A Novel Method for Source-Specific Hemoglobin Adducts of Nitro-Polycyclic Aromatic Hydrocarbons

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Abstract

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous air pollutants associated with negative impacts on growth, development and behavior in children. Source-specific biological markers of PAH exposure are needed for targeting interventions to protect children. Nitro-derivatives of PAH can act as markers of exposure to diesel exhaust, gasoline exhaust, or general combustion sources. Using a novel HPLC-APCI-MS/MS detection method, we examined four hemoglobin (Hb) adducts of nitro-PAH metabolites and the Hb adduct of a benzo[a]pyrene (BaP) metabolite in 22 umbilical cord blood samples. The samples were collected from a birth cohort with comprehensive data on prenatal PAH exposure, including prenatal personal air monitoring and DNA adducts in maternal and umbilical cord blood. Using non-parametric analyses, heat maps, and principal component analysis (PCA), we analyzed the relationship between the five Hb adducts and previous PAH measurements, with each measurement representing a different duration of exposure. We found that Hb adducts derived from several diesel-related nitro-PAHs (2-

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Conflict of Interest

The authors have no conflicts of interest to disclose

nitrofluorene and 1-nitropyrene) were significantly correlated ($r=0.77$, $p<0.0001$) and grouped together in PCA. Nitro-PAH derived Hb adducts were largely unrelated to previously collected measures of exposure to a number of PAH parent compounds. These measures need to be validated in a larger cohort.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are common air pollutants formed during incomplete combustion of gasoline, fossil fuels, or organic materials including food. Animal studies have found associations between prenatal PAH exposure and alterations in brain development and functioning (1, 2). In the Columbia Center for Children's Environmental Health (CCCEH) birth cohort, prenatal PAH exposure has been associated with lower birth weight, smaller head circumference, developmental delay, obesity, reduced IQ, symptoms of anxiety/depression, attention problems, Attention Deficit Hyperactive Disorder (ADHD) behavior problems, and anatomical brain changes (3–9).

Exposure to PAH in the CCCEH cohort has been assessed through 48 hour personal air monitoring of mothers during the third trimester of pregnancy and PAH-DNA adducts measured in maternal blood at birth and umbilical cord blood. DNA adducts measured in blood reflect the biologically effective dose of the chemicals, integrating exposure with individual metabolic and DNA repair capacities. In the current study we leverage archived samples from the CCCEH cohort to evaluate novel biological markers of PAH exposure that we hypothesize to be specific to either diesel or other combustion sources, which integrate medium term exposures of 3–4 months, and may be more closely related to external exposure than DNA adducts (10).

In the polluted atmosphere, nitro-PAH compounds are present through two main mechanisms, thermal and photochemical additions. Thermal formation occurs via the addition of a nitro functional group to a parent PAH. For example, combustion occurring in a diesel engine results in thermal formation of 1-nitropyrene, 2-nitrofluorene, and 9-nitrophenanthrene (11, 12). Thus, these nitro-PAH are indicators of primary diesel combustion. Photochemical formations occur through the addition of a nitro functional group to the parent PAH via a photochemical reaction initiated by a hydroxyl ($\text{OH}^\bullet$) or nitrate ($\text{NO}_3^\bullet$) radical (13). Interestingly, a thermal vs. a photochemical addition of a nitro functional group can result in distinctly different nitro-PAH isomers. For example, in the case of pyrene, a thermal addition forms 1-nitropyrene and a photochemical addition forms 2-nitropyrene, representing primary and secondary diesel emissions, respectively. Furthermore, certain nitro-PAH (e.g., 1-nitronaphthalene) are predominantly formed via photochemical reactions in the atmosphere and are secondary pollutants mainly reflecting gasoline combustion, which is the major source of these pollutants in U.S. urban centers (14).

Although it has been well-established that nitro-PAH isomers in the atmosphere can be used as specific source markers (15, 16), measurement of nitro-PAH in the atmosphere presents technical challenges due to low concentrations and sampling artifacts (17–20).

Biomonitoring of hemoglobin (Hb) adducts provides a direct means to measure human

exposure to these compounds that are easier to measure, reflect longer exposure periods, and are typically more mutagenic and/or toxic than their PAH parent compounds and hence potentially more biologically relevant (21, 22). After inhalation, nitro-PAH can be metabolically reduced to reactive nitroso intermediates, and subsequently react with the thiol (-SH) group of cysteine in Hb. This results in an intramolecular rearrangement that gives sulfinic acid amide. This Hb adduct is usually stable *in vivo* but can be readily hydrolyzed *in vitro* to give corresponding amino-PAH (23–25). The resulting amino arenes are extracted and quantified using a GC-MS method (12, 26). We modified the previously published GC-MS method into a more sensitive HPLC-MS-MS method. The hydrolysis products (amino-PAH) that are derived from source-specific nitro-PAH not only reflect the level of exposure to nitro-PAH but can also be used to trace the exposure to certain combustion sources (12). Benzo[a]pyrene (BaP) is bioactivated to BaP diol-epoxides (BPDEs) that can bind to hemoglobin to form protein adducts. Disruption of the tertiary structures of the protein releases BaP tetrol metabolites, which are used as a quantitative measurement of BaP-Hb adducts.

In this study, we examined the relationships among four different nitro-PAH derived Hb adducts reflecting diesel (1-aminopyrene, 2-aminofluorene and 9-aminophenanthrene) and other combustion (1-aminonaphthalene) sources, and a benzo[a]pyrene (BaP) derived Hb adduct, reflecting multiple combustion sources and dietary intake, in 22 umbilical cord blood samples. To gain a better understanding of the relationship between nitro-PAH and their parent PAH, we examined the correlation between specific nitro-PAH-Hb adducts and PAH measured in the same CCCEH cohort via personal PAH air monitoring during the third trimester of pregnancy, BaP-DNA adducts measured in maternal blood at birth and umbilical cord blood, and bulky aromatic DNA adducts measured in umbilical cord blood.

Methods

Sample Selection

Samples were collected from the CCCEH Mothers and Newborns cohort, designed to examine the impact of prenatal and early life environmental exposures, including exposure to air pollution, on various health outcomes. From 1998–2006, Dominican and African-American women (ethnicity classified by self-report) residing in Washington Heights, Harlem, and the South Bronx, New York, and who registered at the obstetrics/gynecology clinics at New York Presbyterian Medical Center and Harlem Hospital by the 20th week of pregnancy, were recruited in the clinics and informed consent was obtained. The study was approved by the Institutional Review Board (IRB) of Columbia University in accordance with the U.S. Department of Health and Human Services Code of Federal Regulations, Title 45, Part 46, Protection of Human Subjects. Eligible women were nonsmokers during the current pregnancy; were free of diabetes, hypertension, and known HIV; had no documented or reported drug abuse; and had resided in the area for at least 1 year. For this initial study, biospecimens were selected from participants with no reported active smoking or secondhand tobacco smoke exposure (defined as an active smoker living in the home) and with high air monitored PAH exposure compared to the overall cohort, as measured by

prenatal air monitoring, in order to increase the chances of detection of Hb adducts unrelated to tobacco smoke exposure.

Air Monitoring

In the cohort from which the study sample was drawn, mothers in the third trimester of pregnancy were asked to wear a small backpack containing a personal monitor during daytime hours for 2 consecutive days and to place the monitor near the bed at night. The personal air sampling pumps operated continuously over this period, collecting vapors and particles $2.5\mu\text{m}$ in diameter on a heat-treated, precleaned quartz microfiber filter and a precleaned polyurethane foam (PUF) cartridge backup. The samples were analyzed at Southwest Research Institute (SwRI) for eight carcinogenic PAH (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, BaP, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[ghi]perylene) and pyrene, as described previously (27–29).

Biospecimen Collection and Analysis

Following delivery, maternal blood and umbilical cord blood samples were collected. Within hours of sample collection, they were transported to the CCCEH Molecular Epidemiology Laboratory where the buffy coat, packed red blood cells, and plasma were separated and stored at -70°C . BaP-DNA adducts were measured in maternal and cord blood as previously described (30, 31).

Red blood cells were washed three times with PBS and then lysed with 10^{-4} M EDTA (pH 7.5). After centrifugation, the supernatant was transferred to a 50mL centrifuge tube, washed three times with ice cold acetone and air dried, followed by hydrolysis with 1 M sodium hydroxide to release the corresponding amino-PAH. The resulting amino-PAH from Hb adducts of the four target nitro-PAHs (1-nitronaphthalene, 2-nitrofluorene, 9-nitrophenanthrene and 1-nitropyrene) were analyzed on our HPLC-MS-MS system. We used a Thermo TSQ Quantum Access Max system (Thermo Fisher Scientific Inc., Hudson, NH) equipped with a Phenomenex Luna HPLC column ($3\mu\text{m}$, C18 100 A, $50 \times 2.00\text{ mm}$) (Phenomenex Inc., Torrance, CA) and a gradient mobile phase involving 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Due to the poor response of ionization under ESI source, BaP tetrol was analyzed using APCI ionization source. All the other metabolites were analyzed using ESI ionization source.

The experimental conditions for the mass spectrometer were as follows: Source: APCI/ESI; spray voltage at 3000 V; Ion polarity: Positive; Vaporizer Temperature: 300°C ; Ion Transfer Capillary Temperature: 350°C ; Source skimmer Offset: 10 V; Scan Mode: Selective Reaction Monitoring; Q2 Pressure: 1.0 mTorr argon; Scan Width: 0.01 (m/z); Scan Time: 0.1 s (each SRM transition) Q1, Q3 Resolution: Unit (0.7 u FWHM); SRM Transitions: m/z 144.1/127.2 for 1-aminonaphthalene, 182.1/163.1 for 1-aminofluorene, 194.1/176.1 for 9-aminophenanthrene, 218.1/189.1 for 1-aminopyrene, 151.1/132.1 for 1-aminonaphthalene-d7, and 303.1/256.9 for BaP tetrol. The collision energy was optimized for each analyte. All the fragment ions used for quantitation for the analytes are summarized in Supplemental Table 1. The method had detection limits of 25 pg/g Hb for 1-aminopyrene, 7.5 pg/g Hb for each of the other amino-PAHs, and 250 pg/g Hb for BaP tetrol.

The method statistics are described in Supplemental Table 2. The limit of detection (LOD) was calculated as 3 times the standard deviation of 5 repeated injections of the standard with the lowest concentration. The recovery of each metabolite was obtained by calculating the ratio of the detected amount of the standard spiked hemoglobin over the actual amount spiked. The samples were measured once since the LC-MSMS method used isotope-labeled internal standard for calibration, which provided satisfying precision for the coefficient of variation of 6 repeated injections smaller than 12.6%. The correlation coefficients R^2 (average $\pm$ SD) for each calibration curve were 0.9973 ± 0.0026 , 0.9939 ± 0.0112 , 0.9966 ± 0.0022 , 0.9967 ± 0.0031 , and 0.9995 ± 0.0004 .

Target analytes included the following:

1-Aminopyrene, 2-aminofluorene, and 9-aminophenanthrene. These hydrolysis products originate from 1-nitropyrene, 2-nitrofluorene, and 9-nitrophenanthrene that are considered specific molecular markers of ambient diesel exhaust (16, 32).

1-Aminonaphthalene. This compound originates from 1-nitronaphthalene resulting from photochemical conversion of naphthalene. In the urban outdoor environment, a main source of naphthalene is traffic emissions (33).

Benzo[a]pyrene (BaP) tetrol. BaP tetrol is a hydrolysis product of BaP derived adducts. In the body, BaP can be enzymatically activated to form BaP diol-epoxides (BPDEs). BPDEs can then form Hb adducts via alkylation of the carboxylate groups of the protein. Although BPDE-Hb adducts are stable in the body, they can be readily hydrolyzed under either acidic or basic conditions in the laboratory to release BaP tetrol. Human exposure to BaP is mainly from inhalation of combustion generated particles and from ingestion of char-broiled meats that have undergone pyrolysis or direct combustion processes (34).

Previously measured DNA adducts. As part of the larger cohort study, maternal and umbilical cord blood samples were analyzed for PAH-DNA adducts using a HPLC-fluorescence method which detects BaP tetraols (30, 35) in white blood cell DNA. The method has a coefficient of variation of 12% and a lower limit of detection of 0.25 adducts per 10^8 nucleotides.

Umbilical cord blood samples were also analyzed for DNA adducts using the nuclease P1 digestion enhancement procedure of the ^{32}P -postlabeling assay that detects bulky/hydrophobic DNA adducts including PAH-, nitro-PAH-, and aromatic amine- adducts. The ^{32}P -postlabeling assay is highly sensitive and can be used to detect an array of DNA adducts having multiple structures (36).

Statistical Analysis

All data were analyzed with SAS software, version 9.3 (SAS Institute Inc., Cary, NC). Two values for 9-aminophenanthrene were below the limit of detection (LOD) and the LOD/2 was substituted in. Distributions of individual Hb adducts were examined and non-parametric Spearman correlations were used to examine the relationship between the 5 Hb adducts, as well as to PAH-DNA adducts and prenatal PAH air concentrations.

To visualize the differences between the hemoglobin adducts, a heat map based on z-scores was created to view the variation among participants' individual and summed Hb adducts. The sum of hemoglobin adducts was calculated by summing z-scores of each individual's Hb adduct values, where z-scores were calculated for each Hb adduct as the individual Hb adduct value minus the mean of the respective Hb adduct, divided by the sample standard deviation of the Hb adduct. Seasonal variation of nitro-PAH concentrations has been observed, with certain nitro-PAH levels higher in the winter and others in the summer due to enhanced photochemistry (32). In the heat map participants were ordered by the season of their air monitoring, summer (April–September) or winter (October–March), to visualize any seasonal differences.

To further determine how the various Hb adducts were related to each other, Principal Component Analysis (PCA) was conducted first for all five Hb adducts, and then including the five Hb adducts plus the 3 previously measured DNA adducts.

Results

The characteristics of the study sample are shown in Table 1. To maximize chances of detection of Hb adducts, the subset of 22 mother child dyads included in the analysis was selected from those with high prenatally monitored PAH concentrations and hence had a significantly higher PAH level compared to the parent population ($p=0.024$). However, the two groups did not differ in child gender, ethnicity, maternal PAH-DNA adducts or ^{32}P -postlabeled DNA adducts from those who were not included in the current analysis ($N=703$). However, the mean cord PAH-DNA adduct level as measured by HPLC fluorescence was lower for those included in the analysis ($p=0.01$).

Table 2 presents the distribution of values for hemoglobin adducts, DNA adducts, and PAH by air monitoring. The dominant Hb adduct was non-diesel related 1-aminonaphthalene. The Hb adducts showed a wide range of concentrations (3 to 24 fold). Hb adducts were right-skewed for 2-aminofluorene, 9-aminophenanthrene, and 1-aminopyrene, while 1-aminonaphthalene and BaP tetrol were more normally distributed.

Table 3 presents the results of correlation analyses. Two of the 3 diesel-related Hb adducts (2-aminofluorene and 1-aminopyrene) were significantly correlated ($r=0.77$, $p<0.0001$). However, neither 2-aminofluorene nor 1-aminopyrene was correlated with 9-aminophenanthrene, which is also considered to be diesel-related ($r=0.12$, $p=0.59$; $r=0.19$, $p=0.40$ respectively). Interestingly, two diesel-related Hb adducts (2-aminofluorene and 1-aminopyrene) were significantly negatively correlated with non-diesel related 1-aminonaphthalene ($r=-0.59$, $p=0.0036$; $r=-0.55$, $p=0.008$, respectively). Individual Hb adducts were not significantly correlated with any of the previous PAH measurements (prenatal PAH air monitoring or PAH-DNA adducts).

The heat map, in which subjects were ordered by season of PAH air monitoring, was based on the z-score of individual and summed Hb adducts. The largest variation between individuals was seen in the sum of the hemoglobin adducts and there appeared to be

variation in hemoglobin adduct levels by season, with greater variation seen in the summer. (Figure 1).

The PCA of Hb adducts resulted in three principal components (PCs) with eigenvalues >1, indicating three primary groupings. In PC 1, 2-aminofluorene, 9-aminophenanthrene, and 1-aminopyrene load in the same direction, suggesting the presence of a diesel-related component. Additionally, 2-aminofluorene had the highest loading in PC 1. PC 2 has the highest loading for BaP-tetrol, suggesting the presence of exposure to multiple combustion related pollutants, and PC 3 has the highest loading for 9-aminophenanthrene (Table 4). Expansion of the PCA to include both nitro-PAH Hb adducts and PAH-DNA adducts resulted in 3 eigenvalues > 1. Similar to the PCA of Hb adducts alone, in PC 1, 9-aminophenanthrene, 2-aminofluorene, and 1-aminopyrene loaded in the same direction, suggesting these adducts co-vary and indicating a diesel traffic-related factor. PC 2 has the highest loadings for BaP-tetrol and PC 3 has highest loading for HPLC PAH-DNA adducts and ³²P-postlabeled DNA adducts (Table 5). Figures 2a and 2b show the relationship between the PCs and indicate a slight separation by season of air monitoring.

Discussion

To the best of our knowledge, this is the first study using the HPLC-APCI-MS/MS method of nitro-PAH Hb adduct detection and the first study of nitro-PAH Hb adducts in cord blood samples from an urban population. We hypothesized that this novel method would differentiate between adducts related to diesel, non-diesel combustion exposure and indeed found that the three diesel-related adducts behaved differently than the others. Two of the three diesel-related adducts (2-aminofluorene and 1-aminopyrene) were highly correlated, but 9-aminophenanthrene (also thought to be diesel-related) was not correlated with either. However, the three diesel-related compounds grouped together in PCA. In the heat map 9-aminophenanthrene stood out as differing by the season of air monitoring. As expected, the previously acquired PAH measures (air concentrations and adducts) were not correlated with the more source-specific Hb adducts. This is not surprising given that they differ in time period reflected and in biology. It appears that source-specific Hb adducts are therefore able to complement, but not replace, exposure measurements through personal monitoring and DNA adducts by differentiating between various sources of nitro-PAH exposure, especially diesel vs. non-diesel.

Other cohort studies have measured BaP-Hb adducts using only the Hb adduct BaP tetraol, but the range of BaP-Hb adducts in this study is higher than that seen in other non-occupational cohorts. In a birth cohort from Louisville KY, the mean BaP tetrol levels measured in cord blood from non-smoking women was 3.46 ± 1.81 pmol/g Hb, lower than the mean in the present study (37). A study of newspaper vendors exposed to vehicle exhaust reported an average BaP tetrol hemoglobin exposure range of <0.01–3.3 pmol/g Hb, surprisingly lower than the present environmental sample (38). In a study examining associations between BaP biomarkers in non-occupationally exposed adults, BaP tetrol Hb adducts in nonsmokers ranged from non-detectable to 0.49 pmol/g Hb, which is also lower than seen in our sample (39). Our participants all live in inner city neighborhoods which are

known to have high levels of traffic and PAH which could contribute to the higher levels observed.

The correlation between 2-aminofluorene and 1-aminopyrene, both hypothesized to be diesel-related, was statistically significant, supporting our initial hypothesis that these adducts are generated by the same source. Lack of correlation between 9-aminophenanthrene (also thought to be diesel-related) and 1-aminopyrene could be due to different relative contributions of photochemical formation and thermal formation during diesel combustion. The negative correlation between 1-aminonaphthalene (combustion) and 1-aminopyrene may be attributable to the seasonal differences in their formation. Diesel exhaust concentration is generally higher in the winter due to decreased atmospheric dispersion, which leads to increased levels of 1-nitropyrene, while photochemical activity is lower in the winter leading to lower concentrations of 1-nitronaphthalene.

The lack of correlation between the Hb adducts and previously collected measures of PAH exposures (prenatal PAH air monitoring and PAH/aromatic-DNA adducts) is not unexpected, given that they measure different steps in a continuum (external exposure, biologically effective dose without DNA repair, biologically effective dose with DNA repair). Differences between Hb and DNA adducts could be attributed to variation in biomarker half-lives and individual variation in DNA repair capabilities (40). In addition, the correlation between diesel generated nitro-PAH and total PAH is generally not high because some nitro-PAH are formed via photochemical reactions and photochemical intensity has large temporal variability (32, 41). In addition, adducts may result from multiple sources that do not necessarily correlate. For example, BaP tetrol measures the total amount of BaP bound to Hb or DNA resulting from inhalation of airborne BaP and from ingestion of PAH-containing food. If the ingested dose is substantial, we would not expect to see a significant correlation between the monitored level of airborne BaP and BaP tetrol. The lack of correlation between the measures could also be due to the different time periods reflected: the air monitoring reflects a 48-hour period in the third trimester, the Hb adducts represent a 3-month period prior to sample collection at birth, and the DNA adducts measured in white blood cells have a half-life of 4 months (27).

The heat map is a useful way to visualize Hb adducts. There was a distinct variation observed in 9-aminophenanthrene when separated by those with air monitoring in the winter season and the summer season. The precursor of 9-aminophenanthrene is 9-nitrophenanthrene. This compound is related to diesel combustion and is therefore more prominent in the winter. In the heat map, 9-aminophenanthrene showed large variation by season. However, we did not observe significant seasonal variability in the other two diesel-related Hb adducts.

The PCA, both with and without the PAH-DNA adducts, showed all 3 diesel-related Hb adducts grouping together, further supporting that these adducts share a common source. In the PCA with DNA adducts, the DNA adduct measurements loaded in the direction of both the diesel and the general combustion compounds in PC 2 and 3. Diesel and non-diesel sources (including gasoline combustion, general combustion, and diet) contribute to PAH-DNA adducts, so it is expected they would load in the direction of each of those compounds.

The correlations between PCAs indicate separation by season of air monitoring, consistent with seasonal differences in exposure to these compounds.

A strength of this study is that the samples were taken from a birth cohort which is well characterized with respect to exposure to PAH; and all of the participants were non-smoking mothers who reported having no exposure to environmental tobacco smoke. Although the personal air monitoring measurements represented a 48-hour period, these measures have been shown to be significantly correlated with 2 week residential monitoring completed in a subset ($n = 84$) of the participants' homes (8). Additionally, personal air monitoring measurements have shown significant associations with adverse developmental outcomes including decreased Mental Development Index at age 3, decreased IQ at age 5, Anxious/Depressed and Attention problems at age 6–7 (3–5), and (42). We did not include maternal report of dietary PAH exposure in the present analysis because previous analyses in this cohort have shown there is not a significant association between self-reported consumption of charbroiled or grilled foods and umbilical cord or maternal adducts.

A limitation is that the subset analyzed was not representative of the overall cohort, having been selected as having relatively high PAH exposure measured by prenatal air monitoring in order to increase the chance of results detection using a novel method. Future studies are needed to thoroughly validate this measure in a larger sample with a wider range of exposure. Additionally the association between Hb adducts and birth and early neurodevelopmental outcomes is of interest and can be examined in the larger cohort.

Conclusion

This novel method of nitro-PAH hemoglobin adduct analysis allows for the use of Hb-adducts as a biomarker of source specific nitro-PAH exposure. The present data indicate that the method has the potential to differentiate between diesel and other combustion sources of air pollution if thoroughly validated in a larger sample. Identification of specific exposure sources is important in targeting regulatory and other interventions to reduce exposure to PAH and associated air pollutants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding was provided by the National Institute for Environmental Health Sciences (NIEHS) and the U.S. Environmental Protection Agency (US EPA): NIEHS/EPA P01ES09600/RD82702701, NIEHS/EPA P01ES09600/RD832141, NIEHS/EPA P01ES09600/RD834509, NIEHS/EPA P50ES09600/RD83615401, NIEHS/EPA P01ES022845/RD83544101, NIEHS R01ES08977, NIEHS P30ES007048 NIH UG3OD023290. Funding from all the institutions listed was used towards the design and conduct of the study; collection, management, analysis, and interpretation of the data; and the preparation, review, and approval of the manuscript. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the US EPA. Further, the US EPA does not endorse the purchase of any commercial products or services mentioned in the publication.

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Figure 1.
Heat map of z-scores of individual and summed hemoglobin- DNA adducts by air monitored PAH exposure. (grouped by season of prenatal air monitoring)

Figure 2a

Figure 2b

Figure 2.

Figure 2a. Pairwise Scatterplot of Principle Components by Season of Monitoring for Hemoglobin Adducts

Figure 2b. Pairwise Scatterplot of Principle Components by Season of Monitoring for Hemoglobin and DNA adducts

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Table 1

Comparison of the characteristics of children included (N=22) and excluded (N=703) in the present analysis, among 725 fully enrolled women.

Variable	Subjects in the analysis (n=22)	Subjects NOT in the analysis (n=703)	P-value
Percent female	63.64	51.21	0.25
Percent African American	22.73	35.42	0.22
Percent with Air Monitoring in Winter	77.27	57.04	0.06
Total air PAH (sum of 9 parent compounds)	10.54 ± 9.62	6.81 ± 7.56	0.02 *
Maternal PAH-DNA adducts [†] [‡]	0.20 ± 0.12	0.21 ± 0.12	0.66
Cord PAH-DNA adducts [†] [‡]	0.17 ± 0.09	0.23 ± 0.13	0.01 *
³² P-postlabeling adducts [‡] [○]	2.49 ± 3.94	2.43 ± 2.40	0.95

* P < 0.05

[†] Adducts formed by benzo[a]pyrene (BaP)

[‡] Measurement taken in blood samples

[○] Bulky/hydrophobic DNA adducts including PAH-, nitro-PAH-, and aromatic amine- adducts.

Table 2

Distribution of PAH Exposure Measured via Hemoglobin Adducts, DNA Adducts, and Air Monitoring

Variable	N	Median	Minimum	Maximum
Hemoglobin adducts (pmol/g Hb)				
1-aminonaphthalene ³	22	11.63	6.16	21.39
2-aminofluorene ⁴	22	1.86	0.55	5.76
9-aminophenanthrene ⁴	22	0.43	0.03	1.24
1-aminopyrene ⁴	22	1.24	0.04	9.26
BaP-tetrol ⁵	22	4.09	1.17	7.95
DNA adducts (per 10 ⁸ nucleotides)				
HPLC maternal PAH-DNA adducts ¹	21	0.13	0.13	0.52
HPLC cord PAH-DNA adducts ¹	19	0.13	0.13	0.38
³² P cord bulky/hydrophobic DNA adducts ²	20	1.50	0.60	19.00
Air PAH measurements (ng/m ³)				
Total PAH (9 compounds)	22	6.61	3.64	43.84
Benz[a]anthracene	22	0.27	0.13	1.81
Benzo[a]pyrene	22	0.42	0.09	6.20
Benzo[b]fluoranthene	22	0.56	0.15	4.22
Benzo[ghi]perylene	22	1.15	0.30	14.14
Benzo[k]fluoranthene	22	0.14	0.04	1.59
Chrysene	22	0.33	0.05	1.79
Dibenz[a,h]anthracene	22	0.05	0.02	0.18
Indeno[1,2,3-cd]pyrene	22	0.79	0.17	7.41
Pyrene	22	2.62	1.60	13.50

¹ DNA adducts measured using the high performance liquid chromatography (HPLC)/fluorescence method, measures BaP-DNA adducts² Bulky/aromatic DNA adducts measured using the ³²P- postlabeling assay³ Hypothesized to be non-diesel combustion related⁴ Hypothesized to be diesel related⁵ Hypothesized to be non-diesel combustion related

Table 3
Spearman Correlation Coefficients between PAH-Hemoglobin Adducts and Additional PAH measures in the Cohort

	1-amino naphthalene	2-aminofluorene	9-amino phenanthrene	1-amino pyrene	BaP-tetrol
1-aminonaphthalene ¹	1.00				
2-aminofluorene ¹	-0.59*	1.00			
9-aminophenanthrene ¹	0.09	0.12	1.00		
1-aminopyrene ¹	-0.55*	0.77**	0.19	1.00	
BaP-tetrol ¹	0.20	0.06	0.03	0.35	1.00
Benzo[a]anthracene ²	-0.06	-0.13	-0.17	-0.23	-0.22
Benzo[a]pyrene ²	0.01	-0.33	-0.20	-0.15	0.00
Benzo[b]fluoranthene ²	0.33	-0.47*	-0.03	-0.25	0.08
Benzo[ghi]perylene ²	0.14	-0.27	-0.21	-0.04	0.07
Benzo[k]fluoranthene ²	0.05	-0.27	-0.01	-0.13	0.00
Chrysene ²	-0.12	-0.05	-0.04	-0.07	-0.24
Dibenzo[a,h]anthracene ²	0.02	-0.41	-0.04	-0.10	0.08
Indeno[1,2,3-cd]pyrene ²	0.13	-0.40	-0.14	-0.24	-0.01
Pyrene ²	-0.11	0.15	-0.21	0.14	0.04
HPLC maternal PAH-DNA adducts ³	0.14	-0.21	0.19	-0.24	-0.02
HPLC cord PAH-DNA adducts ³	-0.01	0.07	0.17	0.20	-0.02
³² P cord bulky/hydrophobic DNA adducts ⁴	0.43	-0.24	-0.25	-0.33	0.15

* p< 0.05

** p< 0.0001

¹ Nitro-PAH hemoglobin adducts

² Air monitored PAH

³ DNA adducts measured using HPLC/fluorescence

⁴ DNA adducts measured using the ³²P-postlabeling assay

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Table 4Loading of Three Principal Components for PAH-Hemoglobin Adducts[/]

Hemoglobin Adduct	Principal Component		
	1	2	3
1-aminonaphthalene	−0.81	0.46	0.18
2-aminofluorene	0.84	0.14	0.08
9-aminophenanthrene	0.14	0.19	0.96
1-aminopyrene	0.67	0.58	−0.13
BaP-tetrol	−0.18	0.92	−0.21

[/]Correlation values between the original data and each principal component

Table 5Loading of Three Principal Components for PAH-Hemoglobin and PAH-DNA Adducts¹

Adducts	Principal Component		
	1	2	3
Hemoglobin Adducts			
1-aminonaphthalene	0.75	-0.41	-0.14
2-aminofluorene	-0.69	0.14	0.37
9-aminophenanthrene	-0.11	0.58	-0.17
1-aminopyrene	-0.61	-0.27	0.54
BaP-tetrol	0.24	-0.83	0.26
DNA Adducts			
HPLC maternal PAH-DNA adducts ²	0.45	0.61	-0.05
HPLC cord PAH-DNA adducts ²	0.36	0.40	0.73
³² P cord bulky/hydrophobic DNA adducts ³	0.60	0.14	0.62

¹Correlation values between the original data and each principal component²DNA adducts measured using HPLC/fluorescence³DNA adducts measured using the ³²P-postlabeling assay