

## Article

# Association between Polycyclic Aromatic Hydrocarbon Exposure and Diarrhea in Adults

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**Abstract:** Objective: Polycyclic aromatic hydrocarbons (PAHs) are not only natural but also anthropogenic contaminants that exist in many places in the environment. Human beings often accidentally ingest PAHs via smoking. Furthermore, smoking may increase the risk of bowel disorder, including diarrhea and other gastrointestinal problems. Therefore, PAH exposure is hypothesized to be related to diarrhea risk. This study discusses the association between diarrhea and PAH exposure in the United States adult population. Method: 10,537 participants from the National Health and Nutrition Examination Survey (NHANES 2001–2006) were involved in this cross-sectional analysis. Bowel disorders were assessed via examination of stool frequency and stool type. The concentrations of urinary PAH metabolites were used to evaluate PAH exposure. The association between bowel habits and PAH exposure was assessed using a multivariate logistic regression model with covariate assessment of gender, age, race, liver function, kidney function, and common chronic health diseases. Results: All PAH metabolites except 1-hydroxynaphthalene, 1-hydroxypyrene, and 9-hydroxyfluorene were substantially correlated with an increased risk of diarrhea ( $p < 0.05$ ) after modification of relevant covariables. This study also revealed significant association in the group of females ( $p < 0.05$ ). Furthermore, all PAH metabolites except 1-hydroxynaphthalene, 2-hydroxyphenanthrene, 1-hydroxypyrene, and 9-hydroxyfluorene show significantly positive association in the non-obesity group (BMI  $< 30$ ,  $p < 0.05$ ). Conclusions: PAH exposure is highly associated with risk of bowel disorders among the adult population in the United States, especially in female and non-obesity populations. More research is necessary to shed light on the pathophysiological mechanisms associated to PAH exposure and diarrhea.

**Keywords:** polycyclic aromatic hydrocarbons; diarrhea; bowel health

## 1. Introduction

PAHs are found in populations worldwide mainly due to long-term human exposure to environmental pollution. The intrinsic characteristics of PAHs, such as aromatic ring constructions, hydrophobicity, and thermostability, have made them prolonged and constant in the environment [1]. PAHs are universal environmental toxins primarily generated by the incomplete burning of organic substances (e.g., oil, grease, gas, and wood) [2]. Combustion of petroleum and other fossil fuels are the main and typical sources of PAHs (especially two-ring PAHs) [3,4]. PHAs damage human health and cause many kinds of systematic diseases, such as cardiovascular diseases [5] and carcinogenic diseases [6]. Furthermore, it

is considered that the more aromatic rings PAHs have, the more toxic they are. Therefore, more attention should be paid to PAHs [3]. The PAH with the most aromatic rings in this study is 1-hydroxypyrene (4 rings).

Diarrhea is a critical public health problem in many areas globally, especially in places where poverty prevails [7]. One of the causes of diarrhea is related to the maintenance of gut microbiome [8]. Smoking causes alterations to the oral and gastrointestinal microbiome, leading to a variety of diseases, such as periodontal disease, chronic obstructive pulmonary disease, ulcerative colitis, asthma, Crohn's disease, and cancers [9]. Since benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH) of environmental pollutants, is one of the main ingredients in cigarettes and an agonist of the aryl hydrocarbon receptor (AhR) [10], cigarette smoking easily affects the gut microbiome via PAHs intake, which may lead to the damage of bowel health, including diarrhea. Furthermore, users of cigarette products had higher urinary PAH biomarker concentrations compared to never users [11]. Therefore, it is critical to know whether smoking is related to an increased risk of diarrhea due to a higher level of PAHs or not.

To date, no study has focused on the relationship between diarrhea and PAH exposure. Therefore, this study investigated the relationship between urinary PAH metabolite and bowel health in a cross-sectional analysis of a nation-wide general adult sample in the United States.

## 2. Materials and Methods

### 2.1. Study Design

All characteristic databases were attained from the National Health and Nutrition Examination Survey (NHANES), a program of studies designed to evaluate the health status of adults in the United States. It is a cross-sectional survey on a nationally representative sample that collects demographic, clinical, behavioral, dietary, social, and laboratory data about the health and nutritional status of non-institutionalized individuals in the United States. All participants were requested to sign an informed consent form before the initiation of the research. Adults aged  $\geq 20$  years from the NHANES 2001–2006 were involved in our study. After eliminating those with lost data, such as demographic and laboratory information, 10,537 eligible applicants were selected for further analysis.

### 2.2. Study Sample Characteristics

5225 males and 5312 females were enrolled in our study. Their demographic and laboratory data are shown in Table 1. The mean age was 32.60 years. The study population characteristic data are presented in Table 1. Each gender of the population was almost equal (male:female = 49.6%:50.4%). In addition, the prevalence of lifelong cigarette smoking was only slightly lower than non-smoker prevalence.

### 2.3. Urinary Polycyclic Aromatic Hydrocarbon Analysis

Oxygenated PAHs (oxy-PAHs) are an important group of polar PAHs that have carcinogenic effects without enzymatic stimulation. A rise in reactive oxygen species (ROS) is also triggered by them in living cells, which generates oxidative stress and other effects on cells, such as abnormal gene expressions. The outcomes include altered protein behaviors, carcinogenesis, and mutagenesis [12]. Urinary PAH data were accumulated to measure PAH content in the human body. Experienced technicians collected spot urine samples and kept them at a temperature of  $-20^{\circ}\text{C}$  storage, which was based on the laboratory protocols. High-resolution mass spectrometry combined with capillary gas chromatography was presented for urinary metabolite quantification. The limit of detection for 1-hydroxynaphthalene is 33.9 (ng/L); for 2-hydroxynaphthalene, it is 9.3 (ng/L); for 3-hydroxyfluorene, it is 3.5 (ng/L); for 2-hydroxyfluorene, it is 6.6 (ng/L); for 3-hydroxyphenanthrene, it is 3.5 (ng/L); for 1-hydroxyphenanthrene, it is 3.5 (ng/L); for 2-hydroxyphenanthrene, it is 3.5 (ng/L); for 1-hydroxypyrene, it is 3.5 (ng/L); and for 9-hydroxyfluorene, it is 9.3 (ng/L). In the present study, where the result was below the

limit of detection, the value for that variable is deleted. The comprehensive procedure is depicted on the internet site of NHANES (CDC 2012) [13].

**Table 1.** Study sample characteristics.

Variables	Number	Mean (S.D.) or Percentage
Age	10,537	32.60 (24.91)
Male	5225	32.24 (24.94) or 49.6%
Young (<65 year old)	4526	86.6%
Elderly ( $\geq 65$ year old)	699	13.4%
Female	5312	32.95 (24.87) or 50.4%
Young (<65 year old)	4581	86.2%
Elderly ( $\geq 65$ year old)	731	13.8%
Laboratory Data Creatinine	6860	0.88 (0.45)
ALT	6859	24.89 (17.84)
Glucose	6860	99.07 (35.17)
Race		
Mexican American	2384	22.6%
Other Hispanic	1133	10.8%
Non-Hispanic White	4420	41.9%
Non-Hispanic Black	1957	18.6%
Other Race—Including Multi-Racial	643	6.1%
BMI		
Male	4673	25.52 (7.12)
Female	4739	26.32 (8.28)
Congestive Heart Failure		
Yes	174	2.8%
No	6025	96.9%
Coronary Heart Disease		
Yes	254	4.1%
No	5936	95.5%
Myocardial Infarction History		
Yes	261	4.2%
No	5940	95.5%
Thyroid Problem		
Yes	608	9.8%
No	5597	90.0%
Smoking History		
Yes	2866	46.1%
No	3352	53.9%

#### 2.4. Diarrhea Assessment

Diarrhea is an alteration in normal bowel movement, characterized by increased frequency, volume, and water content of stools. It is defined as an increase in stool frequency to three or more liquid or semi-formed motions in a day [14]. The data were gained from the NHANES: the Bowel Health (BHQ\_F) section, which was administered in the Mobile Examination Center (MEC) during the MEC Interview by trained interviewers using a computer-assisted personal interviewing (CAPI) system. Hand cards showing response categories were used for some questions. Details regarding the procedure are available on the NHANES website.

#### 2.5. Covariate Assessment

The questionnaire data, including gender, age, race/ethnicity, congestive heart failure, myocardial infarction, coronary heart disease, thyroid problem and smoking history, were collected by skilled examiners utilizing computer-program-assisted individual questioning methodology. Race/ethnicity was classified into Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and others. Patients with medical histories were defined as participants who had been diagnosed with congestive heart failure (CHF), my-

ocardial infarction, coronary heart disease (CHD), and thyroid problems. Smoking history was defined as smoking at least 100 cigarettes in the participant's entire life. Obesity was defined as BMI level > 30. Laboratory data were collected using the respective standard procedures. Serum creatinine (Cr), ALT, and glucose levels were assessed using the requirements of a multi-rule quality control system using Beckman Synchron LX20 for each analyte. All of the data were available on the NHANES data list.

## 2.6. Statistical Analysis

A *p*-value less than 0.05 (typically  $\leq 0.05$ ) is defined as statistically significant. Analyzed models were adjusted with multivariable covariates as follows: Model 1 was unadjusted; Model 2 adjusted for gender, age, and race/ethnicity; Model 3 additionally adjusted for creatinine, ALT, and glucose; and Model 4 further adjusted for congestive heart failure, coronary heart disease, myocardial infarction, thyroid problem, and smoking history. Multivariate logistic regression analysis was used to analyze the relationship between PAHs and diarrhea. The Statistical Package for Social Sciences (IBM SPSS) 22.0 version (International Business Machines United States. IBM Corp., Armonk, NY, USA) was used to conduct analyses in the present study.

## 3. Results

### 3.1. Urinary PAH Data List Characteristics

Nine hydroxylated urinary PAH metabolites, namely, 1-hydroxynaphthalene, 2-hydroxynaphthalene, 3-hydroxyfluorene, 2-hydroxyfluorene, 3-hydroxyphenanthrene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 1-hydroxypyrene, and 9-hydroxyfluorene, were included to be analyzed in the present study. There are two, three, and four aromatic rings in hydroxynaphthalene, hydroxyfluorene, and hydroxypyrene, respectively. Their numbers and means are shown in Table 2.

**Table 2.** The number and mean of urinary PAHs.

Variables	Number	Mean (S.D.) (ng/L)
1-hydroxynaphthalene	2747	21.61 (328.26)
2-hydroxynaphthalene	2747	7.34 (9.47)
3-hydroxyfluorene	2744	0.26 (0.50)
2-hydroxyfluorene	2747	0.55 (0.95)
3-hydroxyphenanthrene	2746	0.12 (0.18)
1-hydroxyphenanthrene	2746	0.20 (0.26)
2-hydroxyphenanthrene	2744	0.10 (0.14)
1-hydroxypyrene	2746	0.25 (0.47)
9-hydroxyfluorene	2747	0.54 (1.62)

### 3.2. Associations between PAHs and Diarrhea

Figure 1 and Table 3 display the associations between PAHs and diarrhea. A significantly positive relationship was observed in the group of 2-hydroxynaphthalene, 3-hydroxyfluorene, 2-hydroxyfluorene, and 1-hydroxyphenanthrene over all kinds of models. However, no significant association was observed in the group of 1-hydroxynaphthalene, 2-hydroxyphenanthrene, 1-hydroxypyrene, and 9-hydroxyfluorene. 3-hydroxyphenanthrene showed significantly positive relationships only in Model 2 and Model 3 adjusted data.

### 3.3. Gender Difference in Association between PAHs and Diarrhea

Table 4 shows the sex-based relationships between PAH metabolites and diarrhea. The significantly positive relationship between PAH metabolites (2-hydroxynaphthalene, 3-hydroxyfluorene, 2-hydroxyfluorene, 3-hydroxyphenanthrene, 1-hydroxyphenanthrene, and 2-hydroxyphenanthrene) and diarrhea was observed in the female group after adjustment of pertinent covariables. However, the significantly positive relationship between

1-hydroxynaphthalene and diarrhea was only observed in the male group for Model 3 and Model 4.

### 3.4. Association between PAHs and Diarrhea Divided by Obesity

Table 5 shows the association between PAH metabolites and bowel health in different BMI level groups. The significantly positive relationship between PAH metabolites (2-hydroxynaphthalene, 3-hydroxyfluorene, 2-hydroxyfluorene, 3-hydroxyphenanthrene, and 1-hydroxyphenanthrene) and diarrhea was observed in the non-obesity group across all of the models. However, no significant association was observed between 1-hydroxynaphthalene, 2-hydroxyphenanthrene, 1-hydroxypyrene, and 9-hydroxyfluorene and diarrhea divided by obesity. The significantly positive relationship between 2-hydroxyphenanthrene and diarrhea was only observed in the non-obesity group for Model 2 and Model 3.

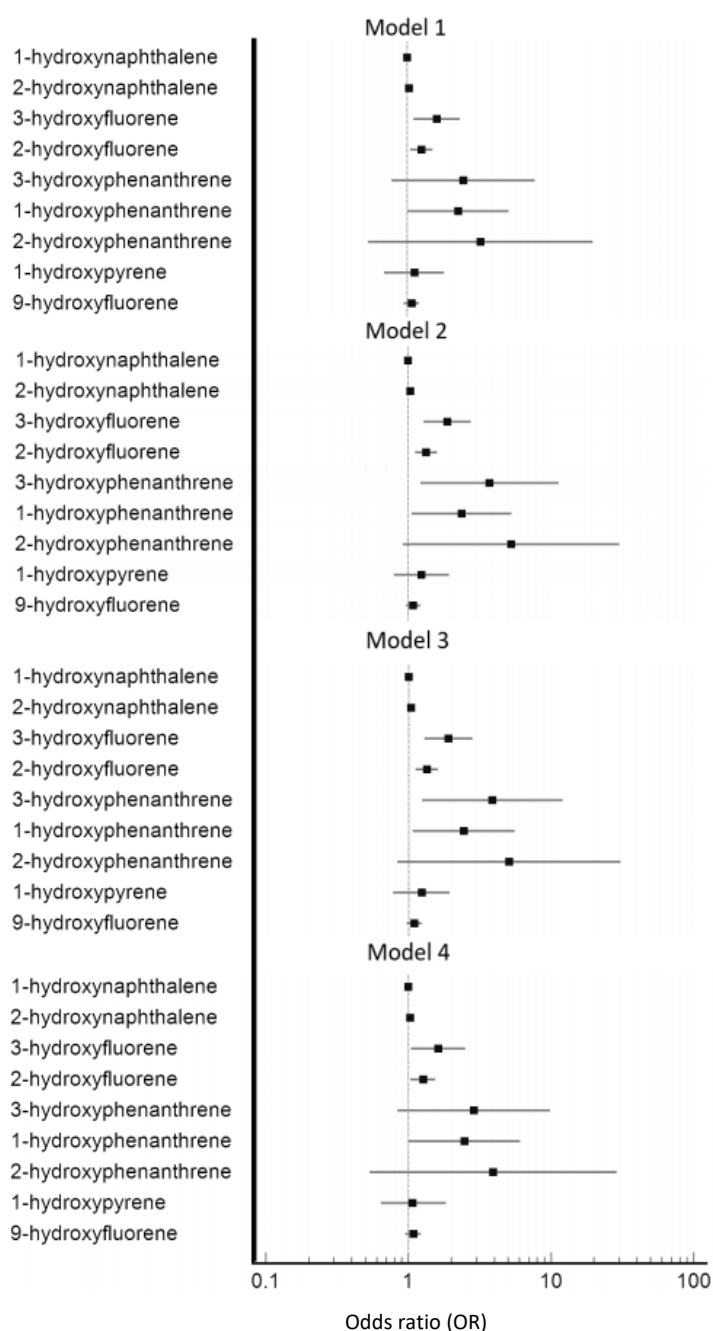


Figure 1. Forest plot of association between PAHs and diarrhea.

**Table 3.** Association between PAHs and diarrhea.

Variables	Model 1	<i>p</i> Value	Model 2	<i>p</i> Value	Model 3	<i>p</i> Value	Model 4	<i>p</i> Value
	Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)	
1-hydroxynaphthalene	1.000 (0.999, 1.001)	0.866	1.000 (0.999, 1.001)	0.932	1.000 (0.999, 1.001)	0.982	1.000 (0.999, 1.001)	0.973
2-hydroxynaphthalene	1.029 (1.007, 1.052)	0.009	1.036 (1.013, 1.060)	0.002	1.036 (1.013, 1.060)	0.003	1.030 (1.005, 1.056)	0.020
3-hydroxyfluorene	1.618 (1.107, 2.365)	0.013	1.900 (1.290, 2.798)	0.001	1.904 (1.287, 2.818)	0.001	1.621 (1.045, 2.515)	0.031
2-hydroxyfluorene	1.263 (1.055, 1.511)	0.011	1.343 (1.122, 1.608)	0.001	1.344 (1.121, 1.611)	0.001	1.266 (1.034, 1.549)	0.022
3-hydroxyphenanthrene	2.486 (0.775, 7.971)	0.126	3.791 (1.227, 11.714)	0.021	3.874 (1.238, 12.122)	0.020	2.876 (0.840, 9.848)	0.093
1-hydroxyphenanthrene	2.285 (1.002, 5.211)	0.049	2.402 (1.059, 5.449)	0.036	2.433 (1.067, 5.548)	0.035	2.468 (1.004, 6.068)	0.049
2-hydroxyphenanthrene	3.301 (0.530, 20.557)	0.201	5.401 (0.918, 31.774)	0.062	5.065 (0.831, 30.885)	0.079	3.935 (0.536, 28.900)	0.178
1-hydroxypyrene	1.123 (0.691, 1.824)	0.641	1.243 (0.792, 1.951)	0.344	1.231 (0.780, 1.943)	0.371	1.068 (0.643, 1.837)	0.757
9-hydroxyfluorene	1.074 (0.947, 1.218)	0.268	1.088 (0.965, 1.226)	0.170	1.095 (0.970, 1.236)	0.143	1.080 (0.950, 1.227)	0.238

Adjusted covariates: Model 1 = unadjusted, Model 2 = Model 1 + gender + age + race/ethnicity, Model 3 = Model 2 + creatinine + ALT + glucose serum, Model 4 = Model 3 + medical histories of congestive heart failure, coronary heart disease, myocardial infarction, thyroid problems, and smoking before.

**Table 4.** Gender difference in association between PAHs and diarrhea.

Variables	Gender	Model 1	<i>p</i> Value	Model 2	<i>p</i> Value	Model 3	<i>p</i> Value	Model 4	<i>p</i> Value
		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)	
1-hydroxynaphthalene	Male	1.001 (1.000, 1.003)	0.073	1.001 (1.000, 1.003)	0.067	1.002 (1.000, 1.004)	0.023	1.002 (1.000, 1.004)	0.021
	Female	1.000 (0.998, 1.002)	0.805	1.000 (0.998, 1.002)	0.816	1.000 (0.997, 1.002)	0.764	1.000 (0.997, 1.002)	0.800
2-hydroxynaphthalene	Male	1.003 (0.944, 1.066)	0.921	1.003 (0.943, 1.066)	0.935	1.002 (0.943, 1.064)	0.948	0.990 (0.919, 1.066)	0.793
	Female	1.042 (1.014, 1.071)	0.003	1.052 (1.023, 1.083)	0.000	1.052 (1.022, 1.083)	0.001	1.044 (1.012, 1.078)	0.007
3-hydroxyfluorene	Male	0.559 (0.086, 3.631)	0.542	0.527 (0.077, 3.588)	0.513	0.498 (0.073, 3.407)	0.477	0.223 (0.019, 2.564)	0.228
	Female	2.088 (1.363, 3.198)	0.001	2.497 (1.606, 3.881)	0.000	2.479 (1.584, 3.880)	0.000	2.063 (1.256, 3.388)	0.004
2-hydroxyfluorene	Male	0.912 (0.437, 1.903)	0.807	0.899 (0.424, 1.908)	0.782	0.887 (0.420, 1.873)	0.753	0.678 (0.252, 1.823)	0.442
	Female	1.436 (1.155, 1.786)	0.001	1.540 (1.227, 1.933)	0.000	1.530 (1.217, 1.922)	0.000	1.418 (1.112, 1.809)	0.005
3-hydroxyphenanthrene	Male	0.050 (0.000, 66.735)	0.414	0.034 (0.000, 64.445)	0.379	0.030 (0.000, 62.866)	0.370	0.005 (0.000, 20.243)	0.212
	Female	6.439 (1.756, 23.611)	0.005	9.212 (2.474, 34.298)	0.001	9.125 (2.456, 33.905)	0.001	6.532 (1.632, 26.147)	0.008

Table 4. Cont.

Variables	Gender	Model 1	<i>p</i> Value	Model 2	<i>p</i> Value	Model 3	<i>p</i> Value	Model 4	<i>p</i> Value
		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)	
1-hydroxyphenanthrene	Male	0.002 (0.000, 7.263)	0.137	0.002 (0.000, 6.523)	0.128	0.002 (0.000, 6.867)	0.133	0.000 (0.000, 3.374)	0.091
	Female	3.347 (1.389, 8.069)	0.007	3.643 (1.457, 9.106)	0.006	3.878 (1.537, 9.783)	0.004	3.946 (1.543, 10.093)	0.004
2-hydroxyphenanthrene	Male	0.005 (0.000, 164.563)	0.316	0.003 (0.000, 152.784)	0.295	0.004 (0.000, 186.132)	0.315	0.000 (0.000, 34.855)	0.176
	Female	12.472 (1.754, 88.711)	0.012	16.957 (2.339, 122.941)	0.005	15.391 (2.093, 113.168)	0.007	11.728 (1.441, 95.487)	0.021
1-hydroxypyrene	Male	0.163 (0.002, 14.771)	0.430	0.136 (0.001, 14.311)	0.401	0.138 (0.001, 12.722)	0.390	0.032 (0.000, 5.620)	0.191
	Female	1.260 (0.791, 2.008)	0.330	1.474 (0.935, 2.323)	0.095	1.445 (0.910, 2.293)	0.118	1.262 (0.742, 2.146)	0.390
9-hydroxyfluorene	Male	0.533 (0.101, 2.824)	0.459	0.496 (0.087, 2.809)	0.428	0.494 (0.084, 2.913)	0.436	0.335 (0.049, 2.299)	0.266
	Female	1.140 (0.977, 1.331)	0.095	1.179 (1.012, 1.373)	0.035	1.185 (1.016, 1.383)	0.031	1.172 (0.998, 1.377)	0.053

Adjusted covariates: Model 1 = unadjusted, Model 2 = Model 1 + gender + age + race/ethnicity, Model 3 = Model 2 + creatinine + ALT + glucose, Model 4 = Model 3 + medical history of congestive heart failure, coronary heart disease, myocardial infarction, thyroid problems, and smoking before.

Table 5. Association between PAHs and diarrhea divided by obesity.

Variables	BMI	Model 1	<i>p</i> Value	Model 2	<i>p</i> Value	Model 3	<i>p</i> Value	Model 4	<i>p</i> Value
		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)	
1-hydroxynaphthalene	<30	1.000 (0.998, 1.002)	0.985	1.000 (0.998, 1.002)	0.975	1.000 (0.998, 1.002)	0.978	1.000 (0.997, 1.002)	0.880
	>30	1.000 (0.999, 1.001)	0.908	1.000 (0.999, 1.001)	0.967	1.000 (0.999, 1.001)	0.967	1.000 (0.999, 1.001)	0.940
2-hydroxynaphthalene	<30	1.044 (1.015, 1.073)	0.002	1.050 (1.020, 1.080)	0.001	1.050 (1.020, 1.081)	0.001	1.040 (1.007, 1.073)	0.017
	>30	1.009 (0.968, 1.052)	0.666	1.011 (0.963, 1.061)	0.666	1.013 (0.965, 1.063)	0.606	1.008 (0.955, 1.063)	0.781
3-hydroxyfluorene	<30	2.057 (1.347, 3.143)	0.001	2.287 (1.484, 3.524)	0.000	2.312 (1.490, 3.587)	0.000	1.850 (1.138, 3.006)	0.013
	>30	0.724 (0.162, 3.239)	0.673	0.937 (0.231, 3.802)	0.927	0.927 (0.220, 3.901)	0.918	0.701 (0.133, 3.689)	0.675
2-hydroxyfluorene	<30	1.478 (1.160, 1.884)	0.002	1.525 (1.198, 1.941)	0.001	1.532 (1.198, 1.958)	0.001	1.354 (1.034, 1.773)	0.028
	>30	1.071 (0.739, 1.552)	0.717	1.164 (0.798, 1.698)	0.430	1.175 (0.801, 1.724)	0.409	1.137 (0.719, 1.799)	0.582
3-hydroxyphenanthrene	<30	4.362 (1.198, 15.879)	0.025	5.585 (1.558, 20.024)	0.008	5.590 (1.544, 20.038)	0.009	3.831 (0.957, 15.326)	0.058
	>30	0.621 (0.019, 20.003)	0.788	1.416 (0.069, 29.182)	0.822	1.468 (0.067, 32.369)	0.808	1.202 (0.040, 36.117)	0.916
1-hydroxyphenanthrene	<30	3.324 (1.238, 8.923)	0.017	3.596 (1.322, 9.783)	0.012	3.586 (1.312, 9.800)	0.013	3.939 (1.307, 11.873)	0.015
	>30	1.179 (0.197, 7.062)	0.857	1.262 (0.259, 6.138)	0.773	1.307 (0.267, 6.411)	0.741	1.259 (0.213, 7.429)	0.800

Table 5. Cont.

Variables	BMI	Model 1	<i>p</i> Value	Model 2	<i>p</i> Value	Model 3	<i>p</i> Value	Model 4	<i>p</i> Value
		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)		Odds Ratio (95% CI)	
2-hydroxyphenanthrene	<30	7.527 (0.902, 62.825)	0.062	9.706 (1.203, 78.275)	0.033	9.322 (1.123, 77.395)	0.039	6.529 (0.598, 71.223)	0.124
	>30	0.712 (0.016, 31.838)	0.861	1.703 (0.050, 58.408)	0.768	1.657 (0.046, 59.678)	0.782	1.267 (0.023, 69.837)	0.908
1-hydroxypyrene	<30	1.400 (0.859, 2.281)	0.177	1.481 (0.925, 2.373)	0.102	1.448 (0.897, 2.338)	0.130	1.248 (0.726, 2.147)	0.423
	>30	0.214 (0.009, 5.082)	0.340	0.283 (0.013, 6.248)	0.424	0.317 (0.014, 7.089)	0.469	0.259 (0.009, 7.075)	0.423
9-hydroxyfluorene	<30	1.089 (0.964, 1.230)	0.172	1.091 (0.967, 1.231)	0.157	1.099 (0.972, 1.241)	0.131	1.072 (0.943, 1.219)	0.287
	>30	0.855 (0.368, 1.985)	0.715	0.977 (0.527, 1.810)	0.940	0.999 (0.542, 1.841)	0.997	1.011 (0.514, 1.988)	0.974

Adjusted covariates: Model 1 = unadjusted, Model 2 = Model 1 + gender + age + race/ethnicity, Model 3 = Model 2 + creatinine + ALT + glucose, Model 4 = Model 3 + medical history of congestive heart failure, coronary heart disease, myocardial infarction, thyroid problems, and smoking before.



#### 4. Discussion

In the present study, a secondary analysis of NHANES data, urinary PAH metabolites demonstrated a substantial association with increased diarrhea risk in a nationally representative sample of the U.S. adult population. A strong association between PAHs and diarrhea was observed, especially in the female group and non-obesity group. To the best of our knowledge, this is the first study to examine the relationship between environmental PAH exposure and bowel health in a population-based cross-sectional survey.

Overall, the analysis revealed that urinary PAHs are associated with diarrhea. However, not all of PAHs had statistically a significant association with diarrhea, especially in the 1-hydroxypyrene group, which had no association with diarrhea in any of the tables. Furthermore, patients who had a history of heart problems, thyroid function problems, and cigarette smoking were thought to be more easily affected by PAHs and, therefore, at an increased risk of diarrhea. However, to our surprise, some Model 4 adjusted groups (Table 3: 3-hydroxyphenanthrene data; Table 4: 9-hydroxyfluorene data; Table 5: 2-hydroxyphenanthrene data) revealed no statistical significance, while Model 2 groups and Model 3 groups showed the opposite trend. Therefore, in our study, the association between smoking and diarrhea is still controversial. Even though some associations were weak, the association of PAHs and the risk of diarrhea was statistically significant, because this analysis included a relatively large sample size and most of the data had low  $p$  values ( $<0.05$ ). After adjustment for variables, including gender and BMI, the female and non-obesity groups became more statistically significant.

It was proven that many PAHs have toxic properties. PAHs have highly lipid solubility and are thus readily absorbed into the gastrointestinal tract of mammals [2]. Therefore, the gastrointestinal tract is an important organ that PAHs can easily approach or affect. This indicates the importance of research on the relationship between environmental PAH exposure and bowel health [15].

Furthermore, PAHs have been proved to alter human gut microbiota functions. [16] This means that exposure of PAHs in the gastrointestinal tract may lead to an imbalance in microbial activity [17], which can possibly lead to the re-modification of a pro-inflammatory state in the gastrointestinal system. Consequently, diarrhea might happen due to this kind of pro-inflammatory state [18]. In addition to epidemiological findings, dietary intake of PAHs in animal models, humans, and cell cultures has been examined in gastrointestinal cancer studies [19]. Long-term gastrointestinal exposure to PAHs can have negative health impacts on the gastrointestinal tract. However, the impact of mid-term (months) gastrointestinal exposure to PAHs has not yet been explored. Our findings provide epidemiologic evidence for future research to determine the mid-term direct impact of PAH exposure on bowel health.

The mechanisms of toxicants, including PAHs triggering the aryl hydrocarbon receptor (AhR), are well established [20]. AhR, which is a receptor that is easily inducible by several small molecular weight chemicals, is responsible for the toxicity of PAHs. It exists in almost all human organs, such as the heart and liver, and tissues, including those of the ectoderm, muscle, and gastrointestinal tract [21]. It is also a ligand-activated transcription factor, which plays an essential role in intestinal barrier function and intestinal immune cells, as well as in intestinal homeostasis [22]. As is well known, AhR is essential for the maintenance of IL-22 (interleukin-22) producing lymphoid cells and intraepithelial lymphocytes in the gastrointestinal tract. AhR-delivered signals that are altered by PAHs may lead to the amplification of gastrointestinal tissue destructive immune-inflammatory responses [23]. That is, PAHs may lead to colitis, making humans more likely to have diarrhea.

The present study has some limitations. First, it is a cross-sectional design; therefore, the direct causal relationships between PAHs and diarrhea were not assessed. A long-term observation period should be considered in future studies. Second, the feeling of diarrhea is self-reported and subjective. It may vary from person to person, which might cause changes in the count of diarrhea and results. Third, PAH metabolites are removed from the body via urine within one day. Therefore, with this consideration, urinary PAH

metabolites can only indicate recent exposures and do not deliver evidence about long-term exposure [24]. Finally, all the population data in this study were acquired from NHANES 2001 to 2006. This analysis might not precisely reflect the modern global population.

## 5. Conclusions

Our study highlighted the significant association between PAH exposure and the risk of diarrhea among the adult population in the United States. In addition, urinary PAHs were significantly correlated with bowel disorder in female and non-obesity populations. It was suggested that guts microbiota and the aryl hydrocarbon receptor play a role in the mechanism of this relationship. Understanding more information about the impact of PHAs on the human body is a critical issue due to the great population of patients suffering from bowel disorders. More research is necessary shed light on the pathophysiological mechanisms associated with PAH exposure and diarrhea.

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## Abbreviations

NCHS: National Center for Health Statistics; CDC: Centers for Disease Control and Prevention; NHANES: National Health and Nutrition Examination Survey; SD: standard deviation; CI: confidence interval; PAH: polycyclic aromatic hydrocarbon; PAHs: polycyclic aromatic hydrocarbons.

## IUPAC Name of Polycyclic Aromatic Hydrocarbons

1-hydroxynaphthalene	naphthalen-1-ol
2-hydroxynaphthalene	naphthalen-2-ol
3-hydroxyfluorene	9H-fluoren-3-ol
2-hydroxyfluorene	9H-fluoren-2-ol
3-hydroxyphenanthrene	phenanthren-3-ol
1-hydroxyphenanthrene	phenanthren-1-ol
2-hydroxyphenanthrene	phenanthren-2-ol
1-hydroxypyrene	pyren-1-ol
9-hydroxyfluorene	9H-fluoren-9-ol

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