Generation and Characterization of Four Dilutions of Diesel Engine Exhaust for a Subchronic Inhalation Study

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Exposure atmospheres for a rodent inhalation toxicology study were generated from the exhaust of a 2000 Cummins ISB 5.9L diesel engine coupled to a dynamometer and operated on a slightly modified heavy-duty Federal Test Procedure cycle. Exposures were conducted to one clean air control and four diesel exhaust levels maintained at four different dilution rates (300:1, 100:1, 30:1, 10:1) that yielded particulate mass concentrations of 30, 100, 300, and 1000 μg/m^3. Exposures at the four dilutions were characterized for particle mass, particle size distribution (reported elsewhere), detailed chemical speciation of gaseous, semivolatile, and particle-phase inorganic and organic compounds. Target analytes included metals, inorganic ions and gases, organic and elemental carbon, alkanes, alkenes, aromatic and aliphatic acids, aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH), oxygenated PAH, nitrogenated PAH, isoprenoids, carbonyls, methoxyphenols, sugar derivatives, and sterols. The majority of the mass of material in the exposure atmospheres was gaseous nitrogen oxides and carbon monoxide, with lesser amounts of volatile organics and particle mass (PM) composed of carbon (~90% of PM) and ions (~10% of PM). Measured particle organic species accounted for about 10% of total organic particle mass and were mostly alkanes and aliphatic acids. Several of the components in the exposure atmosphere scaled in concentration with dilution but did not scale precisely with the dilution rate because of background from the rodents and scrubbed dilution air, interaction of animal derived emissions with diesel exhaust components, and day-to-day variability in the output of the engine. Rodent-derived ammonia reacted with exhaust to form secondary inorganic particles (at different rates dependent on dilution), and rodent respiration accounted for volatile organics (especially carbonyls and acids) in the same range as the diesel exhaust at the lowest exhaust exposure concentrations. Day-to-day variability in the engine output was implicated partially for differences of several components, including some of the particle bound organics. Though these observations have likely occurred in nearly all inhalation exposure atmospheres that contain complex mixtures of material, the speciations conducted here illustrate many of them for the first time.

Introduction

The National Environmental Respiratory Center (NERC) was established to conduct laboratory research improving our understanding of the contributions of individual air contaminants and their combinations to the health effects associated with exposures to complex air pollution mixtures. The NERC research strategy (described at www.nercenter.org (*1*)) involves conducting a series of inhalation toxicology studies of common source emissions having different, but overlapping, compositions. Both the composition of the exposure atmospheres and a range of health effects are measured in detail using identical protocols for all source emissions, allowing the combination of results from all studies into a single composition-concentration—response database. The NERC strategy will provide contemporary comparisons among the health hazards of key source emissions (e.g., diesel and gasoline engine exhaust, wood smoke, coal emissions, etc.), and analysis of the combined database from all studies will point toward the roles of individual compounds, classes of compounds, and combinations of compounds in the health effects of complex exposures. The first exposure, termed "Contemporary Diesel Emissions" (ca. 2000), is completed. The design of the generation and exposure system and the composition of the exposure atmospheres at four dilutions of fresh emissions are described here. The health responses are being reported in separate papers.

Exposures included four dilutions of diesel engine exhaust (DEE) and one clean air control. Exposure concentrations were selected to span a range that would likely yield some biological effects at the highest exposure level down to the lowest level that was considered practical to maintain over a 6-h exposure duration. It was also desirable to keep the highest exposure level in the range of plausible human exposure concentrations. For DEE, the highest known human exposures occur in occupational environments, especially underground mines where concentrations of DEE particulate matter (PM) in the range of 1000 μg/m^3 are common (e.g., ref *2*). Based on this, the high exposure level used for this study was DEE diluted to 1000 μg/m^3 PM. The lowest level (30 μg/m^3 PM) during this study was higher than typical DEE contributions to ambient PM (*3*) but in the range (~30—60 μg/m^3 DEE PM) of what has been observed for environmental exposures in microenvironments on or near roadways (e.g., refs *3*—*5*). The intermediate exposure levels were chosen based on log-dilutions between the high and low exposure level, resulting in DEE exposure levels containing PM concentrations of 30, 100, 300, and 1000 μg/m^3. Although PM mass was selected as the metric for specifying exposure concentrations, all components of the exposure atmosphere were of interest.

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Exposure chamber composition was assessed for several parameters, including a range of physical and chemical characteristics selected on the basis of components that have been targeted as potentially important for health effects studies (*6*) and that have been identified in comprehensive studies of source emissions (e.g., (*7*−*14*)), microenvironments such as road tunnel studies (e.g., refs *15*−*17*), and ambient assessments (e.g., ref *18*). Measurements were made to characterize PM composition, particle number count (reported elsewhere) and size distribution (reported elsewhere), elements (metals and associated analytes), inorganic sulfate, nitrate, and ammonium, PM organic and elemental (also termed black carbon) carbon (EC), particle size distribution, and speciated organic compounds (including alkanes, alkenes, alkynes, cycloalkanes/cycloalkenes, furans, phenols, sugars, acids, carbonyls, volatile aromatic compounds, polycyclic aromatic hydrocarbons (PAH), oxygenated PAH, hopanes, and steranes. Gaseous ammonia, oxides of nitrogen (NO_x), sulfur dioxide (SO_2), carbon monoxide (CO), and carbon dioxide (CO_2) were also measured. Several of the components were semivolatile organic compounds (SVOCs) that existed simultaneously in the gas and particle phase.

Because this characterization resulted in thousands of compositional data points, it was not practical to publish the data in their entirety. Instead, this paper describes the experimental methodology and summarizes major findings. The entire data set for the study is available on the NERC Web Site (*1*). Data were evaluated in the context of previous DEE compositional studies (on-road and laboratory) as well as previous/future DEE inhalation studies.

Experimental Setup

This work utilized a wide range of techniques for detailed characterization of exposure atmospheres. Most of these techniques are not novel, though for many their use for inhalation toxicology is novel, and the details of their implementation and operation are critical if this work were to be reproduced. Due to page limitations, the text of the experimental has been limited to describe the general approach for the setup and characterization of these exposure systems. To provide adequate detail for those interested in repeating this work or placing specific method in context, a more detailed account of the methods is available as Supporting Information.

Exhaust Generation and Dilution. Diesel exhaust was generated from either of two 2000 model Cummins ISB Turbo Diesel 5.9-L engines using Number 2 certification diesel fuel (Chevron Phillips Chemical Co., Borger, TX, see the Supporting Information for specifications) and 15 w/40 lubrication oil (Rotella T, Shell, Houston, TX). Lubrication oil and filters (Fleetguard LF3349, Cummins, Columbus, IN) were changed after every 200 h of engine operation. Engines were mated to eddy current dynamometers (Alpha-240, Kiel, FRG) and operated on repeated transient engine cycle that was based on the U.S. Environmental Protection Agency Engine Dynamometer Schedule for Heavy-Duty Diesel Engines (*19*)). Further details on the engine test system (including dilution design considerations and system schematics) and the specific dynamometer cycle are given in the Supporting Information. During exposures, exhaust passed through a muffler and tailpipe and was immediately diluted (5:1) with charcoal and HEPA-filtered cool (∼15 °C) ambient air. Immediately after extraction from the primary dilution tunnel, the exhaust was further diluted and cooled using a series of dilution/dilution dump ports. To provide similar material to each exposure atmosphere, all delivery lines were constructed with the same dimensions, material (stainless steel), and lengths. In each exposure line, an in-line flow-through muffler was used to reduce sound in the exposure chambers to less than 85 dB.

Exposure Atmospheres. Exposures were conducted in 2-m^3 flow-through exposure chambers (H2000, Hazleton Systems, Maywood, NJ). Material passed through these chambers at approximately 500 L/min, yielding a material residence time within the chamber of about 4 min. These chambers were designed to enhance the uniformity of the distribution of test material to ensure similar exposures are encountered throughout tests (*20*). As described by McDonald et al. (*21*), the homogeneity of DEE PM within these chambers was good, with a maximum of 6.5% variability throughout. The exposures included five treatment groups: four exposed to different dilutions of engine exhaust and one to clean air as a control group. The target exposure levels were 30 (low), 100 (mid low), 300 (mid high), and 1000 (high) $\mu g/m^3$ of DEE PM. At the highest exposure level, the dilution from the tailpipe was determined to be ∼10:1 by measuring CO directly from the tailpipe and in the exposure chamber.

Exposures were conducted daily for 6 h plus an additional 8 min that was the amount of time it took the concentration in the chambers to rise to 90% of the target concentration once the DEE was diverted to the chambers. Because several different health models were used in this study, the durations of exposure varied from several days to 6 months, and, because of staggered exposures, the study duration was nearly 8 months (10/23/2000−6/10/2001). The summary data also include some additional measurements that were obtained as part of a follow-on experiment (3/22/03−6/20/03) to repeat some health exposures from the first group. Exposure concentrations were maintained at target levels by monitoring NO_x and PM throughout the exposure period and manually adjusting dilution air to achieve the targets.

Characterization of Exposure Atmospheres. Because they were used to monitor and control dilution, PM and NO_x concentrations were measured each day of the study. Due to practical and cost limitations, detailed characterizations of exposure atmospheres were conducted only periodically (two to four times/level), and these "intensive" sampling/monitoring periods were only conducted at one exposure level (chamber) per day. Samples for daily monitoring and many of the detailed measurements were drawn from the breathing zone of the animals through one of four sampling ports on the front panel of the exposure chambers (see schematics in the Supporting Information). A limitation of these experimental setups is that a sample flow above 10 L/min will disturb the homogeneous spatial distribution of airborne material in the chamber. To accurately measure low concentrations of some analytes, a larger volume of sample was necessary. To accommodate this, some samples were drawn from the chamber exhaust in a stainless steel sampling plenum. This approach was validated as described in the Supporting Information.

Sampling Approach. The range of measurements utilized in the study, the collection/analysis technique, sampling conditions, sample location (plenum versus chamber), and analysis allocation (supporting laboratories) are summarized in Table 1 (particle size distribution measurements were taken but are not discussed here). Sample transit lines were composed of either stainless steel (for PM) or Teflon (for gases), and all sample flow rates were monitored per National Institute of Standards and Technology (NIST) standards. Flow rates < 2 L/min were monitored with a Dry-Cal flow meter (Gillian, Sensidyne Inc., St. Petersburg, FL). Flows between 2 and 30 L/min were measured with a bubble flow meter (Gillian, Sensidyne Inc., St. Petersburg, FL), and higher flow rates were measured with a hot wire anemometer (TSI, Inc., Model 4000).

Particle Mass. PM was collected on 47-mm Pallflex (Pall-Gelman, East Hills, NY) filters. Pre- and postexposure filter weights were measured gravimetrically immediately prior to and after exposures using a Mettler MT5 microbalance

TABLE 1. Summary of Exposure Atmosphere Characterization Measurements and Measurement Conditions[a]

analysis	collection device	collection media	collection point	sample flow rate (L/m)	analytical instrument	analysis allocation
gravimetric mass	aluminum in-line filter holder	TIGF	chamber/ plenum	4	MB	LRRI
continuous mass	Dust-Trak nephelometer	NA	chamber	2	NA	LRRI
nitric oxides	chemiluminescence analyzer	NA	chamber	0.4	NA	LRRI
CO/CO_2/THC	photoacoustic analyzer	NA	chamber	1	NA	LRRI
THC	flame ionization detector	NA	chamber	1	NA	LRRI
organic/black carbon	aluminum in-line filter holder	quartz filter (1)	plenum	20	TOR	DRI
ions (sulfate/nitrate/ ammonium)	aluminum in-line filter holder	quartz filter (2)	plenum	20	IC, AC	DRI
SO_2	aluminum in-line filter holder	K_2CO_3-impregnated filter	chamber	2−5	IC	DRI
NH_3	URG denuder (20 cm, 4 channel)	citric acid-coated	chamber	5	AC	DRI
metals and other elements	Teflon in-line filter holder	Teflon filter (2)	plenum	20	ICPMS/ICPOES	WSLH
volatile hydrocarbons (C_1−C_{12})	volatile organic sampler	electropolished canister	chamber	0.1	GCMS/GCFID	DRI
volatile carbonyls	volatile organic sampler	DNPH cartridge	chamber	0.3	LC/DAD/MS	DGA, Inc.
volatile organic acids	aluminum in-line filter holder	KOH-impregnated quartz filter	chamber	1−2	LC/DAD/MS	DGA, Inc.
semivolatile/fine particle organics (1)	Tisch environmental PUF sampler	quartz filter/PUF/ XAD-4/PUF	plenum	80	GCMS	LRRI
semivolatile/fine particle organics (2)	URG denuder (60 cm, 8 channel)	XAD-coated/filter/ PUF/XAD-4/PUF	plenum	80	GCMS	LRRI

[a] List of abbreviations: *Chemical species*: CO = carbon monoxide; CO_2 = carbon dioxide; SO_2 = sulfur dioxide; NH_3 = ammonia; THC = total hydrocarbons. *Instruments/collection devices*: MB = microbalance; NA = not applicable; TOR = thermal/optical reflectance; IC = ion chromatography; ICPMS = inductively coupled plasma mass spectrometry; ICPOES = inductively coupled plasma optical emission spectroscopy; GCMS = gas chromatography mass spectrometry; GCFID = gas chromatography flame ionization detector; DNPH = dinitrophenylhydrazine; LC/DAD/MS = liquid chromatography/diode array/mass spectrometry; PUF=polyurethane foam; GCMS/LCMS = gas chromatography mass spectrometry/liquid chromatography mass spectrometry; URG = URG Corporation; MOUDI = micro orifice uniform deposit impactor; SMPS = scanning mobility particle sizer; CPC = condensation particle counter. TIGF = Teflon impregnated glass fiber filter. AC= automated colorimetry. *Organizations*: LRRI = Lovelace Respiratory Research Institute; DRI = Desert Research Institute; WSLH = Wisconsin State Laboratory of Hygiene; DGA, Inc. = Daniel Grosjean and Associates, Inc.

located in the exposure laboratory. A static discharger was used prior to weighing the filters to avoid any interference from electrical charge on the filters. Filter samples were collected three times daily (every 2 h) from the high and mid high exposure levels and twice (every 3 h) from the low and mid low levels. One filter sample per day was collected from the control chamber. Because filter based PM data were utilized for monitoring and controlling PM concentrations during exposures, it was not practical to equilibrate the filters prior to and after sampling in a conditioning chamber at a specified temperature and humidity. Some samples were taken to assess the difference in measured PM concentration after filter conditioning for 24 h at 23 °C and 40% relative humidity prior to and after sampling, and results did not vary more than 10%.

Real-Time Particle Mass. Real-time PM was measured using a Model 8520 DustTrak Nephelometer (TSI, Inc., Minneapolis, MN) to assess system stability and serve as an indicator of chamber concentration in order to adjust dilutions for achieving the target mass concentration. The DustTrak response was calibrated for DEE PM by making simultaneous gravimetric measurements and deriving a correction factor for the DustTrak response based on filter weights.

Gas Analysis (NO_x, CO, CO_2, VOC). Chambers were monitored continuously for the concentration of NO_x (NO + NO_2) and periodically for the concentration of CO, CO_2, and total VOCs. The measurement techniques are shown in Table 1 and described in detail in the Supporting Information.

Carbon. Organic carbon (OC) and EC were measured by the IMPROVE technique (*22*) on quartz-fiber filters (Pall-Gelman, QAO, East Hills, NY, 47-mm diameter, heat treated) collected using the same type of probe as for PM sampling. Details on precleaning procedures and analytical technique are described both in the Supporting Information and in Chow et al.[22] The OC measurement only quantified the carbon component of organic carbon species; thus, mass concentration reported by this method did not reflect mass contributions from hydrogen, oxygen, and other elements associated with OC species in the air. To estimate the actual OC mass concentration, a correction factor of 1.2 was applied as recommended by Pierson and Brachaczek (*23*) for organic mass (OM) emitted from vehicles. The OC concentration reported here is thus defined as OM.

Inorganic Ion Analysis. Inorganic ions were analyzed either by ion chromatography (*24*) for sulfate (SO_4^{2-}) and nitrate (NO_3^-) or the indolphenol colorimetric method for ammonium (NH_4^+), all collected on the same quartz-fiber filters described for carbon. The inorganic ion analyses for SO_4^{2-} and NH_4^+ were also used for the analysis of SO_2 and NH_3 that were collected and extracted from an impregnated filter or citric acid coated denuder as described in the Supporting Information.

Elements (Metals and Associated Analytes). Samples for metal determination were collected through a 15.2-cm long by 1.3-cm i.d. probe onto Teflo membranes (Pall-Gelman, 2.0-μm pore size, 47-mm diameter) filters that were housed in a Teflon-coated filter cartridge (URG, Inc.). All filters were precleaned at the Wisconsin State Laboratory of Hygiene (WSLH) using a flow-through rinsing protocol that incorporated sequential leaches with high-purity 2 N HCl, 2 N HNO_3, and purified (Milli-Q) water. Filters were handled with Teflon forceps to minimize contamination from metallic materials. After collection, samples were immediately placed in a freezer at −20 °C prior to shipment (priority overnight)

to the WSLH under "cold" conditions (frozen blue ice in an ice chest).

Elemental analysis was performed using inductively coupled plasma mass spectrometry (ICP-MS) and ICP-optical emission spectroscopy (ICP−OES) techniques. ICP−OES provided a more robust measurement for a select few high concentration light elements and complemented the very large element menu attainable with ICP-MS. While the methods were slightly different for this work, the general strategy for the analysis and sample handling were as described previously (*25*). Specific details on the extraction/analysis procedures used here and the target analyte list are given in the Supporting Information.

Speciated Volatile Organics. Volatile organic speciation of alkanes, alkenes, alkynes, isoalkane/isoalkene, alcohols, and aromatic compounds were measured in collaboration with the DRI. Sampling and canister handling was done as described by Zielinska et al. (*26*). Methane was analyzed as described by the Zielinska et al. (*26*). Analysis of the remaining target analytes was conducted by two-dimensional gas chromatography (GC) including flame ionization detection (FID) and mass spectrometric (MS) detection (*27*). All quality assurance procedures outlined in both (*26*) and (*27*) were followed during this work, including analysis of canister blanks and at least 10% sample replication. The data reported from the canister method accounted for ~232 total analytes.

Volatile carbonyls (~120 analytes) were collected and derivitized using the dinitrophenylhydrazine (DNPH) method (*17*, *28*). Briefly, carbonyl-DNPH derivatives were analyzed as described by Grosjean et al. (*17*) using a benchtop liquid chromatograph/diode array/mass selective detector (Hewlett-Packard Model 1100, Palo Alto, CA) that allows for independent acquisition of both ultraviolet and mass spectral data. Authentic standards (Aldrich Chemical Co., Lancaster Synthesis, Milwaukee, WI; Wiley Organics, Fluka Chemical Corp., Milwaukee, WI) of carbonyls were used to produce the DNPH derivative standards for analysis. The carbonyl compounds measured ranged in size and volatility from formaldehyde (C_1) to tetradecanal (C_{14}). Most of the dicarbonyl and other hydroxyl/acid substituted carbonyls were less volatile than tetradecanal. Though the particle phase of the heaviest molecular weight carbonyls reported here was not measured directly, it is possible that a portion of the compounds in the range of C_{14} were lost during sampling to the prefilter. Standards not commercially available have been synthesized as described by Grosjean and Grosjean (*29*). Each analysis set included at least 10% sample replicates and analysis of field blank cartridges. Blank values were subtracted from analysis results.

Volatile organic acids (~36 analytes) were collected in series (with backups to assess sample breakthrough) on potassium hydroxide (KOH)-impregnated quartz filters downstream of Pallflex (Pall-Gelman) pre-filters. The KOH filters were prepared according to the methods described by Kawamura et al. (*30*) and Chien et al. (*31*). Immediately after collection filters were placed in double-lined containers with KOH coated filters (to absorb potential contaminants) and stored at −20 °C until shipment to DGA, Inc., along with several (10% of total sample count) field blanks. Acids were analyzed by liquid chromatography/diode-array/MS (same instrument used for carbonyl analysis) after derivatization with pentafluorobenzyl bromide as per Grosjean and Grosjean (*32*). The LCMS was calibrated with authentic standards purchased commercially (Aldrich Chemical Co., Milwaukee, WI). Each analysis set included at least 10% sample replicates and analysis of field blank cartridges. Blank values were subtracted from analysis results.

Speciated Semivolatile/Particle Bound Organics. PM and SVOCs were reported here were collected with an XAD-4 coated annular denuder (URG, Inc., Chapel Hill, NC, 600-mm long)/Teflon coated glass fiber filter (Pallflex, Pall-Gelman)/PUF/XAD-4 (Sigma-Aldrich)/PUF train for investigating gas/particle partitioning as described by Gundel et al. (*33*). The denuder filter/sorbent pack was a Teflon holder (URG, Inc.). The sampling media were precleaned as described previously (e.g., refs *34*). The denuder was coated by Restek, Inc. (Bellefonte, PA) using their patented technique. Sample flow rates for the denuder sampling approaches are listed in Table 1. Flow rates were targeted to provide a residence time less than 0.3 s as recommended by Kamens et al. (*35*). After collection, the denuder was spiked with a suite of deuterated internal standards (described in the Supporting Information) and extracted with a dichloromethane/acetone/hexane (2:3:5) solvent mix. Separately, filters and sorbents were spiked with internal standards and Soxhlet extracted for 12 h with either dichloromethane (filters) or hexane/ether (90:10, for sorbents) followed by acetone.

After extraction, solvents were concentrated by rotary evaporation and filtered through a 0.2-μm acrodisc filter (Waters, Inc., Milford, MA). Extracts were then evaporated to ~100 μL under a gentle nitrogen stream and reconstituted in acetonitrile prior to analysis by GC/MS (details in the Supporting Information). Each sample was then split into separate fractions. The first fraction was analyzed directly by GC/MS without derivitazation. This injection was used to assay the alkanes, PAHs and PAH derivatives, hopanes, steranes, and phenols. The analysis of the polar fractions was explored via two methods in two separate aliquots of the extract. First, diazomethane derivitization was used to convert organic acids to their methyl ester analogues as described by Mazurek et al. (*36*). A trimethylsilation derivitization was conducted on the second aliquot to investigate sterols and sugars as described by Nolte et al. (*37*). Further descriptions on sample handling and calibration are given in the Supporting Information.

Data Analysis. Nearly all data presented in this manuscript are averages of multiple measurements and include all valid measurements collected from each exposure level. As mentioned above, the entire data set is available for public assess on the NERC Web Site (*1*). Each individual data point included in the NERC database has an associated uncertainty (error) that places the significance of the measured values in context. For all data except the elements and bacterial mutagenicity, measurement uncertainties were calculated as previously described (e.g., refs *2*, *8*, *11*, and *14*) based on the following formula

$$\text{uncertainty} = \sqrt{(a*b)^2 + (c)^2}$$

where a = analysis precision, b = analyte concentration, and c = limit of quantitation.

When possible, analytical precision was measured directly based on replicate analysis. The precision of destructive or flow-through instruments such as a gas analyzer can be assessed using a standard reference material. Nondestructive techniques (e.g., filter weights, GC/MS analysis) were usually assessed with representative samples. Measured precisions were typically rounded to the nearest whole number multiple of five. Precision assessments were not made directly for real-time mass, particle number, or canister analysis of VOCs, so precision estimates were set at 10%. Limits of quantitation were set based on the ability to discern analyte concentration from sample background with reasonable precision (typically a coefficient of variation < 20%). Considering the breadth of techniques utilized during this study, the details on the determination of limit of quantitation for each assay are not reported. Concentration data with uncertainty values higher or equal to their concentrations had a low degree of measurement confidence and were considered below quantifiable

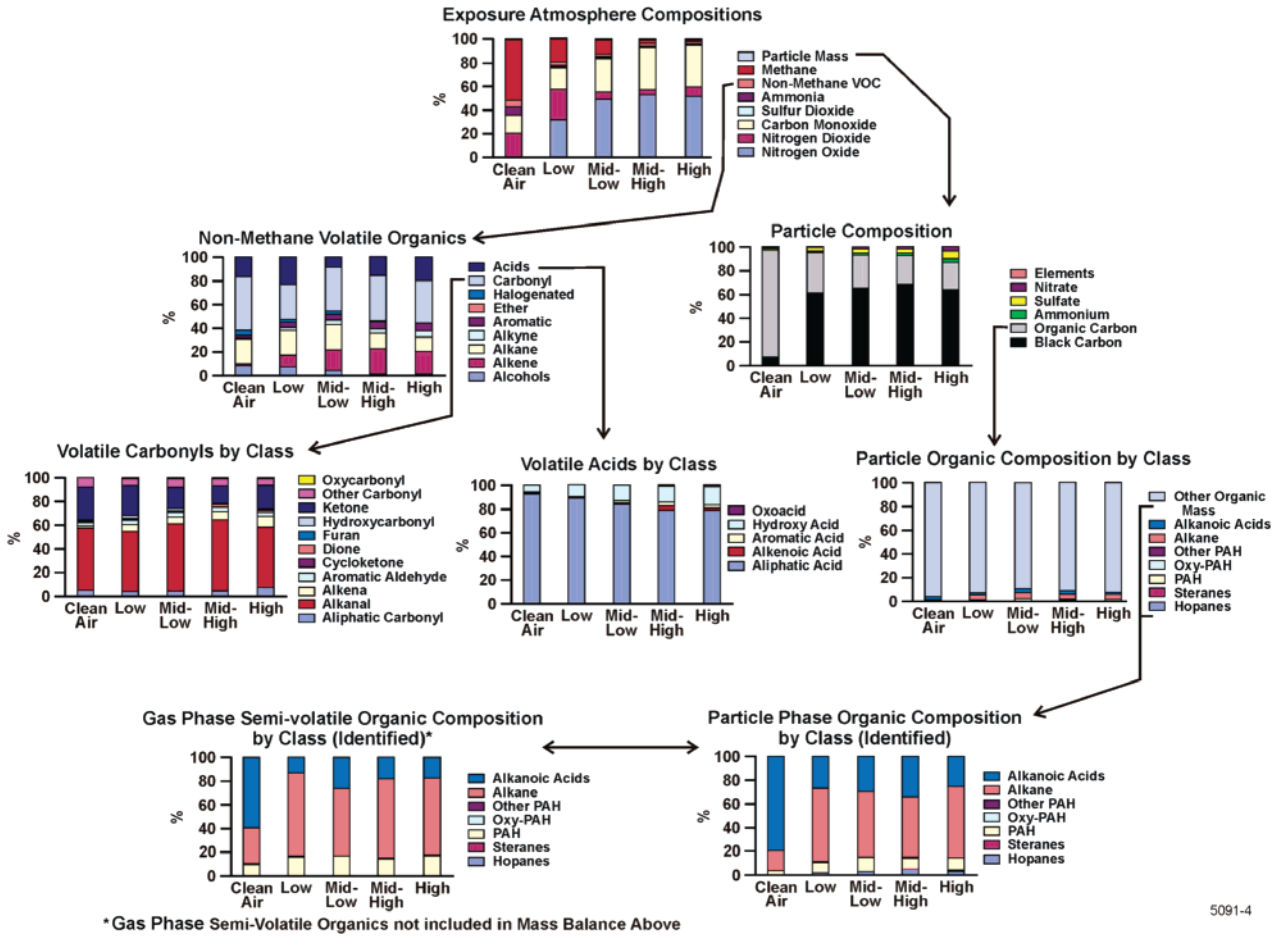

FIGURE 1. Composition (weight fraction) of diesel exhaust and clean air exposure atmospheres.

limits if detected. Measurements are not considered to be statistically significant unless they are > 2 times their analytical uncertainty.

Uncertainty estimates for the elements and associated ICP analytes were calculated by the collaborating laboratory (WSLH) using a slightly different technique. The uncertainty for those data was propagated by the sum of squares technique and included the one-sigma standard deviation obtained from multiple analyses ($n = 3$) of samples, sample blanks, and SRM.

The uncertainty descriptions above apply to data that are in the NERC database. Here summary data are shown, and the uncertainty values are propagated from the individual component uncertainty or they are the one-sigma standard deviation for all measurements. One-sigma standard deviation was applied for data when the number of samples for each exposure level was greater than 2 (PM, CO, NO, NO2, THC, particle number). For other compositional data, uncertainty values are the root-mean-squared error of the uncertainty estimates described above.

Results and Discussion

Figure 1 and Table 2 show the summary (average) compositions at each exposure level. Table 2 contains the data that were used to create the plots in Figure 1 (plus FID measured VOCs and total particle number) and are organized as such. All averages are composites of repeated 20-min transient engine cycles, during which the composition within the exposure chambers was constantly changing. To illustrate this, Figure 2 shows the change in PM concentration in real time over the course of several (six shown) repeated engine cycles at the high exposure level. PM concentrations ranged from about 1/2 to 2 times the target during each engine cycle. Real-time data are not available for all analytes, so all data are reported as exposure averages. As indicated previously, the exposure targets were set to achieve DEE PM concentrations of 30 (low), 100 (mid low), 300 (mid high), and 1000 $\mu g/m^3$ (high). While air entering the clean air (control) chamber was particle free, there were small amounts of PM due to the presence of the animals. To account for background PM in the DEE exposure chambers, the targets are actually set at DEE PM plus historical background PM in clean air chamber, so DEE PM is considered to be equal to DEE PM minus the clean air PM, and the averages were within 10% of the target values.

Composition of Exposure Atmospheres. DEE contains PM, NO_x, CO, SO_2, and hydrocarbons in the gas, semivolatile, and particle phase. The majority of the health-related research has focused on the PM component of DEE, most likely due to ambient exposure associations between PM and several health indicators, laboratory associations with cancer in some rodents with DEE PM at high concentrations, and a common misperception that PM is the most abundant constituent of DEE. PM is commonly reported in units of $\mu g/m^3$, while gaseous constituents are typically reported in units of parts per million (ppm). These units yield higher numbers for PM than for the gases, leading to a misperception that PM comprises the majority of the mass. In Table 2 the concentration of the most commonly reported constituents of DEE in each exposure atmosphere are given both in units of ppm and $\mu g/m^3$. This, along with Figure 1, shows that the majority of the mass of DEE in these exposure atmospheres was composed of gaseous material, especially NO_x (~90% NO and ~10% NO_2) and CO. SO_2 was present in relatively low

TABLE 2. Summary of Exposure Atmosphere Concentrations (by Class)

	units	clean air	low	mid-low	mid-high	high	notes[a]
Exposure Atmosphere Composition							
particle mass	$\mu g/m^3$	8.7 ± 8.5	43.6 ± 8.4	109.0 ± 14.5	319.9 ± 28.2	1005.0 ± 74.6	
nitrogen oxide	mg/m^3 (ppm)	0.0 ± 0.1(0.0)	2.2 ± 0.6(2.0)	5.8 ± 1.1(5.2)	17.9 ± 3.4 (16.1)	50.4 ± 0.6 (45.3)	
nitrogen dioxide	mg/m^3 (ppm)	0.2 ± 0.2 (0.1)	0.3 ± 0.6 (0.2)	0.7 ± 0.3 (0.4)	1.3 ± 1.9 (0.8)	6.9 ± 3.3 (4.0)	
carbon monoxide	mg/m^3 (ppm)	0.3 ± 0.1 (0.3)	1.5 ± 0.5 (1.5)	3.6 ± 0.4 (3.4)	10.2 ± 1.3 (9.8)	30.9 ± 4.5 (29.8)	
sulfur dioxide	$\mu g/m^3$ (ppb)	nd[b]	21.8 ± 1.3 (8)	141.1 ± 8.9 (54)	299.9 ± 18.6 (115)	955.2 ± 58.4 (365)	
ammonia	$\mu g/m^3$	176.5 ± 8.8	76.0 ± 0.2	20.7 ± 0.2	12.0 ± 0.6	9.1 ± 0.2	
methane	$\mu g/m^3$	1406.5 ± 253.2	1181.4 ± 231.5	1510.3 ± 233.3	1415.7 ± 332.5	2642.1 ± 455.9	
non-methane volatile organic carbon	$\mu g/m^3$	134.0 ± 52.1	171.8 ± 65.1	255.7 ± 37.3	567.4 ± 67.8	1578.6 ± 256.2	
FID total hydrocarbon	ppm	0.1 ± 0.1	0.1 ± 0.2	0.4 ± 0.3	0.9 ± 0.3	2.2 ± 0.2	
particle number	$particles/cm^3$	6.5×10^1	7.6×10^4	1.9×10^5	5.4×10^5	1.0×10^6	
Particle Composition							
particle organic carbon mass	$\mu g/m^3$	8.2 ± 0.4	13.6 ± 0.5	25.9 ± 0.5	66.0 ± 1.0	192.9 ± 3.0	
particle elemental carbon	$\mu g/m^3$	0.7 ± 0.1	24.6 ± 1.0	60.6 ± 0.6	198.3 ± 2.3	557.1 ± 9.1	
ammonium	$\mu g/m^3$	0.0 ± 0.1	0.5 ± 0.2	1.4 ± 0.1	5.1 ± 0.4	31.0 ± 3.9	
sulfate	$\mu g/m^3$	0.0 ± 0.1	1.2 ± 0.1	3.0 ± 0.1	10.3 ± 0.2	55.3 ± 1.7	
nitrate	$\mu g/m^3$	0.0 ± 0.1	0.1 ± 0.1	1.7 ± 0.1	4.5 ± 0.1	31.9 ± 1.2	
chloride	$\mu g/m^3$	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	
sum of elements	ng/m^3	145.1 ± 3.7	154.1 ± 4.4	405.5 ± 5.9	519.0 ± 17.4	1189.3 ± 34.4	$n = 43$
Non-Methane Volatile Organics							
alcohols	$\mu g/m^3$	11.1 ± 1.5	11.8 ± 1.5	9.3 ± 1.0	5.2 ± 0.6	6.0 ± 0.7	$n = 2$
alkene	$\mu g/m^3$	1.6 ± 0.5	16.9 ± 2.4	44.6 ± 4.8	124.6 ± 15.9	314.9 ± 23.3	$n = 21$
alkane	$\mu g/m^3$	28.7 ± 2.7	36.2 ± 2.2	55.5 ± 2.0	70.0 ± 1.4	184.1 ± 3.7	$n = 15$
alkyne	$\mu g/m^3$	0.7 ± 0.1	4.3 ± 0.2	9.0 ± 0.4	22.8 ± 1.1	81.5 ± 3.3	$n = 2$
aromatic (benzene and methyl benzenes)	$\mu g/m^3$	2.6 ± 0.4	7.0 ± 0.5	12.9 ± 0.6	34.2 ± 1.4	107.3 ± 4.3	$n = 26$
ether	$\mu g/m^3$	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	$n = 1$
halogenated	$\mu g/m^3$	5.8 ± 0.4	4.5 ± 0.4	6.9 ± 0.4	5.2 ± 0.4	5.4 ± 0.4	$n = 21$
carbonyl	$\mu g/m^3$	60.8 ± 7.1	51.3 ± 6.8	94.2 ± 8.4	216.6 ± 18.9	561.1 ± 47.4	$n = 111$
acids	$\mu g/m^3$	22.7 ± 1.8	39.8 ± 2.9	23.2 ± 2.0	88.8 ± 8.2	318.3 ± 29.7	$n = 36$
Volatile Carbonyl by Class							
aliphatic carbonyl	$\mu g/m^3$	2.7 ± 0.3	1.9 ± 0.2	3.4 ± 0.3	8.9 ± 0.7	37.5 ± 3.9	$n = 32$
alkanal	$\mu g/m^3$	32.2 ± 3.0	26.2 ± 2.4	54.3 ± 5.6	130.7 ± 13.6	287.4 ± 32.4	$n = 16$
alkenal	$\mu g/m^3$	1.2 ± 0.1	3.0 ± 0.2	5.1 ± 0.6	15.4 ± 1.3	52.9 ± 4.9	$n = 6$
aromatic aldehyde	$\mu g/m^3$	1.6 ± 0.3	1.6 ± 0.2	3.8 ± 0.4	7.7 ± 0.8	21.5 ± 1.9	$n = 8$
cycloketone	$\mu g/m^3$	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	$n = 2$
dione	$\mu g/m^3$	0.3 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	4.4 ± 0.1	8.7 ± 0.7	$n = 10$
furan carbonyl	$\mu g/m^3$	nd[b]	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	$n = 2$
hydroxycarbonyl	$\mu g/m^3$	0.8 ± 0.1	1.5 ± 0.2	1.2 ± 0.1	1.4 ± 0.2	2.4 ± 0.3	$n = 4$
ketone	$\mu g/m^3$	16.9 ± 2.1	12.8 ± 1.4	17.2 ± 1.9	33.4 ± 3.4	115.7 ± 12.3	$n = 9$
other carbonyl	$\mu g/m^3$	5.0 ± 0.6	3.2 ± 0.3	7.3 ± 0.7	14.0 ± 0.1	32.0 ± 0.3	$n = 13$
oxycarbonyl	$\mu g/m^3$	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	2.6 ± 0.3	$n = 5$
Volatile Acids by Class							
aliphatic acid	$\mu g/m^3$	21.2 ± 1.9	35.4 ± 3.1	19.5 ± 1.8	70.4 ± 7.3	252.3 ± 24.1	$n = 21$
alkenoic acid	$\mu g/m^3$	nd[b]	0.2 ± 0.1	0.2 ± 0.1	2.8 ± 0.3	4.3 ± 0.4	$n = 5$
aromatic acid	$\mu g/m^3$	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	3.1 ± 0.3	8.0 ± 0.7	$n = 6$
hydroxy acid	$\mu g/m^4$	1.3 ± 0.1	3.7 ± 0.4	3.0 ± 0.3	12.0 ± 0.9	49.7 ± 5.2	$n = 2$
oxoacid	$\mu g/m^5$	nd[b]	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	4.0 ± 0.4	$n = 2$
Semivolatile Organic Composition by Class (Identified)							
hopanes	ng/m^3	nd[b]	0.2 ± 5.7	2.4 ± 5.7	11.3 ± 5.7	21.9 ± 7.8	$n = 11$
steranes	ng/m^3	nd[b]	nd[b]	nd[b]	nd[b]	27.7 ± 13.3	$n = 8$
polycyclic aromatic hydrocarbons (PAHs)	ng/m^3	80.5 ± 16.2	1111.3 ± 167.2	2445.0 ± 357.1	5718.6 ± 684.2	16056.2 ± 1500.1	$n = 57$
oxygenated PAHs	ng/m^3	1.3 ± 5.1	31.0 ± 6.1	51.3 ± 7.4	146.8 ± 18.7	297.8 ± 35.6	$n = 6$
sulfur containing PAHs	ng/m^3	2.8 ± 5.2	0.6 ± 5.0	1.6 ± 5.0	3.2 ± 5.0	13.7 ± 5.1	$n = 2$
alkane	ng/m^3	260.8 ± 31.8	5032.5 ± 625.1	8961.6 ± 921.0	28415.1 ± 2500.5	63940.5 ± 6023.2	$n = 20$
alkanoic acids	ng/m^3	514.8 ± 61.3	951.5 ± 98.7	4134.6 ± 461.3	7631.8 ± 741.5	16928.0 ± 1789.2	$n = 9$
Particle Organic Composition by Class (Identified)							
hopanes	ng/m^3	nd[b]	11.2 ± 5.7	63.3 ± 6.7	219.4 ± 25.2	307.8 ± 34.9	$n = 11$
steranes	ng/m^3	nd[b]	nd[b]	nd[b]	79.7 ± 9.7	130.2 ± 14.5	$n = 8$
polycyclic aromatic hydrocarbons (PAHs)	ng/m^3	10.5 ± 5.4	130.0 ± 14.5	325.5 ± 34.1	765.3 ± 86.7	2021.1 ± 234.1	$n = 57$
oxygenated PAHs	ng/m^3	0.4 ± 5.4	2.4 ± 5.4	2.4 ± 5.4	24.3 ± 5.6	52.5 ± 5.9	$n = 6$
sulfur containing PAHs	ng/m^3	nd[b]	nd[b]	0.1 ± 5.0	nd[b]	nd[b]	$n = 2$
alkane	ng/m^3	48.2 ± 8.1	553.1 ± 53.1	1536.0 ± 174.2	2978.8 ± 321.0	8683.5 ± 875.2	$n = 20$
alkanoic acids	ng/m^3	234.9 ± 26.7	243.2 ± 2.7	825.4 ± 89.5	2037.9 ± 254.3	3669.8 ± 398.2	$n = 9$

[a] n = number of individual components composited into average. [b] nd = not detected, defined here as components that were zero or below zero after background (blank) subtraction. In database (reported elsewhere) actual numbers are reflected with their associated uncertainty.

concentrations on a ppm basis, but the mass concentration of SO_2 was nearly identical to the total PM. More abundant than these gases, and not reported here, were water and CO_2, both of which showed little dilution-related difference

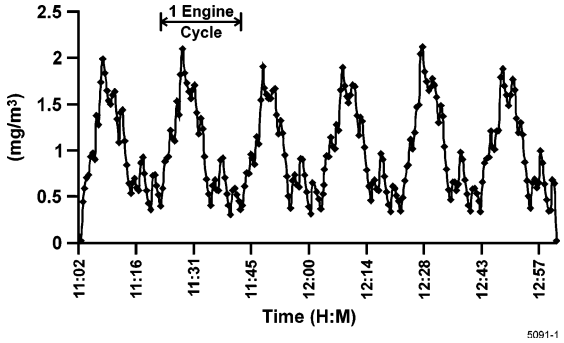

FIGURE 2. Real-time particle mass concentration in the high exposure level chamber.

in concentration because they came from a variety of sources (including dilution air and animal respiration).

Volatile Organic Carbon. The sum of measured volatile organic species (including methane) was in reasonable agreement with the FID measurement of total VOC (conversion from ppm gives ~3500 $\mu g/m^3$ for high level FID response), considering the calibration/response was based on propane and the individual VOC each respond differently to this detector depending on carbon number and the presence of non-carbon functional groups (e.g., oxygen). This suggests that the majority of the VOC present in the chamber were accounted for by the speciated measurements. The sum of nonmethane (NM)VOC is listed separately because the concentration of methane, the most abundant component of VOC, was similar at all exposure levels, likely because the primary sources were the animals themselves and the limited methane removal efficiency of the dilution air charcoal scrubbing. The NMVOC concentrations, even at the highest exposure level, were in the range of those reported for ambient concentrations in polluted urban areas (e.g., ref *26*).

In some cases, there was no difference between the NMVOC concentrations at the two lowest exposure levels, most likely due to the low concentrations in the highly diluted DEE, VOCs in dilution air, and VOCs produced by the animals. VOC samples taken from rodents in individual containers (see the Supporting Information for methodology) contained large amounts of carbonyl and acid compounds similar in composition to that observed in the clean air exposure chamber. As the DEE dilutions decreased, the proportional amount of rodent contributions to the exposure atmosphere decreased, and a dilution-related increase was thus observed at the highest levels.

The contribution of chemical classes to NMVOC ranked carbonyl > alkene > acids > alkane > aromatic > alkyne > halogenated > ethers. The amount of individual compounds that were summed to account for each of these classes differed, but in general all classes were dominated (>80% of total) by the C_1−C_2 compounds (e.g., formaldehyde and acetaldehyde for carbonyls). For the aromatics, benzene and toluene were the most abundant compounds. These proportions showed reasonable agreement to previously published proportions of NMVOC from diesel exhaust (e.g., refs *13* and *38*). Some of the measured compounds are on the EPA's list of Hazardous Air Pollutants (HAPs). Benzene and 1,3-butadiene have received perhaps more attention than other NMVOC HAPs in DEE. Both of these components were present at levels in the range of ambient, even at the high DEE exposure level. The average levels observed in the high level were 27.5 $\mu g/m^3$ for benzene and 1.6 $\mu g/m^3$ for 1,3-butadiene, and average concentrations observed in ambient air in Los Angeles were ~15 $\mu g/m^3$ for benzene (*39*) and <5 $\mu g/m^3$ along the California/Mexico border for 1,3-butadiene (*26*). Saptkota and Buckley (*40*) have reported benzene and 1,3-butadiene near toll booths in the range of <1−30 and <1−20 $\mu g/m^3$. It is possible that 1,3-butadiene may have been emitted from the engine in higher concentrations in this study, but because it is very reactive in the presence of NO_x, it may have reacted and decomposed during dilution in the chambers or during sampling.

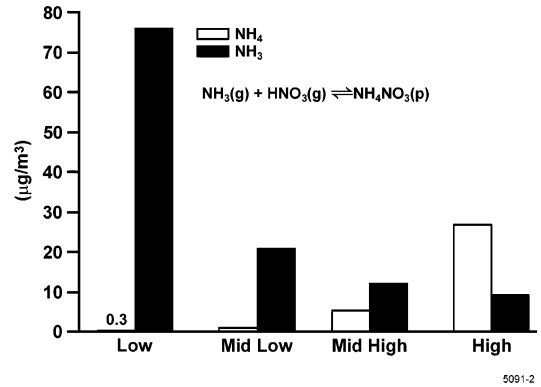

FIGURE 3. Inverse relationship between the concentration of diesel exhaust and the concentration of gas-phase ammonia or particle phase ammonium. Ammonia from the animals reacts with nitrogen oxides (e.g., HNO_3) to form secondary inorganic particles.

Particulate Matter. The fractional abundance of the primary PM constituents (EC, OM, inorganic ions [sulfate/nitrate]) were slightly different among the DEE exposure chambers because of variation in the proportions of particle bound inorganic species in these chambers. The clean air exposure chamber PM was composed of nearly 100% OM that likely consisted of a combination of rodent dander and exhaled organic vapor that adsorbed onto the quartz filter and was weighed as organic carbon. EC in the DEE exposure atmospheres accounted for ~60% of the PM, and OM was approximately 30%. There was about a 10% difference in the proportion of inorganic PM between the high and low exposure chambers (discussed below). Elements (mostly metals) accounted for less than 0.5% of the PM. The average OM/EC proportions were typical for DEE emissions and were consistent with the average composition of "typical" diesel exhaust described in the recent Environmental Protection Agency Assessment of DEE (*41*).

The dilution-related differences in the proportion of inorganic constituents was likely due to the interaction of NH_3, contributed primarily by the rodents (*42*), with gaseous and semivolatile components from the DEE to form secondary PM, primarily ammonium sulfate, and ammonium nitrate. Evidence for this is shown in Figure 3, which illustrates inverse relationships in the concentrations of NH_3 and NH_4^+ in the exposure chambers. The highest exposure level contained the highest amount of inorganic gaseous and semivolatile components, and there was an apparent higher rate of conversion to the particle bound inorganic compounds in that chamber. Because the primary source of NH_3 in the chambers was the rodents, the concentration would be the same in each of the exposure levels if no interaction occurred with the DEE. However, the highest concentration of NH_3 was in the clean air exposure chamber, and the lowest was in the high DEE chamber. The equation presented in Figure 3 shows one route for the conversion of gas-phase NH_3 to secondary inorganic PM when it interacts with HNO_3 (conversion for sulfate also occurs, but not shown). A mole balance of the amount of NH_4^+ in the DEE chambers compared with sulfate and nitrate shows that the sulfate accounts for ~50% and nitrate ~25% of the neutralized ammonia, indicating not only that all of the inorganic acids have been neutralized in these exposure chambers but also that excess NH_4^+ was present, likely from the neutralization of some organic acids. There are several reaction pathways

that can lead to secondary PM, and a detailed discussion of them is beyond the scope of this manuscript (see ref *43*). Ours is the first known study to describe secondary PM formation in multilevel DEE rodent exposure studies, though such reactions have likely occurred in every whole-body DEE exposure study to date. NH_4^+ and other inorganic ions were not measured in the DEE study reported by Cheng et al. (*44*), but NH_3 concentrations in that study did decrease with increased DEE concentrations as well. The formation of secondary PM inside the exposure chamber is consistent with ambient reactions; for example, Mysliwiec and Kleeman (*45*) predicted based on model results that diesel exhaust contributes substantially to airborne secondary inorganic PM when it interacts with ambient NH_3.

Emissions of the elements from diesel vehicles have ranged from a fraction of a percent to up to ~5% of the total DEE PM (*46*). These emissions arise from fuel/lube additives, engine/exhaust system wear, and metals taken into the engine from resuspended soil, asphalt, or other airborne materials (*46*, *47*). Our study used new engines operated indoors with filtered intake air. This avoids the presence of metals observed from on-road emissions that arise from resuspended soil and chassis dynamometer experiments where the air intake on the engine may not efficiently remove contaminants from ambient air. The only metals that were consistently observable above detection limits and increased in concentration with increases in DEE levels were calcium, sodium, zinc, and hafnium. Calcium, sodium, and zinc are all components of additives that are added to diesel fuel and lubrication oil (*46*). Hafnium, which was present in low but detectable amounts in some samples, is not a commonly reported element in DEE but is a component of specialized steels. Samples for elements were collected during two separate sampling periods, with one to two samples collected from each level during each period. Hafnium was only observed in statistically significant amounts (four to five times uncertainty) during one of the sampling periods. All other elements, including iron and silicon (two common constituents observed in DEE emissions), were detected at concentrations at or below the analytical uncertainty or were at background levels (concentration not different from background and did not scale with dilution). Iron, silicon, and other components were likely not observed because of the filtered intake air and new engine because these constituents arise from engine degradation and suspended material brought in through the intake.

Gas and Particle Bound Semivolatile Organics. The most commonly measured fractions of organic material are the VOCs and PM associated OM that are collected on filters. Much of the OM consists of individual SVOCs that can exist at least in part in the gas phase simultaneously with the particle phase. Though the gas phase portion of the SVOCs typically can account for more of the mass of DEE than the particle phase, they are rarely studied, and measurements of total SVOCs (gas plus particle) are not common. Because a conventional measurement of total SVOCs does not exist (there is no conventional way to measure total SVOC mass), SVOCs were only assessed for individual compounds, and these compounds were not included in the first plot in Figure 1 that shows the total DEE exposure atmosphere. Instead, the particle phase organic compounds are shown as a fraction of total OM, and the gas-phase SVOCs are shown in relationship to the particle phase.

The SVOC/PM organic classes included the *n*-alkanes from C_{13}–C_{30}, acids from C_{13}–C_{25}, PAHs, oxygenated/sulfur containing PAHs, hopanes, steranes, methoxylated phenols, sugar derivatives, resin acids, and select other nitrogen compounds such as quinoline and nicotine. These classes were chosen based on components that have been shown in previous studies to be abundant constituents in emissions from air pollution sources, and many of these classes have been used to distinguish among different sources in ambient air by source/receptor models (e.g., refs *48* and *49*). These compounds originate from fuel (e.g., alkanes in petroleum fuels, hopanes in oils, methoxylated phenols in wood, nicotine in tobacco), combustion (e.g., PAHs from all combustion sources, sugar derivatives), or both (e.g., PAHs and acids). All classes mentioned above were assessed, but some are not shown here (but are retained in database) because they were not present at detectable levels. In many cases, the same compound existed simultaneously in the particle and gas phase, and the portion in either phase is included in either the gas-phase SVOC or PM organics portion of Figure 1.

Similar to several other classes of material discussed earlier (e.g., VOC, inorganic ions, elements), the concentrations of several individual organic compounds and summed classes of organics did not scale precisely with dilution (e.g., 3:1 difference in dilution did not necessarily correspond to a 3:1 difference in concentration). For some of the components (e.g., alkanoic acids) this can in part be attributed to the contribution of background air and rodent respiration. However, the most likely explanation for the remainder of the constituents is daily variability in the output of these compounds from the engine. Sampling for comprehensive characterizations were conducted periodically, and because of practical considerations they were not collected simultaneously from every exposure level. Therefore differences in concentrations at different exposure levels result from a combination of factors, including background air and rodent respiration (for some component classes), analytical variability (for low concentrations), and day-to-day variability in the output from the engines. Reports of day-to-day variability in engine output (for detailed organics) are nonexistent (to our knowledge). The average concentrations of PAH, alkanes, and alkanoic acids all showed reasonable agreement with the dilution scale (e.g., 3:1 difference indilution corresponds to 2.6 × difference in particle PAH at two highest levels). Hopanes, which were present at much lower concentrations, did not track well with dilution at the two highest levels (3:1 dilution and 1.4 × increase in concentration), while it tracked reasonably well at the two middle dilution levels (3:1 dilution and 3.4 difference in dilution). Because these compounds are derived from unburned lube oil emissions, it is unlikely that the concentrations reflect day-to-day differences in the crankcase oil derived emissions.

As Figure 1 shows, the majority of the mass of the SVOC/PM compounds was gas phase. Only a relatively small portion of the particle OM (~10%) was accounted for by direct measurement. This poor mass balance was consistent with other comprehensive measurements of DEE PM and is attributed to the fact that DEE extracts contain large abundances of compounds that are difficult to resolve by current gas chromatographic techniques, termed the "unresolved complex mixture" (UCM). Schauer et al. (*13*) quantified the UCM in bulk utilizing a response factor derived from engine oil and was able to account for a large fraction of the unidentified OM. Because the UCM had a similar response factor as engine oil it was postulated by Schauer et al. (*13*) that the UCM contains similar components, especially branched alkane type compounds typical of petroleum products. Recently, Frysinger et al. (*50*) applied two-dimensional chromatography to further resolve the UCM in extracts of diesel fuel-contaminated marine sediments and showed that thousands of individual compounds can be further resolved. Early progress showed that the majority of the UCM was composed of petroleum-based alkanes (straight chain, branched, and cyclic) and hydrocarbon substituted monoaromatics, and the remainder (~15%) was composed primarily of multiring aromatics. A large portion of the OM

in the present study likely contained unburned fuel and lube oils and was likely consistent with the composition described above. The quantified OM consisted of a large portion of alkane and straight chain aliphatic acids (likely combustion products of alkanes). Because these are largely derived from the fuel and to some extent lube oils, it can be assumed that many components derived from unburned or partially burned fuel that are similar in chemical structure likely account for a large remainder of the unidentified OM.

Table 2 shows the total concentration of measured gas and particle PAHs. These sums represent a total of 65 PAHs ranging in volatility from naphthalene (molecular weight = 128) to coronene (molecular weight = 300). Although PAHs are one of the most studied class of diesel exhaust constituents, the total amount of particle PAHs only represent a fraction of the DEE PM (around 0.2% in the high chamber). Concentrations of many of the most studied particle-phase PAH compounds, such as benzo[*a*]pyrene, were at or below the analytical uncertainty during this study (~3−4 ng/m^3 at the high level). Benzo[*a*]pyrene has received considerable attention as a component of diesel exhaust because of its promutagenic potential, but it is actually emitted in low (and somewhat variable) amounts relative to other PAHs and has been shown to be present at levels close to the analytical detection limits in emissions from many diesel vehicles (e.g., refs *2* and *11*).

Implications

The diesel system was designed to produce inhalation exposure atmospheres consistent in composition with contemporary (ca. 2000) emissions as they have been characterized in previous on-road and laboratory emmisions characterization studies. Evaluations of the chemical composition showed that this was achieved. In addition, the detailed assessment revealed several characteristics about animal exposures to combustion emissions that have not been reported before, such as the formation of secondary PM by interaction of exhaust with ammonia from the animals and the failure of the concentrations of some components to scale exactly with dilution ratios.

The concentrations of most components scaled precisely with dilution rate. However, the presence of background material (from the rodents and dilution air) plus small variations in the daily composition of the exhaust caused concentrations of some components to vary from what was expected from the dilution ratios. These observations illustrate several phenomena (e.g., volatile organics contributed from rodent respiration/interactions of rodent excreta with components of the atmosphere) that are likely common to all complex mixture exposure atmospheres conducted to date and point out that it cannot be assumed that components in multilevel exposure atmospheres scale precisely with single components (e.g., PM) that are used to control dilution levels.

Acknowledgments

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Supporting Information Available

Additional details on the engine cycle, exposure system, exposure system sampling validation, and analytical methods and a description of the measurement of rodent emissions to confirm their contribution to the exposure atmospheres is described.

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