Identification of Surrogate Measures of Diesel Exhaust Exposure in a Controlled Chamber Study

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · JANUARY 2009

Impact Factor: 5.33 · DOI: 10.1021/es800813v · Source: PubMed

6 AUTHORS, INCLUDING:

Jon R Sobus
United States Environmental Protection A…
40 PUBLICATIONS **425** CITATIONS
SEE PROFILE

Joachim D Pleil
United States Environmental Protection A…
136 PUBLICATIONS **1,833** CITATIONS
SEE PROFILE

Stephen Rappaport
University of California, Berkeley
257 PUBLICATIONS **6,965** CITATIONS
SEE PROFILE

Environ. Sci. Technol. **2008,** *42,* 8822–8828

Identification of Surrogate Measures of Diesel Exhaust Exposure in a Controlled Chamber Study

JON R. SOBUS,[†] JOACHIM D. PLEIL,[*,‡] MICHAEL C. MADDEN,[§] WILLIAM E. FUNK,[†] HEIDI F. HUBBARD,[‡] AND STEPHEN M. RAPPAPORT[∥]

School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27599, Human Exposure and Atmospheric Sciences Division, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, Human Studies Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Chapel Hill, North Carolina 27599, and School of Public Health, University of California, Berkeley, California 94720

Received March 21, 2008. Revised manuscript received June 28, 2008. Accepted September 18, 2008.

Exposure to diesel exhaust (DE) has been associated with acute cardiopulmonary and vascular responses, chronic noncancer health effects, and respiratory cancers in humans. To better understand DE exposures and eventually their related health effects, we established a controlled chamber experiment wherein human volunteer subjects were exposed to approximately 100 μg/m^3 DE. In general, human exposure assessment for DE is based on ambient air measurements of surrogates such as elemental carbon (EC) or total organic carbon (OC) collected on filters. As specific health effect mechanisms and dose−response are obscured by the complex composition of DE, the linkage from exposure to internal dose can presumably be improved by use of specific biomarkers and metabolites in blood, breath, or urine. Because EC and OC are not suitable as biomarkers, in this study, we focus on identifying compounds that are demonstrated indicators of DE and can also be found in biological fluids. We measured an assortment of volatile, semivolatile, and particle-bound aromatic compounds in the chamber air and report their airborne concentrations in DE and purified air, as well as the estimated values of the corresponding exposure ratios (mean DE air concentration:mean purified air concentration). These estimated exposure ratios were used to identify naphthalene (Nap) and phenanthrene (Phe) as potentially useful surrogates for DE exposure that could also serve as biomarkers. Estimated mean levels of Nap and Phe associated with the nominal 100 μg/m^3 DE were 2600 and 765 ng/m^3 with estimated exposure ratios of 252 and 92.4, respectively. Nap levels were significantly correlated with OC and total particle-bound polycyclic aromatic hydrocarbons (PAHs); Phe levels were significantly correlated with total volatile + semivolatile PAHs. These results suggest that Nap and Phe may be particularly useful surrogates for DE concentrations. While Nap and Phe are not validated here as internal biomarkers of DE exposure, we are currently assessing human biological specimens collected during this study and will discuss those results in ensuing papers.

1. Introduction

Diesel exhaust (DE) is composed of a mixture of gaseous and particulate contaminants, the exact composition of which depends upon the type of fuel, engine operating conditions, and other factors. Vapor-phase constituents of DE include atmospheric gases, oxides of sulfur and nitrogen, and volatile organic compounds. Particulate matter emitted from diesel engines consists of an elemental carbon core to which a variety of organic and inorganic compounds are adsorbed. Controlled human exposures to DE have been associated with cardiopulmonary changes including lung inflammation, altered vascular response, and cardiac ischemia (*1*−*3*). Chronic DE exposure has been associated with respiratory tumors in rats (*4, 5*) and lung cancers in occupational epidemiological studies (*6, 7*). Thus, DE is considered to be a probable human carcinogen (*4, 8*).

Health effect mechanisms and dose−response relationships remain unclear due to the chemical complexity of DE and limited quantitative exposure data (*4*). Additionally, identification of susceptible subpopulations is not yet clear, further clouding the issue of effective dose (*9*). To simplify the process of assessing exposures to DE, investigators have employed surrogate measures of particulate diesel emissions, including organic carbon (OC), elemental carbon (EC), and fine particulate matter (PM2.5 = particulate matter with aerodynamic diameter less than 2.5 μm) mass (*10*−*12*). These particulate measures may not reflect the many potentially toxic aromatic constituents of DE, notably benzene (Ben) and a host of polycyclic aromatic hydrocarbons (PAHs) in the volatile fraction [e.g., naphthalene (Nap)], the semivolatile fraction [e.g., phenanthrene (Phe) and fluorene (Flu)], and the particle-bound fraction [e.g., benzo[*a*]pyrene (Bap) and chrysene (Chr)]. Therefore, the goal of this study was to characterize volatile, semivolatile, and particle-bound aromatic compounds in DE to identify useful exposure surrogates. Here we present our sampling design and measurement techniques, descriptive statistics, and a correlation analysis to evaluate selected DE surrogates.

2. Materials and Methods

2.1. Study Design. All controlled chamber exposures took place at the EPA National Health and Environmental Effects Research Laboratory (NHEERL) Human Studies Facility in Chapel Hill, NC, with approval from the University of North Carolina at Chapel Hill Biomedical Institutional Review Board (IRB 99-EPA-283; title, Physiological, Cellular, and Biochemical Effects of Diesel Exhaust in Healthy Young Adults). Ten volunteer subjects were exposed once to purified air and once to DE in a random and double-blind fashion, with 2-h exposure sessions separated by a minimum of 3 weeks [further details of volunteer selection are given in Supporting Information (Part 1)]. Purified air used in control exposures was drawn across activated charcoal to remove gaseous organic constituents and high-efficiency particle absolute (HEPA) filters to remove particulates. For DE exposures, exhaust was introduced into the chamber after an approximate 1:30 dilution with purified air. The DE was generated from an idling six-cylinder, 5.9 L-displacement

* Corresponding author e-mail: pleil.joachim@epa.gov.
[†] University of North Carolina.
[‡] National Exposure Research Laboratory, U.S. EPA.
[§] National Health and Environmental Effects Research Laboratory, U.S. EPA.
[∥] University of California.

10.1021/es800813v CCC: $40.75

Published on Web 10/25/2008

TABLE 1. List of Measured Analytes with Respective Analytical Parameters

chemical name	abbreviation	SIM ion (*m*/*z*)	LOQ[a] (ng/sample)	LOQ[b] (ng/m^3)
		Measured by TD GC-MS[c]		
benzene	Ben	78	1.48	123
toluene	Tol	91	3.19	264
ethylbenzene	Etb	106	0.63	52.3
m,*p*-xylene	MP-xyl	106	4.93	408
styrene	Sty	104	2.36	195
o-xylene	O-xyl	106	1.86	154
4-ethyltoluene	Etol	120	2.04	169
1,3,5-trimethylbenzene	Tmb	120	2.28	189
1,2,4-trimethylbenzene	Tmb*	120	10.7	885
naphthalene	Nap	128	0.32	26.6
acenaphthalene	Anap	152	0.09	7.09
acenaphthene	Ace	154	0.12	9.79
fluorene	Flu	166	0.25	20.4
phenanthrene	Phe	178	0.24	20.0
anthracene	Ant	178	0.18	15.0
fluoranthene	Fla	202	0.10	8.31
pyrene	Pyr	202	0.12	9.56
		Measured by LE GC-MS[d]		
benz[*a*]anthracene	BaA	228	0.000 04	0.000 02
chrysene	Chr	228	0.000 12	0.000 06
benzo[*b*]fluoranthene	BbF	252	0.001 47	0.000 76
benzo[*k*]fluoranthene	BkF	252	0.001 38	0.000 72
benzo[*e*]pyrene	BeP	252	0.001 57	0.000 81
benzo[*a*]pyrene	BaP	252	0.000 27	0.000 14
indeno[1,2,3-*cd*]pyrene	Ind	276	0.002 66	0.001 38
benzo[*g*,*h*,*i*]perylene	BgP	276	0.003 03	0.001 57
dibenz[*a*,*h*]anthracene	DaA	278	0.000 03	0.000 02

[a] Limit of quantitation in nanograms per sample is calculated as 3 times the standard deviation of field-blank values. [b] Limit of quantitation in nanograms per cubic meter, with the assumption of a 0.012 m^3 air sample (average volume) for TD GC-MS samples, and a 1.9 m^3 air sample (average volume) for LE GC-MS samples. [c] Thermal desorption coupled with gas chromatography−mass spectrometry. [d] Liquid extraction coupled with gas chromatography−mass spectrometry.

diesel engine (Cummins, Columbus IN), mounted in a vehicle located outside the human studies facility, which burned a certified diesel fuel (Chevron Phillips Chemical Co., Borger, TX; 0.05 LS Certification Fuel, type II). DE particulate levels were feedback-controlled via an exhaust dilution manifold by use of real-time measurements given by a tapered element oscillating microbalance (ThermoFisher Scientific, Franklin, MA) and monitored on a DataRAM aerosol monitor (ThermoFisher Scientific, Franklin, MA). Particle size was measured on a scanning mobility particle sizer (TSI Inc., Shoreview, MN), and PM2.5 exposure concentration was determined on a versatile air pollutant sampler (VAPS) (URG, Chapel Hill, NC). The volume median diameter (±standard deviation, SD) particle size over 10 DE exposure periods was 0.10 ± 0.02 μm, and the estimated mean PM2.5 concentration was 106.3 ± 8.6 μg/m^3. This concentration is comparable to levels encountered at busy intersections in large urban areas (*4*). Further details of pollutant gas and environmental exposure data are available in the Supporting Information (Part 1).

2.2. Collection and Analysis of Volatile Aromatic Compounds and 2−4-Ring PAHs. Volatile aromatic compounds and 2−4-ring PAHs were sampled with custom-made 89 mm × 6.4 mm ($^1/_4$ in.) o.d. aluminum tubes containing 350 mg of 60−80 mesh TenaxTA (Scientific Instrumentation Specialists, Inc., Ringoes, NJ) that was held in place with stainless steel screens. The day prior to each chamber experiment, all adsorbent cartridges were cleaned by thermal desorption at 290 °C for 60 min with a constant helium flow (99.999% purity) of 100 mL/min. After cleaning, tubes were sealed by use of metal Swagelok fittings and were stored overnight at room temperature. Three adsorbent tubes were sampled in parallel inside the chamber for each 2-h exposure period at an air flow of 100 mL/min. At the conclusion of each experiment, adsorbent tubes were removed from the chamber, sealed with metal Swagelok fittings, and stored at −20 °C prior to analysis (up to 2 weeks). Air flow rates were calibrated both before and after air sampling, by use of a DryCal DC-1 flow calibrator (Bios International Corp., Butler, NJ).

Adsorbent tubes were thermally desorbed by use of a Markes Unity thermal desorber coupled to an Ultra autosampler (Markes International, Ltd., Llantrisandt, U.K.), and analyzed on an Agilent 6890N gas chromatograph (GC) coupled to a 5973I mass spectrometer (MS; Agilent, Santa Clara, CA). Sample batches included two reagent blanks, three chamber samples, three field blanks, and two external standards containing all analytes of interest. Each sample tube was thermally desorbed at 260 °C for 10 min, focused on a secondary trap at 0 °C for 3 min, and ballistically desorbed at a maximum temperature of 310 °C for GC injection. A 0.7-mm i.d. injector liner was used in the injection port of the GC, which was held at 250 °C and operated in the splitless mode with a pulse pressure of 103 kPa (15 psi). An RTX-5SILMS (Restek Corp., Bellefonte, PA) fused silica capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness) was used with helium as the carrier gas at a flow rate of 1.1 mL/min. After injection of the sample into the GC, the oven was held at 40 °C for 2 min and then ramped at a rate of 12 °C/min to a final temperature of 300 °C, where it was held for 8 min. The MS transfer line was held at 280 °C, the source temperature at 200 °C, and the quadrupoles at 100 °C. The MS operated with electron ionization (EI) at an ionization voltage of 70 eV. Analytes were identified in the selective-ion monitoring (SIM) mode at *m*/*z* values summarized in Table 1 (first 17 analytes). Quantitation was based on conversion of raw area counts into nanograms per sample values by use of external calibration standards with correction for field blanks. Air concentrations (nanograms per cubic meter) were determined from the volume of air sampled by each

tube. Limits of quantitation (LOQ) were defined as 3 times the standard deviation of field-blank levels for each analyte.

2.3. Collection and Analysis of Particle-Bound PAHs. A VAPS was used to sample the chamber inflow airstream at an average rate of 15 L/min for each 2-h exposure period. Particulate matter, for analysis of particle-bound PAHs, was collected with a preweighed (±10% mass accuracy) 47-mm o.d. 2-μm poly(tetrafluoroethylene) (PTFE) filter (Pall Life Sciences, Ann Arbor, MI). At the end of each exposure period, the filter was reweighed to determine total mass. Samples were stored at −20 °C for up to 6 months prior to liquid extraction and GC-MS analysis. Particle-bound PAHs were analyzed according to a previous method (*13*) with minor modifications. Each sample filter was excised from its polypropylene ring and placed in an 8-mL extraction vial to which a 4-mL solution of HPLC-grade dichloromethane (Burdick & Jackson, Morristown, NJ), containing 4 ng of both ($^2H_{10}$)pyrene (Sigma−Aldrich, St. Louis, MO) and ($^2H_{12}$)benzo[*e*]pyrene (Cambridge Isotope Laboratories, Andover, MA) as internal standards was added). Vials were capped, vortexed for 20 s, and then agitated at 300 rpm for 90 min on an orbital shaker. After agitation, filters were removed from the vials, and the extracts were reduced to 1 mL under high-purity nitrogen gas (National Specialty Gases, Durham, NC). The extracts were transferred into conical high-recovery autosampler vials (Agilent, Santa Clara, CA) and further reduced to 50 μL. For increased storage stability, a solvent exchange was performed; 50 μL of HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA) was added and the remaining dichloromethane was removed under nitrogen gas purge, leaving approximately 50 μL final volume. Samples were sealed with Teflon-lined septum crimp caps and were stored at −20 °C under aluminum foil (light sensitivity) for up to 1 month prior to analysis by GC-MS. An aliquot (2 μL) of each sample extract was injected into an Agilent 6890N GC via a 7683 autoinjector connected to a 5973 MS (Agilent, Santa Clara, CA). Samples were injected in splitless mode with pulse pressure of 138 kPa (20 psi); the injection port contained a gooseneck liner with glass wool (Restek Corp., Bellefonte, PA) held at 275 °C. An RTX-5SILMS (Restek Corp., Bellefonte, PA) fused silica capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness) was used with helium as the carrier gas at a flow of 1.0 mL/min. After injection of the sample into the GC, the oven was held at 50 °C for 3 min, ramped at a rate of 25 °C/min to a temperature of 150 °C, and then ramped at a rate of 10 °C/min to 300 °C, where it was held for 24 min. The MS transfer line was held at 280 °C, the source temperature at 200 °C, and the quadrupoles at 100 °C. The MS operated with EI at an ionization voltage of 70 eV. Nine 4−6-ring PAHs were detected in SIM mode as summarized in Table 1. Quantitation was based on peak areas of selected ions relative to the deuterated internal standard closest in molecular weight to the analyte of interest. LOQs for each compound were defined as 3 times the standard deviation of field-blank levels.

2.4. Collection and Analysis of OC, EC, and PM2.5. During each exposure period, a VAPS configured with PTFE filters sampled PM2.5 in the chamber inlet airstream. Filters were weighed before and after sampling to determine total mass loadings. The VAPS sampler operated at a flow rate of 15 L/min. The average chamber concentration determined from duplicate filters was used for statistical analyses. One quartz fiber filter (Sunset Laboratory, Hillsborough, NC) sample was also collected during each exposure period and analyzed for OC and EC by NIOSH Method 5040 (*14*).

2.5. Statistical Methods. All statistical analyses were performed with SAS statistical software (v. 9.1, SAS Institute, Cary, NC), and all graphs were generated with GraphPad Prism software (v. 4, GraphPad Software, Inc., San Diego, CA). For observations below detection limits (purified air exposures only), a value of LOQ/$\sqrt{2}$ was imputed (*15, 16*). A total of 73 out of 1148 total measurements were below detection limits, mainly for 4−6-ring PAHs. These values were used to generate descriptive statistics for control chamber measurements only and were not used in subsequent statistical analyses. Restricted maximum likelihood estimates of within- and between-day variance components were determined for selected analytes via Proc MIXED of SAS. Values used in mixed-effects modeling satisfied assumptions of normality (Shapiro−Wilks *W* test). Mixed-effects model results are discussed in Supporting Information, Part 2. Spearman correlation coefficients, determined via Proc CORR of SAS, were used to estimate pairwise correlations among analytes. For all tests, $p < 0.05$ (two-tailed) was determined to be statistically significant.

3. Results and Discussion

3.1. Volatile Aromatics and PAHs in DE and Purified Air. Descriptive statistics for measured airborne analytes are presented in Table 2. Estimated mean exposure concentrations of measured analytes in DE are arranged in order of increasing magnitude, as shown by the black bars (from left to right), in Figure 1. Air levels were lowest for particle-bound PAHs, while volatile and semivolatile PAHs had intermediate values, and volatile aromatic compounds had the highest concentrations. Air concentrations of the particle-bound PAHs ranged from 0.068 ng/m^3 for dibenz[*a*,*h*]anthracene (DaA) to 0.346 ng/m^3 for Chr; air concentrations for the volatile and semivolatile PAHs ranged from 17.1 ng/m^3 for fluoranthene (Fla) to 2600 ng/m^3 for Nap; and air concentrations for the volatile aromatic compounds ranged from 3160 ng/m^3 for ethylbenzene (Etb) to 62 100 ng/m^3 for 1,2,4-trimethylbenzene (Tmb*).

For most analytes, measured concentrations in purified air were similar to field blank values (Table 2). Under the standard assumptions satisfying least-squares linear regression of our calibration data (particularly linearity and homogeneity of variance), we calculated these low concentrations using comparisons to batchwise analyses of control standards processed exactly as if they were regular samples at nominal "zero" and "span" levels. Ratios were calculated of the mean analyte air concentration in DE to the mean analyte concentration in purified air. Calculation of the ratio parameter is used as one of two discriminators to differentiate a good from a poor potential DE surrogate. Although a relatively high ratio may not be proof of a useful surrogate, a comparatively low ratio suggests discarding the compound, as it is difficult to differentiate even under the best of conditions. The estimated values of these exposure ratios are shown in Figure 1 by the hatched bars. The largest exposure ratios were observed for the particle-bound PAHs, which ranged from 27.2 for DaA to 288 for Chr, and the smallest exposure ratios were observed for the volatile aromatic compounds, which ranged from 0.776 for styrene (Sty) to 58.5 for Ben. In selecting chemical surrogates for DE exposure, it is desirable to select candidate compounds that are both abundant, to maximize detection, and have high exposure ratios, suggesting that they are highly differentiated with respect to purified air. Our results indicate that although the volatile aromatic compounds were the most abundant, they had low exposure ratios and were, therefore, not highly differentiated from purified air. Particle-bound PAHs, on the other hand, had large exposure ratios but had very low abundance and would be difficult to detect in studies of health effects. The compounds having the best combination of large abundance and large exposure ratios were the volatile and semivolatile PAHs, notably Nap and Phe.

3.2. Nap and Phe as Surrogates for DE Exposure. Naphthalene and phenanthrene clearly stand out among volatile and semivolatile PAHs (see Figure 1) due to their

TABLE 2. Mean Levels (± SD) and Ranges of Analytes Measured in Chamber Air

	diesel exhaust (ng/m^3)			purified air (ng/m^3)		
analyte	mean ± SD	min	max	mean ± SD	min	max
			Gas-Phase Compounds[a]			
Ben	3330 ± 790	217	5130	56.9 ± 33.0	24.3	117
Tol	3670 ± 1090	1970	5320	604 ± 376	177	1290
Etb	3160 ± 929	1660	4180	76.6 ± 62.8	24.0	195
mp-xyl	11 800 ± 4660	4720	19 600	624 ± 610	101	1830
Sty	4910 ± 1410	3140	7210	6330 ± 3280	2290	13 800
o-xyl	4470 ± 1550	2080	6890	306 ± 379	33.4	1310
Etol	6520 ± 3120	2190	12 700	381 ± 571	39.6	1940
Tmb	10 500 ± 4060	4440	18 100	717 ± 847	114	2970
Tmb*	62 100 ± 41 000	17 900	164 000	3210 ± 5280	393	18 000
			Gas- + Particle-Phase Compounds[a]			
Nap	2600 ± 658	1690	3630	10.3 ± 4.94	5.14	21.0
Anap	173 ± 48.5	69.8	232	2.21 ± 1.55	0.354	5.01
Ace	118 ± 19.6	96.7	150	3.40 ± 2.60	0.521	6.92
Flu	158 ± 50.8	72.9	236	5.14 ± 5.20	0.613	14.4
Phe	765 ± 181	507	1070	8.28 ± 4.60	1.32	17.7
Ant	78.6 ± 20.6	53.2	114	4.78 ± 4.37	0.392	10.6
Fla	17.1 ± 6.0	8.17	29.3	3.52 ± 2.75	0.291	7.69
Pyr	89.9 ± 35.8	34.0	145	6.86 ± 6.55	1.31	22.2
			Particle-Phase Compounds[b]			
BaA	0.239 ± 0.076	0.160	0.345	0.0010 ± 0.0018	0.000 06	0.0056
Chr	0.346 ± 0.100	0.220	0.525	0.0012 ± 0.0018	0.000 21	0.0056
BbF	0.307 ± 0.066	0.177	0.390	0.0037 ± 0.0062	0.000 68	0.0190
BkF	0.153 ± 0.037	0.0874	0.214	0.0019 ± 0.0033	0.000 11	0.0100
BeP	0.264 ± 0.036	0.195	0.309	0.0028 ± 0.0043	0.000 15	0.0133
BaP	0.341 ± 0.072	0.169	0.410	0.0036 ± 0.0053	0.000 51	0.0163
Ind	0.205 ± 0.066	0.103	0.320	0.0022 ± 0.0033	0.000 92	0.0104
BgP	0.233 ± 0.076	0.132	0.330	0.0023 ± 0.0026	0.000 70	0.0084
DaA	0.068 ± 0.029	0.0348	0.121	0.0025 ± 0.0048	0.000 01	0.0139

[a] Gas- and gas + particle-phase compounds were collected by use of adsorbent cartridges and analyzed by TD GC-MS. [b] Particle-phase compounds were collected by use of active filter sampling and analyzed by LE GC-MS.

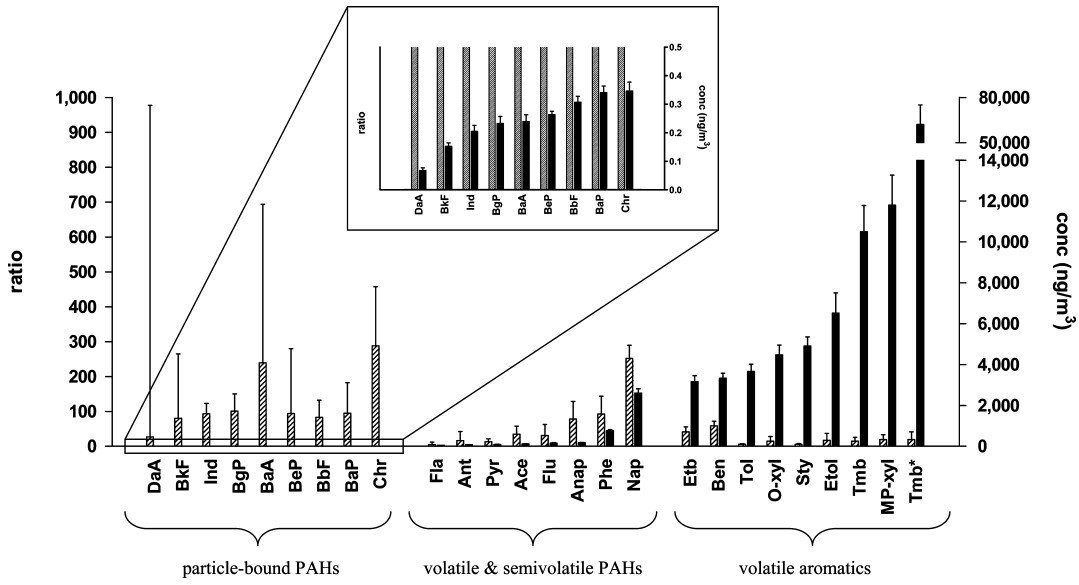

FIGURE 1. Air concentrations of volatile aromatics and PAHs in diesel exhaust, and corresponding exposure ratios. Data are arranged in order of increasing air concentration. Black bars represent estimated mean diesel exhaust exposure concentrations (right *y*-axis); hatched bars represent estimated exposure ratios [(mean diesel exhaust air concentration:mean purified air concentration); left *y*-axis]. Error bars represent estimated SE.

relatively high abundance (mean DE concentrations = 2600 and 765 ng/m^3, respectively) and large exposure ratios (252 and 92.4, respectively). This finding supports results from previous observational studies in which air levels of Nap and Phe were relatively large among populations occupationally exposed to DE (i.e., truck drivers, maintenance and bus garage workers, and toll booth operators *17*−*19*). Table 3 compares the levels of Nap and Phe measured in our chamber experiments with the air concentrations from these earlier investigations. Levels of Nap and Phe in chamber purified air were lower than those measured among literature-reported control subjects (228 and 37 ng/m^3, respectively). Levels of Nap and Phe in chamber DE were very similar to those measured among bus garage workers (1023 and 568

TABLE 3. Comparison of Nap and Phe Levels in Studies Involving Diesel Exhaust Exposure

study	exposure group	air Nap,[a] ng/m^3	air Phe,[a] ng/m^3
Kuusimaki et al., 2002 (*17*)	truck drivers[b]	455 (175−2500)	154 (20−1135)
	maintenance workers[b]	162 (50−476)	53 (<20−395)
	controls[b]	228 (100−510)	37 (<20−215)
Kuusimaki et al., 2003 (*18*)	bus garage workers[b]	1023 (204−3362)	568 (43−2664)
	controls[b]	228 (100−510)	37 (<23−215)
Tsai et al., 2004 (*19*)	toll booth attendants[c]	8850 (5040−12 300)	264 (111−422)
this study	diesel exhaust exposure	2600 (1690−3630)	765 (507−1070)
	purified air exposure	<27	<20

[a] Mean exposure levels and ranges are reported. [b] Mean values of combined winter and summer data; gas-phase measurements only. [c] Values reflect vehicle engine emissions, including those from diesel sources.

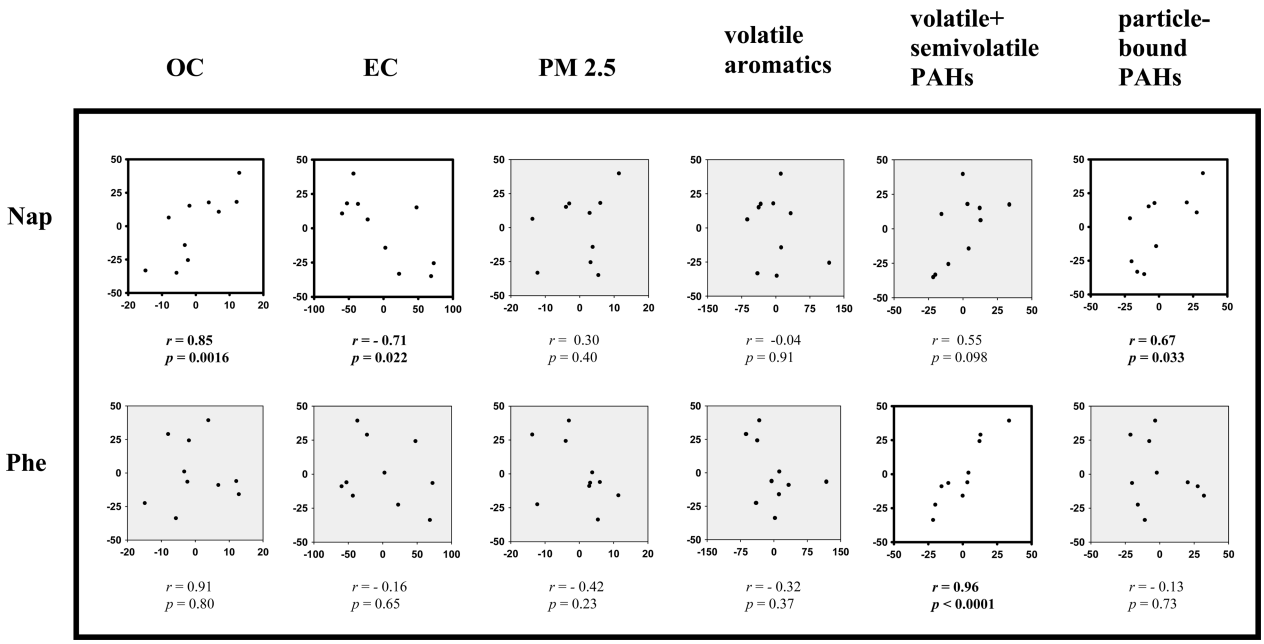

FIGURE 2. Correlations among constituents of diesel exhaust; total DE mass was feedback-regulated to achieve 100 μg/m^3. Nap, naphthalene; Phe, phenanthrene; OC, organic carbon; EC, elemental carbon; PM2.5, fine particulate matter; *r*, Spearman correlation coefficients; *p*, *p*-value. *x*- and *y*-axes represent percent deviation from mean exposure level ($n = 10$).

ng/m^3, respectively), and were higher than those measured among truck drivers (455 and 154 ng/m^3, respectively) and maintenance workers (162 and 53 ng/m^3, respectively). Whereas Nap levels in chamber DE were lower than those measured among toll booth attendants (8850 ng/m^3), Phe levels were higher in chamber DE compared to toll booth attendant exposure levels (264 ng/m^3). We note that Nap and Phe measurements among toll booth attendants reflect exposures from diesel and nondiesel sources (i.e., gasoline-powered engines). As few studies have published individual Nap and Phe measurements from strictly diesel sources, the data are included here for a general comparison with our controlled exposure levels.

Naphthalene and phenanthrene possess physiochemical properties that make them potentially desirable surrogates for DE. Naphthalene is generally the most abundant PAH measured from a given source (*20*). Furthermore, because Nap exposure occurs almost entirely in the vapor phase, it can be measured by passive adsorbent techniques (*21*). Such techniques eliminate the need for cumbersome and expensive sampling equipment, thereby increasing the ability to obtain more air measurements at a given cost for studies of health effects (*22*). Naphthalene also offers several potentially useful biomarkers of PAH exposure, including unmetabolized Nap in urine (*23, 24*), several urinary metabolites (*25*−*27*), and protein adducts of the naphthoquinones (metabolites of Nap) in human serum albumin (*28*). Finally, since Nap is classified as a possible human carcinogen (*29*), it is useful to characterize human exposures to Nap per se. Phenanthrene is also abundant in PAH exposure scenarios, in both vapor and particle-bound phases. Vapor-phase Phe can presumably be measured in air by passive adsorbent techniques. While Phe is not classified as a human carcinogen (*30*), its metabolism closely follows that of carcinogenic PAHs (*31, 32*); numerous Phe metabolites can be measured in human urine as biomarkers of PAH exposure (*32*−*34*).

3.3. Correlations among Surrogate Measures of DE Exposure. Although chamber DE exposures were highly regulated based on real-time particulate measurements, individual analytes associated with DE displayed variability. While this variability was very modest compared to that observed in environmental and occupational settings (*35*), significant daily variations in individual analyte levels were observed (see Supporting Information, Part 2, for a detailed variability assessment for Nap and Phe). In considering Nap and Phe as potential surrogates for DE exposure, it is particularly useful to compare the air concentrations of Nap and Phe with those of accepted measures of particulate exposure, namely, OC, EC, and PM2.5, as well as with the combined air concentrations of PAHs in the particle-bound phase (containing the most carcinogenic compounds (*30*)). Figure 2 shows a correlation matrix for the estimated mean air levels of Nap, Phe, OC, EC, PM2.5, total volatile aromatics, total volatile + semivolatile PAHs (minus Nap and Phe), and

total particle-bound PAHs for the 10 subjects exposed to DE in our study (only measurements made during actual DE exposures were included in this correlation analysis). Air concentrations of Nap were significantly correlated with OC ($r = 0.85$, $p = 0.0016$), EC ($r = -0.71$, $p = 0.022$), and particle-bound PAHs ($r = 0.67$, $p = 0.033$). The negative correlation between Nap and EC mirrors that which was observed between OC and EC ($r = -0.64$, $p = 0.048$; not shown in Figure 2). As DE is composed of an elemental carbon core with adsorbed organics, it stands to reason that when the total mass is held constant (as was the case during our controlled exposures), the EC and OC components of total mass should be negatively correlated. While EC is a useful DE exposure surrogate in occupational studies (*10*−*12, 36*), the positive correlations between Nap and OC and between Nap and total particle-bound PAHs suggest that Nap may be a particularly good surrogate for the organic constituents of DE. This finding is even more interesting when it is considered that air levels of Nap have previously been shown to be highly correlated with those of total PAHs in studies of workers in the steel and aluminum industries, where PAH levels are particularly high (*20*). In addition to the observed Nap correlations, a highly significant pairwise correlation was observed between concentrations of Phe and total volatile + semivolatile PAHs ($r = 0.96$, $p < 0.0001$), suggesting that Phe may also be a useful surrogate for selected organic DE consituents.

3.4. Concluding Remarks. Naphthalene and phenanthrene are promising surrogate markers for the broad spectrum of airborne constituents resulting from DE sources. They are both abundant in DE and highly differentiated in trace-level measurements with respect to purified ambient air. In addition, Nap and Phe are easily measured in air and can also be assayed as biomarkers of exposure. Although the chamber concentrations were highly regulated based on real-time particulate measurements, individual analytes associated with DE displayed variability. We attribute this to small differences in organic enrichment of the particles, stemming from subtle changes in truck engine performance and weather conditions. Despite this observed variability, significant positive correlations between concentrations of Nap and other DE markers (i.e., OC and total particle-bound PAHs) and between Phe and total volatile + semivolatile PAHs suggest that Nap and Phe could serve as quantitative markers for DE concentrations in future health effects studies and epidemiological risk assessments. We caution that all of the common DE markers may have other environmental sources as well and that other metadata will be important for assessing their relative contributions. However, we conclude from this work that Nap and Phe are optimal surrogates if a direct comparison between biomarker and external source is sought. We are currently assessing a series of metabolic biomarkers and response parameters in human biological specimens collected during this study and will discuss those results in ensuing papers.

Acknowledgments

The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and collaborated in the research described here. Chamber operation was conducted under Contract EP-D-05-101 to TRC Environmental Corporation. Analyses of OC and EC, along with aromatic analyte standards preparation, were performed under Contract EP-D-05-065 to Alion Sciences and Technology. Training Grant T32 ES7018 from the National Institute of Environmental Health Sciences to the University of North Carolina, Chapel Hill, provided partial support. Some funding was provided for operational support through EPA's Student Services Contracts Program. This work has been subjected to Environmental Protection Agency review and approved for publication. We appreciate the expert advice, assistance, and review from Jon Berntsen of TRC, Karen Oliver and Hunter Daughtrey of Alion, and Alan Vette, Nicolle Tulve, Myriam Medina-Vera, and Linda Sheldon of U.S. EPA.

Supporting Information Available

Description of volunteer selection, pollutant gas and environmental exposure data, and variability in chamber measurements of Nap and Phe. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Stenfors, N.; Nordenhall, C.; Salvi, S. S.; Mudway, I.; Soderberg, M.; Blomberg, A.; Helleday, R.; Levin, J. O.; Holgate, S. T.; Kelly, F. J.; Frew, A. J.; Sandstrom, T. Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. *Eur. Respir. J.* **2004**, *23*, 82–86.

(2) Mills, N. L.; Tornqvist, H.; Robinson, S. D.; Gonzalez, M.; Darnley, K.; MacNee, W.; Boon, N. A.; Donaldson, K.; Blomberg, A.; Sandstrom, T.; Newby, D. E. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation* **2005**, *112*, 3930–3936.

(3) Mills, N. L.; Tornqvist, H.; Gonzalez, M. C.; Vink, E.; Robinson, S. D.; Soderberg, S.; Boon, N. A.; Donaldson, K.; Sandstrom, T.; Blomberg, A.; Newby, D. E. Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. *N. Engl. J. Med.* **2007**, *357*, 1075–1082.

(4) Health Assessment Document for Diesel Engine Exhaust; U.S. Environmental Protection Agency: Washington, DC, 2002; http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=29060.

(5) Diesel exhaust: a critical analysis of emissions, exposure and health effects; Health Effects Institute: Cambridge, MA, 1995; http://www.healtheffects.org/Pubs/diesum.htm.

(6) Bhatia, R.; Lopipero, P.; Smith, A. H. Diesel exhaust exposure and lung cancer. *Epidemiology* **1998**, *9*, 84–91.

(7) Lipsett, M.; Campleman, S. Occupational exposure to diesel exhaust and lung cancer: A meta-analysis. *Am. J. Public Health* **1999**, *89*, 1009–1017.

(8) Monographs on the evaluation of carcinogenic risks to humans, Volume *46*: *Diesel and gasoline engine exhausts and some nitroarenes*; International Agency for Research on Cancer: Lyon, France, 1989; http://monographs.iarc.fr/ENG/Monographs/vol46/volume46.pdf.

(9) Gilliland, F. D.; Li, Y. F.; Saxon, A.; Diaz-Sanchez, D. Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. *Lancet* **2004**, *363*, 119–125.

(10) Davis, M. E.; Smith, T. J.; Laden, F.; Hart, J. E.; Ryan, L. M.; Garshick, E. Modeling particle exposure in U.S. trucking terminals. *Environ. Sci. Technol.* **2006**, *40*, 4226–4232.

(11) Davis, M. E.; Smith, T. J.; Laden, F.; Hart, J. E.; Blicharz, A. P.; Reaser, P.; Garshick, E. Driver exposure to combustion particles in the U.S. trucking industry. *J. Occup. Environ. Hyg.* **2007**, *4*, 848–854.

(12) Smith, T. J.; Davis, M. E.; Reaser, P.; Natkin, J.; Hart, J. E.; Laden, F.; Heff, A.; Garshick, E. Overview of particulate exposures in the U.S. trucking industry. *J. Environ. Monit.* **2006**, *8*, 711–720.

(13) Pleil, J. D.; Vette, A. F.; Rappaport, S. M. Assaying particle-bound polycyclic aromatic hydrocarbons from archived PM2.5 filters. *J. Chromatogr. A* **2004**, *1033*, 9–17.

(14) Elemental Carbon (Diesel Particulate) 5040. In *NIOSH Manual of Analytical Methods*, 4th ed.; Cassinelli, M. E., O'Connor, P. F., Eds.; National Institute for Occupational Safety and Health: Atlanta, GA, 1998.

(15) Hornung, R. W.; Reed, L. D. Estimation of average concentration in the presence of nondetectable values. *Appl. Occup. Environ. Hyg.* **1990**, *5*, 46–51.

(16) Pleil, J. D.; Lorber, M. N. Relative congener scaling of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to estimating building fire contributions in air, surface wipes, and dust samples. *Environ. Sci. Technol.* **2007**, *41*, 7286–7293.

(17) Kuusimaki, L.; Peltonen, Y.; Kyyro, E.; Mutanen, P.; Peltonen, K.; Savela, K. Exposure of garbage truck drivers and maintenance personnel at a waste handling centre to polycyclic aromatic hydrocarbons derived from diesel exhaust. *J. Environ. Monit.* **2002**, *4*, 722–727.

(18) Kuusimaki, L.; Peltonen, K.; Mutanen, P.; Savela, K. Analysis of particle and vapour phase PAHs from the personal air samples of bus garage workers exposed to diesel exhaust. *Ann. Occup. Hyg.* **2003**, *47*, 389–398.

(19) Tsai, P. J.; Shih, T. S.; Chen, H. L.; Lee, W. J.; Lai, C. H.; Liou, S. H. Urinary 1-hydroxypyrene as an indicator for assessing the exposures of booth attendants of a highway toll station to polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* **2004**, *38*, 56–61.
(20) Rappaport, S. M.; Waidyanatha, S.; Serdar, B. Naphthalene and its biomarkers as measures of occupational exposure to polycyclic aromatic hydrocarbons. *J. Environ. Monit.* **2004**, *6*, 413–416.
(21) Egeghy, P. P.; Hauf-Cabalo, L.; Gibson, R.; Rappaport, S. M. Benzene and naphthalene in air and breath as indicators of exposure to jet fuel. *Occup. Environ. Med.* **2003**, *60*, 969–976.
(22) Rappaport, S. M. Assessment of long-term exposures to toxic substances in air. *Ann. Occup. Hyg.* **1991**, *35*, 61–121.
(23) Waidyanatha, S.; Zheng, Y.; Rappaport, S. M. Determination of polycyclic aromatic hydrocarbons in urine of coke oven workers by headspace solid phase microextraction and gas chromatography-mass spectrometry. *Chem.-Biol. Interact.* **2003**, *145*, 165–174.
(24) Campo, L.; Addario, L.; Buratti, M.; Scibetta, L.; Longhi, O.; Valla, C.; Cirla, P. E.; Martinotti, I.; Foa, V.; Fustinoni, S. Biological monitoring of exposure to polycyclic aromatic hydrocarbons by determination of unmetabolized compounds in urine. *Toxicol. Lett.* **2006**, *162*, 132–138.
(25) Serdar, B.; Egeghy, P. P.; Gibson, R.; Rappaport, S. M. Dose-dependent production of urinary naphthols among workers exposed to jet fuel (JP-8). *Am. J. Ind. Med.* **2004**, *46*, 234–244.
(26) Serdar, B.; Waidyanatha, S.; Zheng, Y.; Rappaport, S. M. Simultaneous determination of urinary 1- and 2-naphthols, 3- and 9-phenanthrols, and 1-pyrenol in coke oven workers. *Biomarkers* **2003**, *8*, 93–109.
(27) Wu, R.; Waidyanatha, S.; Henderson, A. P.; Serdar, B.; Zheng, Y.; Rappaport, S. M. Determination of dihydroxynaphthalenes in human urine by gas chromatography-mass spectrometry. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2005**, *826*, 206–213.
(28) Waidyanatha, S.; Zheng, Y.; Serdar, B.; Rappaport, S. M. Albumin adducts of naphthalene metabolites as biomarkers of exposure to polycyclic aromatic hydrocarbons. *Cancer Epidemiol. Biomarkers Prev.* **2004**, *13*, 117–124.
(29) Monographs on the evaluation of carcinogenic risks to humans, Volume *82*: *Some traditional herbal medicines, some mycotoxins, naphthalene and styrene*; International Agency for Research on Cancer: Lyon, France, 2002; http://monographs.iarc.fr/ENG/Monographs/vol82/volume82.pdf.
(30) Monographs on the evaluation of carcinogenic risks to humans, Volume *32*: *Polynuclear aromatic compounds, Part 1, Chemical, environmental and experimental data*; International Agency for Research on Cancer: Lyon, France, 1983; http://monographs.iarc.fr/ENG/Monographs/vol32/volume32.pdf.
(31) Shou, M.; Korzekwa, K. R.; Krausz, K. W.; Crespi, C. L.; Gonzalez, F. J.; Gelboin, H. V. Regio- and stereo-selective metabolism of phenanthrene by twelve cDNA-expressed human, rodent, and rabbit cytochromes P-450. *Cancer Lett.* **1994**, *83*, 305–313.
(32) Carmella, S. G.; Chen, M.; Yagi, H.; Jerina, D. M.; Hecht, S. S. Analysis of phenanthrols in human urine by gas chromatography-mass spectrometry: potential use in carcinogen metabolite phenotyping. *Cancer Epidemiol. Biomarkers Prev.* **2004**, *13*, 2167–2174.
(33) Hecht, S. S.; Chen, M.; Yagi, H.; Jerina, D. M.; Carmella, S. G. r-1,t-2,3,c-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. *Cancer Epidemiol. Biomarkers Prev.* **2003**, *12*, 1501–1508.
(34) Kim, J. Y.; Hecht, S. S.; Mukherjee, S.; Carmella, S. G.; Rodrigues, E. G.; Christiani, D. C. A urinary metabolite of phenanthrene as a biomarker of polycyclic aromatic hydrocarbon metabolic activation in workers exposed to residual oil fly ash. *Cancer Epidemiol. Biomarkers Prev.* **2005**, *14*, 687–692.
(35) Lin, Y. S.; Kupper, L. L.; Rappaport, S. M. Air samples versus biomarkers for epidemiology. *Occup. Environ. Med.* **2005**, *62*, 750–760.
(36) Zaebst, D. D.; Clapp, D. E.; Blade, L. M.; Marlow, D. A.; Steenland, K.; Hornung, R. W.; Scheutzle, D.; Butler, J. Quantitative determination of trucking industry workers' exposures to diesel exhaust particles. *Am. Ind. Hyg. Assoc. J.* **1991**, *52*, 529–541.

ES800813V