weight PAH pyrene.

Researchers have examined PAHs directly in the blood and tissues of experimental animals, and in humans. The most commonly used biomarkers of PAH exposure are urinary OH-PAH. Urinary OH-PAH has been found to correlate well with levels of exposure to PAHs in the general population (Castano-Vinyals et al., 2004). In addition, there is an evidence that the additional effect of human occupational exposures can be detected, independent of cigarette smoking (Ciarrocca et al., 2013).

Humans are usually exposed to PAHs in either a gas or particulate phase. PAHs with the lower molecular weight (naphthalene,

fluorene, and phenanthrene) are more abundant in the gas phase and are absorbed mainly through inhalation (Maliszewska-Kordybach, 1999). In contrast, PAHs with higher molecular weight (pyrene) have higher vapor pressure and are found in a particulate form (Maliszewska-Kordybach, 1999). They can be absorbed through ingestion, skin contact and inhalation (Elovaara et al., 1995; Warshawsky, 1999).

The mechanisms underlying the positive association between urinary PAH biomarkers and serum CRP and total WBC count remain unknown. Several in-vitro and animal studies have reported a positive association between exposure to PAHs and systemic inflammation (Albert, 1977; Penn, 1981; Curfs, 2005;

**Table 6**Logistic regression: association between urinary OH-PAH levels (ng/g creatinine) and high total WBC count (cells/ $\mu$ L) at or above the 95th percentile.

Chemicals	Sample size	Quartile 1 Multivariable odds ratio (95% confidence interval)*	Quartile 2	Quartile 3	Quartile 4	Log-PAH
1-Hydroxynaphthalene	2620	1 (Referent)	1.44(0.78, 2.64)	1.20(0.60, 2.39)	2.18(1.17, 4.05)	1.18(1.02, 1.37)
2-Hydroxynaphthalene	2628	1 (Referent)	0.70(0.30, 1.59)	0.98(0.49, 1.97)	2.58(1.05, 6.35)	1.33(0.87, 2.03)
2-Hydroxyfluorene	2604	1 (Referent)	1.02(0.44, 2.36)	1.63(0.86, 3.08)	3.07(1.47, 6.43)	1.50(1.17, 1.92)
3-Hydroxyfluorene	2588	1 (Referent)	1.34(0.62, 2.89)	1.90(0.93, 3.87)	4.15(1.93, 8.92)	1.42(1.14, 1.78)
1-Hydroxyphenanthrene	2610	1 (Referent)	1.04(0.51, 2.13)	1.67(0.90, 3.10)	1.52(0.77, 3.02)	1.20(0.91, 1.58)
2-Hydroxyphenanthrene	2587	1 (Referent)	1.94(0.89, 4.23)	1.45(0.69, 3.04)	2.25(1.07, 4.75)	1.32(1.03, 1.70)
3-Hydroxyphenanthrene	2585	1 (Referent)	1.55(0.85, 2.83)	1.19(0.64, 2.20)	1.86(0.92, 3.76)	1.27(0.99, 1.64)
1-Hydroxypyrene	2600	1 (Referent)	1.68(0.80, 3.55)	1.94(0.98, 3.82)	2.27(1.12, 4.61)	1.31(1.03, 1.66)
Summed LMW PAH biomarkers	2505	1 (Referent)	0.98(0.51, 1.90)	1.03(0.59, 1.80)	2.57(1.27, 5.19)	1.34(1.12, 1.60)

\* Adjusted for age (years), sex (male/female), BMI (Normal weight/overweight/obese), race (non-Hispanic White/non-Hispanic Black/all others), alcohol drinking (yes/no), poverty-income ratio, total cholesterol (mg/dL), serum cotinine (ng/mL), diabetes mellitus (absent/ present) and systolic blood pressure (mm Hg).

**Table 7**

Logistic regression: association between summed urinary biomarkers of LMW PAH (ng/g creatinine) and serum inflammatory markers by sociodemographic characteristics.

Subgroups	Multivariable odds ratio of CRP $\geq 3$ mg/L*	P interaction	Multivariable odds ratio of total WBC count in the 95th percentile*	p-interaction
Gender		0.001		0.003
Female	1.08(0.93, 1.25)		1.21(0.95, 1.55)	
Male	1.32(1.12, 1.55)		1.90(1.41, 2.56)	
Race-ethnicity		0.29		0.29
Non-Hispanic white	1.16(1.00, 1.34)		1.34(1.10, 1.64)	
All others	1.18(0.98, 1.41)		1.27(0.92, 1.74)	
Body mass index		0.55		0.71
Normal weight	1.32(0.98, 1.76)		1.41(1.03, 1.93)	
Overweight	1.11(0.89, 1.38)		1.38(0.98, 1.94)	
Obese	1.09(0.93, 1.28)		1.28(1.00, 1.65)	
Smoking		0.27		0.35
Never smokers	1.21(0.98, 1.50)		1.21(0.88, 1.68)	
Former smokers	1.03(0.88, 1.19)		1.15(0.83, 1.59)	
Current smokers	1.10(0.81, 1.49)		1.78(1.28, 2.48)	

\* Adjusted for age (years), sex (male/female), ethnicity (non-Hispanic White/non-Hispanic Black/all others), poverty-income ratio, alcohol drinking (yes/no), diabetes (absent/present), BMI (normal/overweight/obese), total cholesterol (mg/dL), serum cotinine (ng/mL) and systolic blood pressure (mm Hg), except for stratified variables.

Jeng, et al., 2011). Upon exposure to PAHs, detoxification occurs, leading to the formation of highly reactive intermediates that can interact with the DNA, forming PAH–DNA plaques in animal arteries (Curfs et al., 2005; Jeng, 2011). Several studies have suggested that PAHs might exert this atherogenic effect via stimulation of an inflammatory process involving an increased influx of proinflammatory cells into these plaques (Curfs, 2005).

Stratifying by gender, the positive association between urinary LMW PAH biomarkers and serum CRP  $\geq 3$  mg/L and total WBC above the 95th percentile was found to be stronger in males when compared to females, suggesting possible gender differences. Different gender-specific effects of environmental pollutants with outcomes such as inflammatory markers have been previously

reported by others (Künzli et al., 2005). Hoffman et al. have reported a positive association between high levels of urban air pollution and systemic inflammatory markers in men (Hoffmann et al., 2009). Different sources and patterns of exposure could explain the reported gender differences. In addition, endogenous estrogen and post-menopausal hormone replacement therapy (Störk, 2008) have been shown to alter plasma levels of a variety of cytokines and inflammatory markers, possibly contributing to the observed effect modification (Wong, 2008). It is also possible that hormonal differences in the way in which men and women metabolize PAHs may explain this observation. PAHs are known to be metabolized by cytochrome P450 (Kleiner et al., 2004). It has been shown that women have increased lung expression of CYP enzymes compared with men which is related to estrogen (Mollerup et al., 1999; Han et al., 2005). Consequently, accelerated breakdown of PAHs in the lungs may potentiate the pulmonary response to PAHs in females while reducing the circulating PAH concentrations (Van Winkle et al., 2002).

Only two human studies have investigated the association between PAH exposure and serum inflammatory markers, and the results were inconsistent. In a study of 999 participants using NHANES 2003–2004, higher exposure to PAHs was associated with elevated levels of serum CRP (Everett et al., 2010). However, in a study of participants in NHANES 2001–2004, urinary levels of OH-PAH were not associated with other serum inflammatory markers such as total WBC count (Clark III et al., 2012). The differences in the results may be partly due to the fact that some NHANES data on PAHs were withdrawn (and re-released) due to inconsistencies in the laboratory methods used in 2001–2002 and 2003–2004 data cycles. The re-release of the data provides an important opportunity for updated analysis. In addition, previous studies included participants with CRP levels of higher than 10 and total WBC count higher than 12,000 cells/ $\mu$ L, where infection or autoimmune diseases might have confounding effects. Also adjustment for major confounders known to interact with inflammatory markers such as alcohol drinking and total cholesterol and socio-economic status, and stratifying by major characteristics of the study population were not performed in previous studies.

The current study used merged data from NHANES 2001–2002, 2003–2004 and 2005–2006. The strengths of the study include the relatively large multiethnic sample of the US adults, the high quality of NHANES data due to standardized data collection and the ability to adjust for potential confounders and to stratify by major sociodemographic characteristics. In addition, we investigated the association between PAH exposure and serum CRP and total WBC count, independent of the health effects of cigarettes smoking, by adjusting for serum cotinine, an objective measure of cigarette smoke exposure in addition to stratifying by self-reported

smoking status. Cotinine is the principal metabolite of nicotine. (Benowitz et al., 1983) Serum cotinine is considered a more precise measure of exposure to cigarette smoking when compared to self-reported smoking status. (Perezstable et al., 1995; Gorber et al., 2009) and is considered an accurate biomarker of second-hand smoke exposure (Benowitz, 1996).

The study has limitations as well. The cross-sectional nature of NHANES does not allow us to draw temporal or causal inferences regarding the relationship between PAHs and serum inflammatory markers. Urinary PAH biomarker measurements reflect recent exposure to PAHs and do not reflect differences between the current exposure sources and the past exposure sources for each subject. Due to its short half-life, serum cotinine also reflects recent exposure to tobacco smoke. However, these biases are likely to be non-differential biases, which would minimize any associations observed.

In conclusion, lower background exposure to PAHs was found to be positively associated with serum markers of systemic inflammation i.e. serum CRP and total WBC count independent of potential confounders. The association was more evident in adult males when compared to females. Active cigarettes smoking appears to play a significant role in the association of urinary OH-PAH and total WBC count, yet the association persists after adjustment for serum cotinine and self-reported smoking status. There is a need to replicate these findings in future prospective studies with adequate sample size

## Contributors

All the authors contributed to the intellectual development of this paper. OA wrote the paper and performed the statistical analyses. MEA and MZ provided statistical expertise and were involved in critical review and revision of the manuscript. AD and BC was involved in critical corrections of the paper.

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