FINAL REPORT
January 21, 2010

Cardiovascular Health Effects of Fine and Ultrafine Particles during Freeway Travel

Contract Number 04-324

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Prepared for the California Air Resources Board
and the California Environmental Protection Agency

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III. Acknowledgements:

This Report was submitted in fulfillment of ARB contract number 04-324, Cardiovascular Health Effects of Fine and Ultrafine Particles during Freeway Travel, by UCLA - School of Public Health, Department of Environmental health Sciences under the sponsorship of the California Air Resources Board.

The Principal Investigator gratefully acknowledges the contributions of co-investigators Wayne Cascio, Nola Kennedy, William Linn, Daniel Stramm and Yifang Zhu, and technical support staff David Fung, Arantza Eiguren-Fernandez, Laxmansa Katwa, Jessica Matthews, Taylor Blachley, and Trudy Webb.

This work was also partially supported by the Southern California Particle Center; U.S. Environmental Protection Agency grant number R82735201;and the Southern California Environmental Health Center, National Institute of Environmental Health Sciences (NIEHS) grant No. 5 P30 ES07048. We also thank the University of California Los Angeles (UCLA) machine shop for modifying the van, and UCLA fleet service manger Tim Pfrimmer and his staff for maintaining the van.

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VII. Abstract

This report presents the results of a 24-month study of human response to two-hour exposures to freeway air in Southern California. A nine-passenger van was modified with a high-efficiency filtration system that delivered filtered or unfiltered air to an exposure chamber inside the van. State-of-the-art instruments were used to measure concentration and size distribution of fine and ultrafine particles and the concentration of other pollutants associated with motor vehicles. Nineteen volunteer subjects (average age 71 years) rode for two hours each in filtered and unfiltered air on two freeways, I-405 and I-710. Double-blind health assessments included 24-hour ambulatory ECG, blood biochemistry, blood pressure, and lung function. Mean unfiltered particle number concentration was 107,500 particles/cm^3 for I-710 and 77,800 particles/cm^3 for I-405; mean PM-2.5 mass was 51.4 and 44.5 $\mu g/m^3$ respectively. Filtration reduced particle count >95% but did not remove gases. Atrial ectopic beat incidence during and after exposure decreased 20% on average with filtered air compared to unfiltered air ($P<0.05$). Individual responses related most strongly to particle count ($P=0.01$). Blood markers NT pro-BNP and VEGF decreased 30% on average in filtered air compared to unfiltered air ($P<0.05$). This study documents a cardiac and vascular response associated with freeway travel.

VII. Executive Summary

BACKGROUND This report presents results of a 24 month study of human response to two-hour exposures to freeway air in Southern California. Numerous epidemiological studies have linked increases in particulate matter exposure to increases in cardiovascular mortality and morbidity. Some evidence has been published recently that supports the hypothesis that particulate matter and gases associated with diesel exhaust are associated with acute clinical and biological effects. Moreover, although supporting data are more limited than data on PM-2.5, concentrated ambient ultrafine particles (diameter <100 nm) are associated with pulmonary and cardiovascular effects, and ambient ultrafine PM is weakly associated with adverse cardiovascular events. A recent study found that the relative risk of myocardial infarction was increased two to threefold by time spent in traffic one or two hours prior to the onset compared with control periods. There are no systematic measurements of ultrafine particles inside vehicles and their associated human health effects during commuting. However, exposure to ultrafine particles is known to be substantially elevated during travel on freeways. The primary objectives of the present study were to document acute effects in late middle-aged and elderly volunteers riding on Los Angeles freeways, and to relate these effects to specific air pollution components. This study contributes to exposure assessment of freeway commuters and the establishment of standards for ultrafine particles associated with freeways.

METHODS The study used a modified nine-passenger van with an exposure chamber and filtration system to expose a total of 19 clinically healthy subjects, aged 61 to 83, for two hours each to either unfiltered or filtered freeway air on two freeways, one with mostly gasoline vehicles (I-405) and the other with a high proportion of heavy-duty diesel trucks (I-710). The filtration system delivered the unfiltered or filtered (96% of particles removed) air to the exposure chamber inside the van. The exposure chamber could accommodate two subjects. State-of-the-art instruments were used to measure concentration and size distribution of fine and ultrafine particles and the concentration of carbon monoxide (CO), carbon dioxide (CO_2), black carbon (BC), particle bound polycyclic aromatic hydrocarbons (PB-PAHs), PM-10 mass, PM-2.5 mass, and oxides of nitrogen (NOx) in near-real time in the subject's breathing zone inside the exposure chamber.

Measures of cardiovascular health included: 24-hour ambulatory ECG for the assessment of heart rate variability and incidence of ectopic heart beats; blood pressure, heart rate – blood pressure product, lung function, and blood biochemistry to assess systemic inflammatory markers (fibrinogen, IL-6, CRP, MPO, MCP-1); vascular response or injury (sE-selectin, VEGF, sVCAM, sICAM, MMP9, MPO, t-PAI-1), and myocardial response to hemodynamic changes (NT-proBNP). Cardio-respiratory symptoms were monitored before and after exposures to detect immediate and acute health effects. Subjects, and those doing the health monitoring and biochemical and electrical signal analysis, were blinded as to the type of exposure.

RESULTS Average total particle number concentration, as measured by a condensation particle counter (CPC), of unfiltered air inside the enclosure was 77,800 and 107,500 particles/cm^3 on the I-405 and the I-710 freeway, respectively. The highest one-minute averaged particle number concentration was 730,000 particles/cm^3 on the I-710 freeway. Bimodal size distributions were typical for both freeways, with the first mode around 12–20 nm and the second mode around 50–100 nm. BC and particle-bound PAH concentrations were more than two times greater on the I-710 than on the I-405 freeway. Ultrafine particles represented about 36 to 76% of total particle number concentrations on I-405, and 56% to 84% on I-710. A peak in average particle number concentration of 125,000 particles/cm^3 was associated with a traffic speed of 40 to 50 mph. Whereas most health endpoints did not vary significantly by freeway or filter condition, atrial ectopic beat incidence during and after exposure decreased 20% on average with filtered versus unfiltered air ($P<0.05$). Between-freeway differences were non-significant, but individual responses related more strongly to particle count ($P=0.01$), PAH ($P=0.02$) and black carbon ($P=0.04$) than to mass ($P=0.07$). N-terminal pro B-type natriuretic peptide (NT pro-BNP) and vascular endothelial growth factor (VEGF) decreased 30% on average in filtered compared to unfiltered air ($P<0.05$).

DISCUSSION These findings indicate that breathing unfiltered freeway air, as compared to breathing filtered air, triggers an increase in supraventricular ectopic beats, and that this increase is associated with a statistically significant increase in NT pro-BNP relative to NT pro-BNP levels measured in the absence of filtration. The association of an increase in supraventricular ectopic beats with an increase in NT pro-BNP

implicates an increase in intra-atrial pressure, or stretch, as the underlying mechanism for the increase in arrhythmia. Such an effect is speculated to be related to direct or indirect effects on traffic related particles on pulmonary artery pressure or atrial mechanical function. The relative increase in VEGF after breathing unfiltered air suggests an injury response of the vascular endothelium. Such a response is most likely to occur in the lung as this is the point of entry of the particles. A local pulmonary vascular response, e.g. secondary to the release of endothelin-1 might evoke a vasoconstriction with an attendant increase in intrapulmonary pressures and subsequently increased pressures in the right heart and release of NT pro-BNP.

While the absolute number of supraventricular ectopic beats initiated in this study is low and not clinically relevant in healthy individuals, it does suggest that such a trigger when applied to hearts with more advanced disease might yield more complex and sustained arrhythmia like those observed in individuals having coronary heart disease or heart failure. As such, the observation in this study that traffic related particulate pollution increases supraventricular ectopic beats might have important public health implications, in that supraventricular ectopic beats initiate a wide variety of atrial and atrioventricular arrhythmias. For example, atrial fibrillation is the most common serious arrhythmia in late middle age and elderly populations. It contributes to poor quality of life, stroke, myocardial infarction and heart failure. Likewise less serious, yet still debilitating arrhythmia, such as atrial tachycardia, AV nodal reciprocating tachycardia, and AV nodal tachycardias are initiated by premature beats.

Health responses were not clearly different between the diesel-truck-dominated freeway and the gasoline-car dominated freeway, and exposure concentration ranges on the two freeways overlapped considerably. However, the significant association of unfavorable responses with PM number concentration, particularly in the ultrafine size range, PAH and black carbon along with the tendency for more PM emissions from diesel engines, argues that diesel-truck traffic presents the greater cardiovascular health hazard.

CONCLUSIONS This study documents a cardiac and vascular response associated with freeway travel that provides new insight into the association of inhalation of traffic related pollutants to arrhythmia. In our relatively healthy (age > 60) adult volunteers, exposure to particulate matter on freeways was associated with a small but statistically significant increased incidence of atrial arrhythmia, but with generally stable or decreased incidence of ventricular arrhythmia and generally stable heart rate variability. Concurrent relative increases in NT pro-BNP and VEGF suggest that increased intra-atrial pressure triggered atrial arrhythmia and might be mechanistically linked to pulmonary vascular injury and attendant vasoconstriction. Effects appeared to relate to black carbon, PAH, and the ultrafine particulate fraction. Particle count was strongly correlated with arrhythmia incidence while PM-2.5 and PM-10 mass concentrations were not significantly correlated with arrhythmia incidence. Rigorous double-blind conditions and filtered-air controls in this study, rule out other traffic-related stresses or pollutant gases as causes of the particulate matter-associated cardiovascular effects. Because sustained arrhythmias (e.g. atrial fibrillation, AV nodal reentrant tachycardia) are triggered by premature atrial beats, traffic exposure may play a role in their occurrence - a risk that could be mitigated by filtering particulate matter from the vehicle passenger cabins. These results will aid the Air Resources Board in evaluating the importance of motor-vehicle related ultrafine particles. They will also contribute to the evidence needed for the development of a number-based standard for ultrafine particles. These conclusions could be strengthened by additional studies using more subjects, subjects with pre-existing cardiovascular conditions, and by focusing on ultrafine particles and black carbon and particulate PAH compounds.

IX. Body of Report

A. INTRODUCTION Epidemiological studies from all over the world have consistently linked increases in particulate matter (PM) exposure to increases in cardiovascular mortality and morbidity. [Dockery et al,1993; Vedal,1997]. Some evidence (Mills et al. 2008, Lucking et al, 2008). Peretz et al, 2008) in man and in animal models (Upadhyay et al. 2008, Hwang et al. 2008, Sun et al. 2008; Yokota et al. 2008) and in vitro assays (Helfenstein et al. 2008, Totlandsdal, et al. 2008, Sumanasekera et al. 2007) has been published recently that supports the hypothesis that ultrafine (UF) particles (particle diameter <100 nm) might be associated with cardiovascular effects because of their high deposition efficiency in pulmonary regions and their propensity to penetrate the epithelium and reach the blood and other organs [Oberdorster, et al, 2002; Elder, et al, 2004]. The presence of UF particles in the circulation has been shown to affect homeostasis [Nemmar, et al, 2002b] that may lead to adverse reactions in critical organs such as the liver, heart, and even the brain, and play a role in the onset of cardiovascular disease [Kreyling, et al 2002].

Previous in-vehicle exposure studies focused primarily on coarse PM (PM-10) and fine PM (PM-2.5), metals, and gas-phase pollutants such as carbon monoxide (CO), ozone, oxides of nitrogen (NOx), and volatile organic compounds [Jo, et al, 1999; Reidiker, et al, 2003; Gomez-Perales, et al, 2004; and Chan, 2003]. Recently a few mobile laboratories have been developed to study UF particles on streets and highways. These studies have focused on diesel emissions [Cocker, et al, 2004a; Cocker, et al, 2004b; Vogt, et al, 2003], UF particle formation, [Kittelson, et al, 2004a, UF particle spatial and temporal variations [Pirjola, et al, 2004a; Bukowiecki, et al, 2002], and on-freeway particle phase PAH, black carbon, and UF particle concentrations [Westerdahl, et al, 2005]. There are no systematic measurements of UF particles inside vehicles and associated human health effects during commuting.

Ambient particulate matter (PM) is the form of air pollution most consistently associated with cardiovascular and respiratory morbidity and mortality, according to extensive epidemiologic evidence [Mills et al., 2009; Mauderly and Chow, 2008; Brook, 2008; Samet and Krewski, 2007; Pope and Dockery, 2006]. Numerous studies have associated cardiopulmonary morbidity with exposure to motor vehicle exhaust [Adar and Kaufman 2007, Boothe et al. 2008, Salam et al. 2008, Götschi et al. 2008, Brugge et al. 2007]. PM exposure and health effect associations can be demonstrated on short (<1 to several days) as well as long (years) time scales. Although it is not clear what particle size ranges or chemical species are primarily responsible for health effects [Schlesinger et al., 2006; Grahame and Schlesinger, 2007], one likely candidate is UF (<0.1 micrometer) particles emitted by motor vehicles [Delfino et al., 2005]. UF particles have been shown to translocate from the lung into the systemic circulation in animal models [Elder, et al, 2004; Oberdorster, et al, 2004; Nemmar, et al, 2001; Kreyling, et al 2002; Hamoir, et al, 2003; Gilmour, et al, 2004], and in human subjects [Nemmar, et al, 2004; Nemmar, et al, 2002a]. In California cities, and presumably in other motor-vehicle-dominated areas, exposure to these particles is substantially elevated near freeways, but is most intense during actual travel on freeways [Zhu et al., 2002a, 2008; Westerdahl et al., 2005]. Many people experience these high exposures for 1 to 2 hours per day while commuting.

A few experimental studies have been conducted with human volunteers breathing UF particles (not directly from traffic) in controlled exposure chambers. They have shown limited evidence for unfavorable acute cardiopulmonary effects, either with concentrated ambient pollution particles [Gong et al., 2008; Samet et al., 2007; Samet et al., 2009] or with artificially generated carbon particles [Shah et al., 2008; Frampton et al., 2006]. Huang and Ghio [2009] provide a detailed

review of controlled PM exposure studies in susceptible populations. Studies of personal exposure and acute health effects during actual vehicle travel are few in number, because of inherent technical difficulties. They have yielded evidence of altered heart rate variability in aged-adult volunteers [Devlin et al, 2003], on bus excursions [Adar et al., 2007], and in young healthy highway patrol officers [Riediker et al., 2004]. This experimental evidence is limited by artificial and somewhat stressful exposure conditions in the chamber studies, and by lack of blinding and relatively limited exposure characterization in the travel studies.

A daily mortality study in Erfurt, Germany was the first epidemiology study that examined and found significant associations between exposure to UF particles and mortality from respiratory and cardiovascular disease [Wichmann, et al, 2001]. Recent epidemiological studies have addressed the role of UF particles and short-term effects in adults and children with asthma, and daily mortality [Wichmann, et al, 2001; Penttinen, et al, 2001a; Penttinen, et al, 2001; Peters, et al, 1997]. These studies concluded that health effects were more closely associated with the number of UF particles than the mass of the fine particles. However, none of these studies could clearly distinguish UF particle effects from fine particle effects, due to the high correlations between these measures, as well as other pollutant measures, in time-series data. (Clear association of high overall pollution levels with ill health, but uncertainty as to effects of specific pollutants, is the usual outcome in epidemiologic studies of community air pollution.) A recent study found that the relative risk of myocardial infarction onset was increased two to threefold by time spent in traffic one or two hours prior to the onset compared with control periods [Peters, et al, 2004]. Alteration of heart rate variability has been associated with particle number concentration in the general environment [Chan, et al, 2004] and roadway exposure to PM mass [Riediker, et al, 2004].

Very high number concentrations of UF particles (>100,000 particles/cm^3) have been found near major freeways in the Los Angeles Basin [Zhu, et al, 2002a; Zhu, et al, 2002b; Zhu, et al, 2004; Zhu, et al, 2006]. Studies conducted in the vicinity of Interstates 405 (I-405) and 710 (I-710) in Southern California found that the number concentration of UF particles was approximately 25 times greater adjacent to the freeways than upwind background levels and that pollution levels decreased to near upwind (background) levels at approximately 300 m (1000 ft), downwind from the freeway during daytime. Researchers from cities around the world later observed similar decay profiles [Reponen, et al, 2003; Gramotnev, et al, 2004]. For the 40% of Californians that spend one to three hours commuting between home and work each workday, their in-vehicle time may contribute 50% or more of their daily UF particle exposure [Zhu, et al, 2007, Fruin et al, 2008]

The objectives of this study were to (1) develop a mobile laboratory to facilitate concurrent measurements, in near real-time, of in-vehicle concentration and size distribution of UF particles and other traffic-related air pollutants while traveling along a freeway, and (2) to measure cardiovascular indicators of exposure in human subjects, and (3) to relate specific components of PM from motor vehicles to these response measures.

B. MATERIALS AND METHODS

1. On-Freeway Exposure Measurement System

The on-freeway exposure and measurement system was installed in a 2002 Chevrolet Express nine-passenger van. Details of the van exposure system and measurement instruments are described in a recent publication by Zhu et al (2008). Originally the van was configured to seat nine people with seats arranged in four rows of two, two, two, and three seats each. In the first row the passenger seat and its base were removed to accommodate the filtration system, which included a high-efficiency particulate air (HEPA) filter box, air delivery fan, and airflow control valve. The HEPA

filter was used to provide nearly particle-free air for filtered air runs. The auxiliary blower and high-efficiency filtration system drew air from the vehicle's interior forward air supply and delivered it to the exposure enclosure. The filtration system delivered to the exposure enclosure either unfiltered freeway air or freeway air with approximately 96% of particulate matter removed. In either case, normal climate control was maintained, and gaseous pollutants were not removed. The second row of seats occupied their original position, but the seats were installed inside the human subject exposure enclosure. All seats in the third row and the two leftmost seats in the fourth row were removed to accommodate the batteries, power supply, and instrument platform. The floor of the van was flat with some stiffening corrugations. A staff member seated in the rear of the van operated monitoring instruments and controlled the filtration, keeping the driver and passengers blind as to its status.

The final layout is shown in Figure 1. The instrument platform height was 0.81 m (32“) above the van floor, which was 0.13 m (5”) below the mouth height of the subjects when seated in the exposure enclosure. This permitted short, straight sample lines from the subjects’ breathing zone to all instruments. The specific instruments used are described below. The instrument platform was constructed of 25-mm (1-in.)-thick plywood and was 1.12 m wide by 1.58 m long. It was attached with neoprene vibration absorbing mounts to a table frame. The table frame was attached to the van floor. The battery box, inverter, and charger were mounted together, as a separate unit, to the van floor beneath the instrument platform (Figure 1). The instrument operator sat in the rear seat and had access to all instruments and the power supply. The operator could communicate with the driver and subjects by a wired intercom system. Preliminary experiments found inside particle number concentrations to be greater than 90% of concentrations outside the van while traveling on a freeway.

The exposure enclosure was designed to accommodate one or two human subjects for environmental health studies. It was constructed of 6-mm (0.25-in.) thick static-dissipative acrylic sheets attached to an aluminum angle frame. This static-dissipative material was used to prevent any buildup of charge on the enclosure walls that might lead to a loss of particles. In addition, the ducts to the enclosure and the sample lines from the enclosure to the instruments were either metal, plastic with conductive paint, or conductive rubber tubing. The gross dimensions of the enclosure were $1.05 \times 1.25 \times 1.23$ m high. The enclosure had a flow distribution manifold on top with 20 25-mm holes and three layers of window screen at the outlet to provide an evenly distributed gentle flow of air over the subjects. Air exited the exposure enclosure through two 76-mm (3-in.) diameter ducts, located on the back wall beneath each seat. Sampling lines came from a 150×200-mm (6×8 in.) sampling panel located on the back wall of the enclosure, between the two subjects approximately 0.4 m from their mouths. Subject access was provided by a removable one-piece door, which sealed to a steel frame around the enclosure doorway by magnetic seals on the enclosure door.

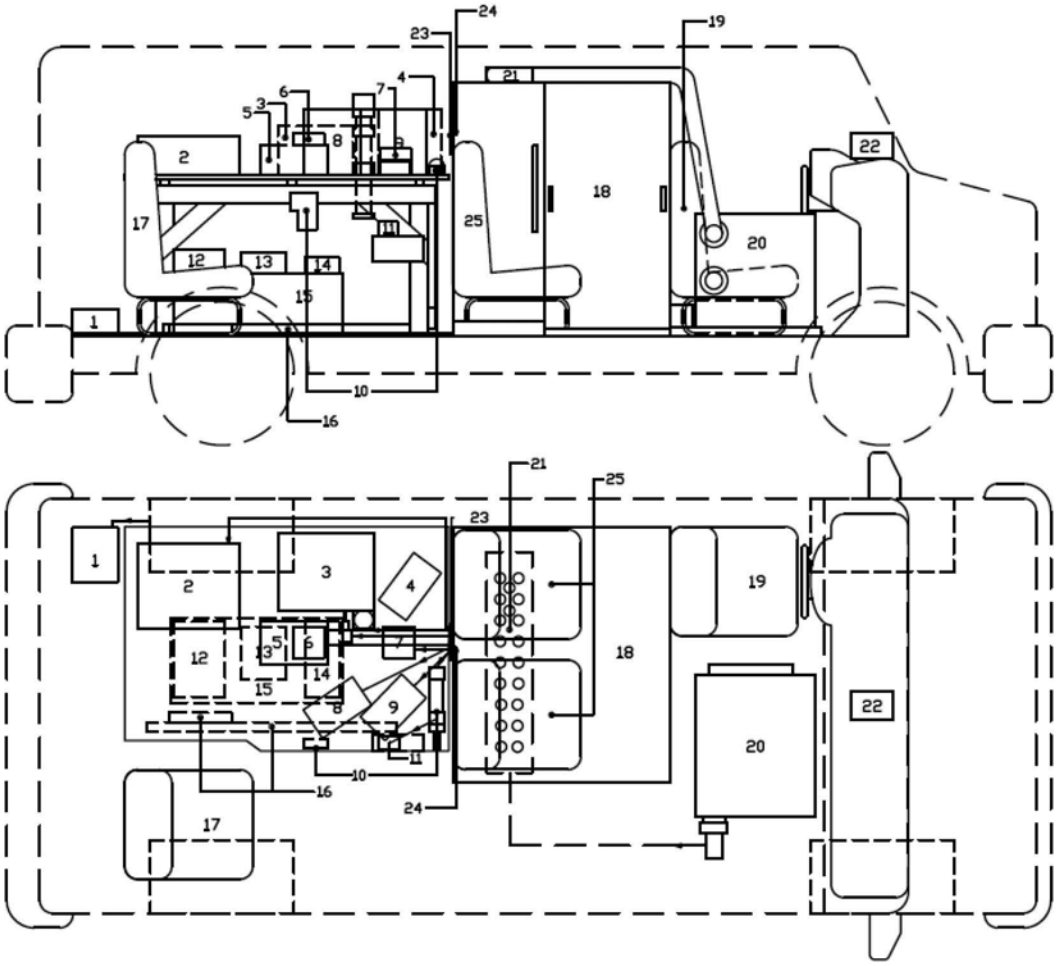


Figure 1. Schematic diagram of the exposure and measurement system (upper panel: side view, lower panel: top view). (1) NOx pump, (2) NOx analyzer, (3) SMPS (electrostatic classifier), (4) SMPS (CPC), (5) PAS 2000, (6) DustTrak (PM-2.5), (7) DustTrak (PM-10), (8) aethalometer, (9) CPC 10, (Q-Trak 11), BGI pump for PEM filter, (12) inverter, (13) switch box, (14) battery charger, (15) battery box, (16) power strips, (17) operator seat, (18) exposure chamber, (19) driver seat, (20) filter box and fans, (21) chamber inlet, (22) camcorder, (23) chamber sampling panel, (24) PEM 2.5 filter, and (25) subject seats.

2. Experimental Design

a. Subject Recruitment and Screening.

A convenience sample of 19 adult Southern California residents aged 60 or older was recruited by word of mouth and advertising. Informed consent was obtained from each volunteer. The project was reviewed and approved by the Institutional Review Boards of UCLA and Rancho Los Amigos National Rehabilitation center. Volunteers were required to be clinically healthy non- or ex-smokers (quit at least 1 yr before study), as determined by medical screening including routine cardiovascular physical examination, medical history, lung function testing, and resting 12-lead electrocardiogram (ECG). Individuals with mild hypertension or on anti-hypertensive medication were accepted, because they are common among the late middle-aged population whose functional status and overall health is good in the conventional sense. (In other words, subjects were not limited to people in a physiologically normal and medication-free state, because doing so might have biased the sample towards atypically healthy individuals, who might also be atypically nonresponsive to pollutants.) Subjects on medication were required to standardize their dose across all four of their exposure sessions. Exclusion criteria included use of medications that might mask responses, as well as history of congestive heart failure, myocardial infarction, asthma,

chronic obstructive pulmonary disease, or significant occupational exposure to dusts and fumes. Volunteers were required to live more than 500 feet from a freeway and not more than 20 miles from the base laboratory, with access to the laboratory by surface-street travel only. Table 1 summarizes subject characteristics. Of the 19 subjects five were taking blood pressure medication and five were taking cholesterol lowering medication including three that were taking both.

Table 1. Subject Characteristics

ID #	Sex	Ethnicity	Height (in)	Weight (lb)	Age (y)	First Expos.	Last Expos.
2676	F	White	62	156	73	6/7/06	6/27/06
2692	F	White	60.5	138	75	7/5/06	7/26/06
2675	F	White	64	232	65	8/2/06	8/23/06
2680	F	White	66	250	69	9/6/06	9/27/06
2683	F	White	66.5	150	77	10/4/06	10/25/06
2705	M	White	73	142	83	10/4/06	10/25/06
2694	M	Hispanic	70	209	70	11/1/06	11/29/06
2674	M	White	67	158	73	12/6/06	12/27/06
2691	M	Asian	67	186	75	1/9/07	1/30/07
2681	F	White	63.5	129	71	2/6/07	2/27/07
2677	F	Asian	60	141	73	2/6/07	2/27/07
2707	M	White	73	223	65	3/6/07	3/27/07
2713	M	White	71	208	64	3/6/07	3/27/07
2710	F	White	61	128	61	4/4/07	4/25/07
2708	M	White	72	225	68	5/1/07	5/22/07
2709	M	White	68	191	70	5/1/07	5/22/07
2716	M	White	68.5	184	74	3/25/08	5/7/08
2842	M	Hispanic	67	184	66	3/25/08	5/7/08
2841	M	White	66	165	76	5/13/08	6/3/08

b. Exposure

An on-freeway exposure system was designed to expose senior subjects (60 or older) to either freeway air or filtered freeway air. Each participant underwent four 2-hr freeway exposures—one on I-405 and one on I-710, and one each on the same freeways, but with filtered air. The I-710 freeway is the major truck-shipping route connecting Long Beach port to downtown Los Angeles and inland distribution centers. It has a total of nine to ten lanes and up to 25% of vehicles are heavy-duty diesel trucks [Zhu, et al, 2002a]. The I-405 freeway is one of the busiest freeways in the United States. It has a total of nine to ten lanes and approximately 5% diesel traffic [Zhu, et al, 2002b].

Figure 2 shows the actual exposure routes on the two freeways, which were used for this study. During the exposure periods, total traffic density on I-710 typically ranged from 180 to 230 vehicles/min. Traffic density on I-405 usually ranged from 200 to 270 vehicles/min.

Ventilation air delivered to the exposure enclosure was filtered until we actually entered the desired freeway. At that time either filtration continued or it was switched to unfiltered air.

Figure 2. Map showing van routes.

Switch-over was done manually from inside the vehicle while the vehicle was under way. Switch-over was not observable by the driver or the subjects riding in the vehicle.

Route on I-710 Starting from Rancho Los Amigos Medical Center (RLAMC), the van entered the I-710 freeway at Imperial and went North to Third Street, reversed directions and went south to Del Amo Boulevard, and them back to Imperial. This route was repeated three times. Exposure started when the van entered I-710 and ended when it exited I-710. The complete route took approximately two hours on the I-710.

Route on I-405 This route included exposure on I-105, approximately 25%, and I-405, approximately 75%. Traffic composition was similar on both freeways. Starting from Rancho Los Amigos Medical Center, the van entered I-105 freeway eastbound at Garfield Avenue. The van traveled to I-405 and went south toward Long Beach. Turn around in the Long Beach area was at either Alameda Street, Long Beach Boulevard, or Cherry Avenue depending on traffic and timing and the northern turn around was at El Segundo. Two round trips were used. Exposure started when the van entered I-105 and ended when it exited I-105.

While the van traveled on all lanes of the freeways, the downwind (easterly) lanes were favored. This gives the most integrated and highest exposure concentrations. To avoid following a high or low emitting vehicle for too long (more than four minutes), the driver deliberately changed lanes when the van was following the same vehicle for four minutes.

c. Experimental Protocol.
All studies began at the base laboratory in Downey, California near the junction of the I-710 and I-105 freeways. The four combinations of freeway and filter status were presented in randomized order. Staff members who measured health responses were blinded to both freeway and filter condition; subjects were blinded only to filter condition. Intervals between exposures were normally seven days (14 days in one instance, 28 in another). Between the base laboratory and the freeway entrance (typically about five min travel time) the exposure atmosphere was always filtered.

Subjects were instructed to minimize potentially confounding air pollution exposures during the 24 hr preceding and following each experimental exposure, by avoiding exposure to tobacco smoke, cooking, freeways, and busy streets, and driving with car windows closed and air conditioning off or in low-flow recirculation mode. They filled out time-activity diaries [Linn et al., 1999; Frazier et al., 2008] over that same time interval. Rough estimates of their ambient PM exposures during the 24 hr before the freeway exposure were determined from diary records of location, and average hourly PM data obtained from the nearest ambient air monitoring stations. Usually the nearest stations were Long Beach for PM-10 and downtown Los Angeles for PM-2.5.

One or two subjects were exposed in each run (i.e. each study day) in each sequence, depending on subject availability. Table 2 summarizes the experimental protocol for one subject/one run. The exposure period began near 10 a.m. and lasted two hours from entering to leaving the freeway. Traffic was usually heavy and free-flowing, conducive to high exposure levels of UF particulate matter. Runs (successive exposures of a given individual or pair) were scheduled 7 days apart, with two exceptions. In sequence 6, runs 3 and 4 were two weeks apart because of the intervening Thanksgiving holiday. In sequence 13, runs 1 and 2 were 4 weeks apart, because the van required repairs. During the time required to travel between the laboratory and the beginning of the freeway route, normally about 5-10 minutes each way, the van air was always filtered. After exposure subjects returned to the base laboratory, underwent post-exposure ("post") health testing, then rested until those tests were repeated approximately 2 hr after the end of exposure ("hour 2" testing). They returned home, with ambulatory cardiovascular monitoring and time-activity recording in operation, until the following morning when they returned to the base laboratory for removal of the ambulatory monitors and repeat health tests ("day 2" testing).

The subjects, the investigators and staff who collected and interpreted health data, and the van driver were blinded to experimental atmospheric condition (filtered vs. unfiltered) The health personnel were also blinded as to which freeway was used on a given day: subjects and van personnel were instructed not to discuss it with them. The order of presentation of exposures was randomly assigned, and was counterbalanced insofar as possible, so that time-dependent effects would not confound the interpretation of exposure atmosphere effects.

One possible confounder is subject's exposure to freeway atmosphere prior to or after their exposure. Subjects were instructed to minimize their freeway exposure the day before and during the 24 hour study period. Detailed time-activity diaries spanning the entire 24-hour interval were kept by each subject. Table 3 provides the information for a typical diary from one of the study subjects. This diary is similar to one previously designed for a volunteer panel with chronic

Table 2. Sequence of Procedures for Each Exposure Session

Day	Time	Activity
Day 1		
	08:00	Arrive at lab; rest in clean air; equipment hookup.
	09:00	Pre-exposure cardiopulmonary examination; symptom recording; SpO_2; spirometry; blood draw; start Holter ECG and ambulatory blood pressure monitor ("pre" testing).
	10:30	Resting exposure for 2 hr in van on freeway; record symptoms every 15 minutes.
	12:30	Return to lab; rest in clean air; symptom recording; SpO_2; spirometry ("post" testing).
	14:30	Symptom recording; SpO_2; spirometry; blood draw ("hour 2" testing); leave laboratory; fill out time-activity diary through the following morning.
Day 2		
	08:00	Arrive at lab; rest in clean air; post-exposure cardiopulmonary examination; symptom recording; SpO_2; spirometry; blood draw ("day 2" testing).
	09:00	Discharge from laboratory.

obstructive pulmonary disease. As shown in Table 3, time-activity information is recorded for each clock hour to allow direct linkage with continuous air monitoring data. The diary was easy to fill out and included questions on time spent indoors, outdoors, and in vehicles; time near vs. away from home; time exercising vs. resting (including a self-assessment of exercise intensity); breathing symptoms experienced; and medications taken. Table 4 summarizes exposure dates, exposure routes (I-405 vs. I-710), and exposure conditions (filtered (F) vs. unfiltered (U)) for all runs.

d. Measured Environmental Parameters

Table 5 summarizes environmental parameters and associated measurement methods used in the on-freeway exposure system. A sampling panel located at the height of subjects' breathing zone in the back wall of the exposure chamber was used to hold sampling lines. No consideration was given to isokinetic sampling because wind speed was minimal inside the exposure chamber. Stainless steel and flexible conductive tubing (Part 3001940, TSI Inc.) were used for sample inlet lines for all particle-measuring instruments to avoid particle losses, due to charge buildup and electrostatic forces. To minimize the residence time and particle loses; all of the sampling lines were kept straight and as short as possible. Sampling line losses due to diffusion were calculated to be less than 5% for 10-nm particles for all instruments [Hinds, 1999].

A scanning mobility particle sizer (SMPS; 3936L85, TSI Inc.) was used to measure fine and UF particle size distribution in the size range of 7.6 to 289 nm. This instrument consists of two components, a Model 3080 Electrostatic Classifier with a Model 3081 Long Differential Mobility Analyzer (LDMA) to select particles of a given size and a water-based condensation particle counter (CPC; 3785, TSI Inc.) to count the particles. The water-based CPC [Hering, et al, 2005] is essential for in-vehicle exposure assessment of particle number concentrations. Butanol-based CPCs are well known to emit butanol vapors and

Table 3. Data from a typical diary filled out on the day of an exposure session*

ID NO	DATE	HOUR	TIME SPENT: AT HOME	NEAR HOME	AT WORK	OTHER	MINUTES OUTDOORS	MINUTES IN VEHICLE	MINUTES ACTIVE	% MAX EXERTION	SYMPTOMS: SOB	COUGH	WHEEZE	OTHER	MEDS	2ND HAND SMOKE	NEAREST STATION
3218	10/12/2007	0	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	1	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	2	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	3	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	4	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	5	all				0	0	0	0	none	mild	none	none		none	SE LA Co
3218	10/12/2007	6	some			some	< 20	< 20	< 20	20	none	none	none	none	thyroid	none	SE LA Co
3218	10/12/2007	7				all	< 20	< 20	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	8				all	0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	9				all	0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	10				all	< 20	20-40	0	0	none	none	none	none		none	[on fwy]
3218	10/12/2007	11				all	0	60	0	0	none	none	none	none		none	[on fwy]
3218	10/12/2007	12				all	< 20	< 20	0	0	none	none	none	none		none	[on fwy]
3218	10/12/2007	13				all	0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	14		some		some	< 20	20-40	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	15	some	some		some	< 20	< 20	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	16		all			< 20	0	20-40	40	none	none	none	none		none	SE LA Co
3218	10/12/2007	17	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	18	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	19	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	20	all				0	0	0	0	none	none	none	none	statin	none	SE LA Co
3218	10/12/2007	21	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	22	all				0	0	0	0	none	none	none	none		none	SE LA Co
3218	10/12/2007	23	all				0	0	0	0	none	none	none	none		none	SE LA Co

***A diary was also filled out during all waking hours of the day prior to the exposure session, and the day following the exposure session until completion of the day-2 laboratory visit.

Table 4. Exposure dates, condition, and subject ID

Seq	Run	Date	ID	Fwy	Filter*
1	1	6/7/2006	2676	710	F
1	2	6/14/2006	2676	405	F
1	3	6/21/2006	2676	710	U
1	4	6/28/2006	2676	405	U
2	1	7/5/2006	2692	710	F
2	2	7/12/2006	2692	405	U
2	3	7/19/2006	2692	710	U
2	4	7/26/2006	2692	405	F
3	1	8/2/2006	2675	710	F
3	2	8/9/2006	2675	710	U
3	3	8/16/2006	2675	405	F
3	4	8/23/2006	2675	405	U
4	1	9/6/2006	2680	710	U
4	2	9/13/2006	2680	405	F
4	3	9/20/2006	2680	405	U
4	4	9/27/2006	2680	710	F
5	1	10/4/2006	2683	710	U
5	2	10/11/2006	2683	405	U
5	3	10/18/2006	2683	405	F
5	4	10/25/2006	2683	710	F
5	1	10/4/2006	2705	710	U
5	2	10/11/2006	2705	405	U
5	3	10/18/2006	2705	405	F
5	4	10/25/2006	2705	710	F
6	1	11/1/2006	2694	405	F
6	2	11/8/2006	2694	710	U
6	3	11/15/2006	2694	405	U
6	4	11/29/2006	2694	710	F
7	1	12/6/2006	2674	405	U
7	2	12/13/2006	2674	405	F
7	3	12/20/2006	2674	710	U
7	4	12/27/2006	2674	710	F
8	1	1/9/2007	2691	405	F
8	2	1/16/2007	2691	710	F
8	3	1/23/2007	2691	405	U
8	4	1/30/2007	2691	710	U
9	1	2/6/2007	2677	405	U
9	2	2/13/2007	2677	710	F
9	3	2/20/2007	2677	405	F
9	4	2/27/2007	2677	710	U
9	1	2/6/2007	2681	405	U
9	2	2/13/2007	2681	710	F
9	3	2/20/2007	2681	405	F
9	4	2/27/2007	2681	710	U
10	1	3/6/2007	2707	710	F
10	2	3/13/2007	2707	710	U
10	3	3/20/2007	2707	405	U
10	4	3/27/2007	2707	405	F
10	1	3/6/2007	2713	710	F
10	2	3/13/2007	2713	710	U
10	3	3/20/2007	2713	405	U
10	4	3/27/2007	2713	405	F
11	1	4/4/2007	2710	710	U
11	2	4/11/2007	2710	405	F
11	3	4/18/2007	2710	710	F
11	4	4/25/2007	2710	405	U
12	1	5/1/2007	2708	405	U
12	2	5/8/2007	2708	710	U
12	3	5/15/2007	2708	710	F
12	4	5/22/2007	2708	405	F
12	1	5/1/2007	2709	405	U
12	2	5/8/2007	2709	710	U
12	3	5/15/2007	2709	710	F
12	4	5/22/2007	2709	405	F
13	1	3/26/2008	2016	405	F
13	2	4/23/2008	2016	710	F
13	3	4/30/2008	2016	710	U
13	4	5/7/2008	2016	405	U
13	1	3/26/2008	2842	405	F
13	2	4/23/2008	2842	710	F
13	3	4/30/2008	2842	710	U
13	4	5/7/2008	2842	405	U
14	1	5/13/2008	2841	405	U
14	2	5/20/2008	2841	405	F
14	3	5/27/2008	2841	710	F
14	4	6/3/2008	2841	710	U

*F= filtered air; U= unfiltered air

Table 5. Environmental Parameters and Measurement methods.

	Species/Parameter	Instrument	Detection Limit	Flow Rate (L/Min)	Response Time (s)
1	Ultrafine Particle Size Distribution	TSI SMPS	Single Particle	1.5	100/200
2	Particle Number Concentration	TSI CPC	Single Particle	0.3	<2
3	PM-10 Real Time Mass	TSI Dusttrak 8520 with 10 µm inlet impactor	1 µg/m^3	1.7	60
4	PM-2.5 Real Time Mass	TSI Dusttrak 8520 with 2.5 µm inlet impactor	1 µg/m^3	1.7	60
5	Elemental Carbon	Portable Aethelometer AE-42	1 µg/m^3	5	60
6	CO, CO2, Temperature, Relative humidity	TSI Q-trak	1 ppm, 0.1°C, 1%	n/a	60
7	Particle-bound PAHs	EcoChem PAS 2000	ng/m^3	2	30
8	NO, NO_2, NOx	API 200AU	<1 ppb	1	60
9	Location and Speed	Camcorder, Garmin GPS18	10 m	n/a	1

are not suitable for human health studies in confined environments. A second water-based CPC (3785, TSI Inc.) was used to measure total particle number concentration in the size range from 5–6 nm to a few micrometers at 1-sec intervals. The high-time-resolution CPC data captured the rapid change in particle number concentration during freeway travel and provided a measure of total particle number concentrations.

A TSI DustTrak photometer (Model 8520 TSI, Inc., St. Paul, MN) with a PM-2.5 inlet impactor was used to continuously monitor particle mass concentration. The same size cut was used for all runs. Powered by an internal battery, the DustTrak samples air at a constant flow rate of 1.7 L/min by means of a built-in diaphragm pump. The sampled airstream passes through a light scattering optical sensing zone. The detected signal is processed by lock-in circuitry followed by high-resolution digitization. The DustTrak covers a concentration range of 1 $\mu g/m^3$ to 400 mg/m^3 and provides an auto-ranging digital display for both real-time and time-averaged concentrations. DustTrak data were calibrated against simultaneous gravimetric measurements using a personal environmental monitor (PEM) that collects PM-2.5 mass. After each run, the PEM filter was weighed, the gravimetric data recorded, and compared to the DustTrak run average. Paired PEM and DustTrak data were pooled to generate a calibration factor which was then used to correct all DustTrak data. For I-405 freeway the relationship between the PM-2.5 filter (F) and the PM-2.5 DustTrak (DT) was F=0.306(DT)+16.38 with r=0.70 and F=0.518(DT)+ 14.50 with r=0.82 for I-710.

A Magee Scientific, Inc. two-channel Aethalometer model AE-42 was used to measure the elemental carbon concentration in near-real time. This device measures the attenuation of a beam of light transmitted through a filter while the filter is continuously collecting an aerosol sample. The instrument measures particulate black carbon (BC) in the near infrared (δ = 880 nm) using a solid-state source. The rate of accumulation of BC is proportional to both the BC concentration in the airstream and the flow-rate. This instrument also produces an output called UVPM. It represents a scaled mass

concentration based on the combined absorption of BC and PAHs at 8 = 370 nm. The mass of additional PAHs cannot be determined from this signal unless the proportions of PAHs are known, so it is expressed as BC equivalent, that is the amount of BC that would give the same absorption at 8 = 370 nm [Hansen, 2005]. Because of saturation effects this signal may actually be slightly lower than the BC signal as was observed in this study. A sample cycle of 1 min was used. Data were continuously logged into an internal data logger. The factory calibration was used for this instrument.

Particle-bound polycyclic aromatic hydrocarbons (PB-PAHs) were measured in near-real time with an EcoChem Analytics PAS 2000. The instrument measures total, non-specific PB-PAHs (with three rings or more) primarily adsorbed to the particle's surface. It is sensitive in the ng/m^3 range. It has built-in data logging storage for 8000 data points (date, time, value). Manufacturer's calibrations were used for this instrument. Measured PB-PAH concentrations were found to be comparable with previously reported levels found on Los Angeles freeways [Westerdahl, et al, 2005].

NOx levels were measured in near-real time with a Teledyne API model 200AU chemiluminescent trace analyzer with an external pump. The instrument covers a range from 5 ppb to 2 ppm. It was calibrated with span gas in the laboratory.

A Q-Trak indoor air quality monitor (Model 8550, TSI Inc.) determined the concentration of carbon monoxide (CO) and carbon dioxide (CO2). An electrochemical cell is used for detecting these species. The detection limit for CO and carbon dioxide (CO2) is 0.1 ppm. Data were continuously logged into an internal data logger and then downloaded to a personal computer. A time resolution of 1 min was used. The instrument was calibrated by sampling known concentrations of CO and CO2. The Q-track was also used to monitor in-vehicle temperature and relative humidity. TrakPro software (version 3.33, TSI Inc.) was utilized for data reduction and analysis of the Q-Trak output.

The speed, direction, and location of the vehicle were monitored every few seconds with a Garmin GPS-18 global positioning system linked directly to the instrument laptop computer. City Select North American map data were loaded onto the laptop to provide a trace of the vehicle's path during each run. An 8-mm, high-resolution video recorder (camcorder) was mounted on the dashboard of the test vehicle to record traffic conditions during all measurement periods. It has been shown that a vehicle's occupants are primarily exposed to the exhaust of neighboring vehicles, particularly those directly ahead of the occupant's vehicle [Westerdahl, et al, 2005; Rodes, et al, 1998]. The camcorder was set to a wide angle to view as much of the freeway traffic as possible.

All instruments, except those with internal batteries, were powered by a battery-operated power supply system. Six batteries, sealed 97 A•hr, 12-V, lead acid, gel-type marine batteries (West Marine, Sea-Gel Deep Cycle 97), supplied power to a 1 kW pure sine wave inverter (120 V output) (Xantrex model 1000) with an associated charging system (Xantrex model TrueCharge 40+). The batteries were housed in a battery box secured to the vehicle deck behind the subject enclosure and under the instrument platform. The inverter, switch box, and charger were mounted on top of the battery box (Figure 1). The unit weighs approximately 230 kg (500 lbs) so Teflon skids were mounted on the bottom to facilitate installing and removing it from the van.

e. Subject Measurements

Exposure Dates
Table 4 summarizes exposure sequence, dates, subjects' IDs, exposure routes (I-405 vs. I-710), and exposure conditions (filtered (F) vs. unfiltered (U)) for all runs. In total, 19 subjects completed the full set of exposures. The study lasted for about two years from 6/7/2006 to 6/3/2008. The first 16 subjects were tested from 6/7/2006 to 5/22/2007. The additional three subjects were tested from 3/26/2008 to 6/3/2008. It usually took four consecutive weeks to finish one subject. For some runs, two subjects were tested on the same day, and consequently shared the same sequence number (5, 9, 10, 12, and 13).

Blinding.
Double-blinded study design was used in this study. Subjects and those doing the health monitoring were blinded to the type of exposure. Although the subjects and the driver could observe the difference between the I-405 and the I-710 exposure routes, only the PI and the instrument operator knew whether a particular run on a particular day was filtered or not. The instrument operator sat in the rear seat and had access to a control lever which switched the HEPA filter unit between providing particle free air for filtered runs or bypassing the filter for the unfiltered runs. The operator followed a prescribed randomized schedule provided by the PI on each run date to conduct either a filtered or a non-filtered run. Similarly, the instrument operator and the PI were blinded as to the health outcomes for each subject. Health data were not released until the subject completed all of his or her four runs.

f. Blood pressure, heart rate, physical activity and symptoms
A digital ambulatory electrocardiogram (H12+ digital Holter ECG recorder, Mortara Instruments, Inc.) was used to record continuous electrocardiographic data on each subject for approximately 24 hours, beginning one hour prior to exposure (see below for details). During every waking hour of Holter monitoring away from the laboratory, the subject recorded physical activity and symptoms in the standard diary. Systolic and diastolic blood pressure (via ambulatory automatic sphygmomanometer, Spacelabs Inc. Model 90217) and heart rate (via Holter ECG) were recorded, and rate-pressure product (an index of overall myocardial work) was calculated. Cardiac-related and non-cardiac symptoms were recorded and scored according to severity on a standardized form during pre-exposure, exposure, and post-exposure (Gong et al. 1998, 2002). As Table 2 indicates, these tests were performed immediately pre-exposure, immediately post-exposure, two hours post-exposure, and on the next morning, approximately 20 hours post-exposure.

g. Respiratory function measurements
Forced vital capacity (FVC), forced expired volume in 1 sec (FEV_1), and maximal midexpiratory flow (MMF) – along with total resistance of the respiratory system (Rrs) determined by forced oscillation at 5 and 20 Hz, and resonant frequency of the respiratory system (Fres), were measured using an MS-IOS integrated testing system (Viasys Inc., Yorba Linda, CA). The system was calibrated daily with a syringe following the manufacturer's procedure. Arterial oxygen saturation (SpO_2) and pulse rate were measured during each testing sequence by a fingertip pulse oximeter (Nellcor, Boulder, CO) with internal calibration standard. Conventional blood pressure measurements were performed with a digital sphygmomanometer (Sunbeam Inc., Hattiesburg, MS). Cardiac-related and non-cardiac symptoms were recorded on a standardized form and scored numerically by the subject according to severity, before, during and following exposure [Gong et al. 1998, 2003]. Total symptom score (TSS) was determined as the sum of scores for specific symptoms, each of which could be scored 0 (none), 1 (mild), 2 (moderate), 3

(severe) or 4 (incapacitating). Peripheral blood samples for analysis of cytokines and other blood markers relevant to systemic inflammatory response or coagulation disturbance (see Results) were drawn from an antecubital vein during "pre", "hour 2", and "day 2" testing sequences.

h. Cardiac function and Heart Rate Variability (HRV) measurements
Twenty-four hour ambulatory electrocardiograms (Holter Monitor)
Digital ambulatory electrocardiograms (Holter monitors) were collected continuously for approximately 24 hours using a Mortara H12+ with a 12-Lead digital ECG Recorder (Mortara Instrument Inc., Milwaukee, WI). The electrocardiographic signals were recorded from the body surface. Digital data sampled at 180 Hz were stored on flash cards prior to processing. Efforts were taken to have the subject remain recumbent and restful for at least 10 minutes before entering the van, after returning from the trip, and the following day. The digital flash cards were sent by express mail from Rancho to the Electrocardiographic Signal Processing Laboratory at the Brody School of Medicine at East Carolina University. The flash cards were downloaded to the Mortara H-scribe system for storage and analysis. Once the data was secure, the card was erased and returned to Rancho by express mail. Electrocardiographic data was backed-up weekly.

Arrhythmia detection and classification
The digitized electrographic signal was displayed to show the sequence of P waves, QRS complex and T waves. The P wave represents the registration of the atrial electrical activation, while the QRS complex represents the electrical activation of the ventricles. The T wave represents the recovery of the electrical activity of the ventricles and the interval between the initial component of the QRS complex and the end of the T wave, i.e. the QT interval, is generally accepted as the time needed for full recovery of the ventricles for repolarization. There is generally a close coupling of the P wave and the QRS complex such that the R-R interval corresponds to the interval between sinus node depolarizations. It is the R-R interval that is used to determine the instantaneous heart rate and heart rate variability. Thus, rapid heart rates are associated with shorter P-P intervals and R-R intervals. Under normal conditions the sinus node demonstrates automaticity and determines the cardiac rhythm. When a beat originates from a location of the heart outside of the sinus node, the resulting rhythm is termed an arrhythmia. Such arrhythmias can be slow or fast, regular or irregular, asymptomatic or symptomatic. A special class of arrhythmia is noted below, i.e. supraventricular premature beats and ventricular premature beats. Moreover, more subtle changes in the P-P and R-R intervals are influenced by autonomic input to the heart a parameter measured and reported as heart rate variability (HRV). Premature beats alone are typically of little clinical significance, yet when a premature beat occurs in vulnerable myocardium a sustained arrhythmia can occur. Such rhythms can be asymptomatic, symptomatic or even life-threatening. In the atrium premature beats can precipitate atrial fibrillation, atrial flutter, AV nodal reentrant tachycardia and atrio-ventricular reentrant tachycardia. Likewise, premature ventricular beats can in the proper environment induce sustained ventricular arrhythmias. In the present study the ambulatory electrocardiogram was utilized to explore the association between traffic related PM and gases and premature beats. If PM is associated with an increase in premature beats then it can be hypothesized that PM might contribute to clinically more important rhythm disturbances in hearts that are conditioned by ischemia, hypertrophy and other clinical conditions that increase the risk of serious arrhythmias.

In the early phase of the study H-scribe software was utilized in the autoscan mode to detect artifact, pauses, premature atrial beats (also referred to as atrial or supraventricular ectopic beats), premature ventricular beats (ventricular ectopic beats) and runs of supraventricular or ventricular beats (i.e. consecutive premature beats). After the release of a new version of H-scribe, all ambulatory ECG files were rescanned with version 4.21 which permitted a more detailed analysis of arrhythmia and in particular the ability to examine templates automatically generated for supraventricular beats. The detection parameters for supraventricular and ventricular beats were set as R-R interval shortening of 20% or greater when compared to the preceding R-R interval. Supraventricular beats were preceded by a P wave and generally had a narrow QRS complex, whereas premature ventricular beats were not preceded by a conducted P wave and had a wide QRS complex.

The technician adhered to the following sequence of analysis. First, all areas of electrocardiographic artifact were coded as such in order to prevent noise from causing the misclassification of R-R intervals, pauses and premature beats. Second, all pauses were inspected and confirmed. They were excluded from analysis if judged to be an artifact. Third, all premature supraventricular and ventricular beats were examined and confirmed. Misclassified beats were reclassified to the correct beat type: normal beat, supraventricular beat, or ventricular beat. In general, the visual inspection and manual analysis were done while looking at two ECG leads simultaneously, i.e. lead II and lead V5. In cases where the classification was still in doubt additional leads were displayed and considered.

No episodes of high-grade atrio-ventricular block were detected, nor was atrial fibrillation or sustained arrhythmia detected. All rhythms, all templates, and most of the premature beats were inspected by a board certified cardiologist with 25 years of experience in the interpretation of electrocardiographic data. A report was subsequently generated showing the number of minutes recorded, the number of beats detected; the mean heart rate, the minimum heart rate, the maximum heart rate, the number of premature supraventricular and ventricular beats, as well as episodes of consecutive premature beats, i.e. couplets (two consecutive beats) or runs (three or greater consecutive beats). The number of pauses and their duration were also displayed. As with most HRV studies provocative measures, for example postural changes or paced breathing, were not undertaken to modulate HRV either before, immediately after exposure, or the following day.

Heart rate indices were calculated and displayed on the report for the entire period of recording and included %R-R>50 (PNN50), rms-SD (root mean square of successive differences in normal-beat intervals), Kleiger SD (SDNN) and the Magid SD (ASDNN5). The report also contains a Rhythm Profile that shows the statistics for minimum, maximum and mean heart rate, PNN50, rmsSD and Magid SD for each hour during the period recorded, as well as the number of supraventricular and ventricular ectopic beats.

Once the dataset was cleaned and finalized, the data were imported to another analysis tool, MISHA. MISHA is a powerful proprietary electrocardiographic waveform software analysis tool provided by Dr. David Mortara at the request of the investigators, that allows for the measurement of multiple electrocardiographic parameters such as the characteristics of the P wave, the QRS complex, the ST segment and the T wave, as well as the QTc, i.e. the QT interval corrected for rate. MISHA also expands the analysis of HRV to include the frequency-domain. Frequency-domain measures are calculated on 5-minute epochs starting with the activation of the digital recorder. Time-domain measures are also available. While MISHA provides significant opportunity to further explore the

impact of exposure of traffic related pollutants on the electrocardiographic parameters, MISHA has certain characteristics that limit its ease of use. It has proven to be time consuming and laborious. MISHA output contains VLF, LF, HF, and total power as well as T wave characteristics such as principal component analysis of the T wave.

Non-linear measures of HRV were calculated with Kubios HRV version 2.0 (http://kubios.uku.fi) provided by Dr. Mika Tarvainen and Juha-Pekka Niskanen from the Biosignal Analysis and Medical Imaging Group of the Department of Physics, University of Kuopion, Kuopio, Finland. Kubios HRV software was used to generate Poincaré plots with SD1 and SD2, where SD1 describes short-term variability and SD2 describes long-term variability. Other calculations included approximate entropy, and sample entropy.

i. Blood biomarkers

Biomarkers were chosen to inform on several possible responses by the cardiovascular system to traffic related pollution. The response of the vasculature to the effects of traffic related air pollution was assessed by measuring: sE-selectin, soluble vascular cellular adhesion molecule (sVCAM), soluble intercellular adhesion molecule (sICAM), matrix metallopeptidase-9, myeloperoxidase (MPO), VEGF, and tissue plasminogen activator inhibitor-1 (t-PAI-1). Pro-inflammatory biomarkers included IL-1beta, IL6, IL8, IL10, MCP-1, IFN gamma, and TNF-alpha. NT-proBNP on the other hand was included to evaluate whether traffic related air pollution has direct or indirect effects on intracardiac pressure or myocardial stretch causing the cardiac secretion of NT-proBNP. NT-proBNP was chosen rather than BNP because of its longer half-life.

A powerful high-throughput xMAP multiplex immunobead based assay technology (Luminex Corp., Austin, TX) was used to simultaneously test human cardiovascular disease (hCVD) panel 1 (6-plex vascular injury panel containing sE-selectin, sVCAM, sICAM, MMP9, MPO, tPAI-1), panel 2 (2-plex containing CRP and fibrinogen) and panel 3 (9-plex cytokine panel containing IL-1beta, IL6, IL8, IL-10, MCP-1, IFN gamma, TNF-alpha, NT-proBNP, VEGF) in human serum samples of 19 volunteers exposed to particle pollution from vehicles. These blood samples were collected before, after two hours of exposure, and 24 hours post-exposure, processed and the serum stored at -80°C until further use. There were four different experimental exposure conditions, and three blood samples were collected per exposure for each subject (4x3= total 12 samples per subject). Human CVD panels 1, 2 and 3 kits were purchased from Millipore corporation (Lincoplex multiplex assay kits) and the above serum samples (25 µl/well in duplicates) were used for these assays either diluted (as per the instructions) or undiluted in 96-well micro plates (supplied with kits) along with standards as per the manufacturer's instructions. After the first eight subjects assays for IL-1beta, IL-8, IL-10, IFN gamma and TNF-alpha were dropped because concentrations were below detection limits.

j. Data Analysis

The basic statistical tool for assessment of health responses was mixed-model analysis of variance/covariance, with repeated measures on subjects, using commercial statistical software (SAS Institute, Cary, NC; BMDP Statistical Software, Cork, Ireland, Microsoft Corporation, Redmond, WA).

Initially, "treatment" analyses were performed to test main and interactive effects of freeway [I-710 (trucks) vs. I-405 (cars)], filter status (filtered vs. unfiltered), and time, ignoring exposure concentration differences between subjects under a given freeway-filter condition.

This analytical design is commonly used in controlled experiments that compare response to a fixed concentration of an artificially generated pollutant against clean air, or an experimental drug against a placebo. The analyses were performed using SAS procedure MIXED, specifying freeway, filter status, and time as fixed factors and subject as a random factor. Thus, in principle, freeway and filter status were considered as uniform experimental states under control of the investigators, while the subjects were considered as a random sample of the late middle-age population at risk from freeway pollution. The analyses were performed on raw data across all four times of measurement, and also on changes in health measures over different intervals before to after exposure. For any health measure, the null hypothesis was that no significant ($P < 0.05$) variation would occur in relation to freeway, filter status, or time of measurement. The alternative hypothesis of interest was that unfavorable changes would occur in response to freeway PM exposure. In that event, a significant interactive effect of filter status and time should be found, or a significant main effect of filter status if response was expressed as a change from pre- to post-exposure. If PM consistently differed in exposure level or toxicity between I-405 and I-710, a significant freeway-filter interaction or freeway-filter-time interaction would be expected. If the experimental exposure protocol itself, or any of the pollutant gases not removable by filters, caused a change in a health measure, this would be detected as a significant main effect of time.

"Treatment"-type analyses were also applied to environmental variables, to test how well actual exposure conditions met the expectations of the experimental design.

"Exposure-response" analyses tested linear relationships between the change in a given health variable pre- to post-exposure and the concentration of a particular pollutant (treated as a covariate) across the four exposure runs, ignoring freeway and filter status. The null hypothesis was that the slope of the estimated regression line relating health-variable change to pollutant concentration was not significantly different from zero. The alternative hypothesis was that the regression slope differed significantly ($P < 0.05$) from zero, in the direction indicating that health response became more unfavorable as exposure concentration increased. These analyses were also performed with SAS procedure MIXED. When statistical significance was found, plots of response vs. exposure and checks of the MIXED results were generated using Microsoft Excel. Exposure-response analyses were expected to be more informative than treatment-type analyses if exposure concentrations varied substantially from one subject to another, and if the response was substantially dose-related within the experienced concentration range. Although treatment and exposure-response effects can be combined in one analytical model, that was not done here because of the relatively small sample size: the model would involve too many degrees of freedom relative to the number of data points. In all analyses, health variables with appreciably skewed distributions were log-transformed before analysis. Additional details of statistical methodology are given in the Results section.

C. RESULTS AND DISCUSSION

1. Environmental

Summary of Measured Pollutant Concentrations

Figure 3 presents a typical one-hour time-series plot for measured pollutants inside the exposure chamber while driving on the I-710 freeway under filter bypass (unfiltered air) mode. Scans were averaged over one-minute intervals. There was a ten minute break in black carbon concentration (Figure 3c) due to an automatic tape-advancing event in the

Aethelometer instrument. In general, two to three tape-advancing events were usually observed on the diesel truck dominated I-710 freeway whereas only one such event usually occurred on the gasoline vehicle dominated I-405 freeway during the two-hour exposure runs. This is likely due to the much greater black carbon concentrations from diesel emissions on the I-710 freeway. Strong concentration variations were observed for all measured pollutants. There is usually a factor of greater than 5 between the highest and the lowest concentrations.

Figure 4 shows the histograms of major air pollutant concentrations based on one-minute-averaged observations. The black bars and the grey bars represent the I-405 and I-710 freeways, respectively. For each figure, the X-axis is the measured pollutant concentrations on a linear scale and the Y-axis is observed frequencies in percent. In general, all pollutant concentrations show a dominant mode with right-skewness. The modes of pollutant concentrations measured on the I-405 freeway were generally to the left of those measured on the I-710 freeway except for CO. This suggests exposure levels on the I-405 freeway were generally lower than those on the I-710. This is particularly true for total particle number concentrations (Fig 4a), black carbon concentrations (Fig 4c), particle-bound PAH concentrations (Fig 4d) and NOx concentrations (Fig 4e).

Table 6 summarizes concentrations of major environmental parameters measured on each

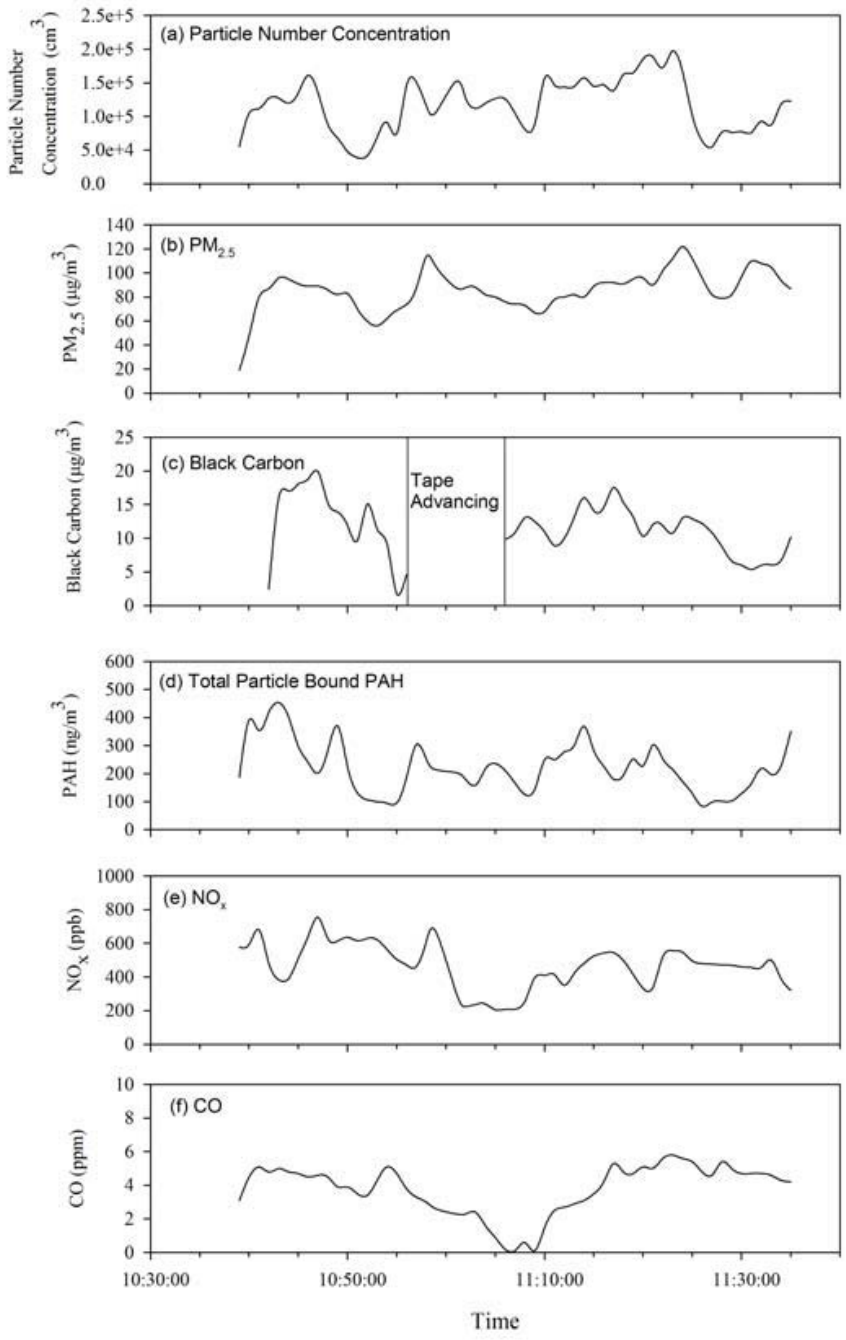


Figure 3. Typical one-hour time-series plots for pollutant concentrations (unfiltered) measured inside the exposure chamber on the I-710 freeway. (a) Particle number concentration (b) PM-2.5 (c) Black Carbon (d) Total Particle Bound PB-PAH (e) NOx and (f) CO.

run. The total particle number concentrations measured by the CPC ranged from 1544 particles/cm^3 on a filtered I-405 run to 175,071 particles/cm^3 on an unfiltered I-710 run. Similarly, much lower concentrations were observed for the filtered runs compared to the unfiltered runs for particle phase pollutants including PM-2.5, PM-10, BC, and particle bound PAH. In contrast, no significant changes in the gas-phase pollutant concentrations were observed between filtered and unfiltered runs. This suggests the HEPA system is

providing an excellent exposure contrast between the gas- and particle-phase

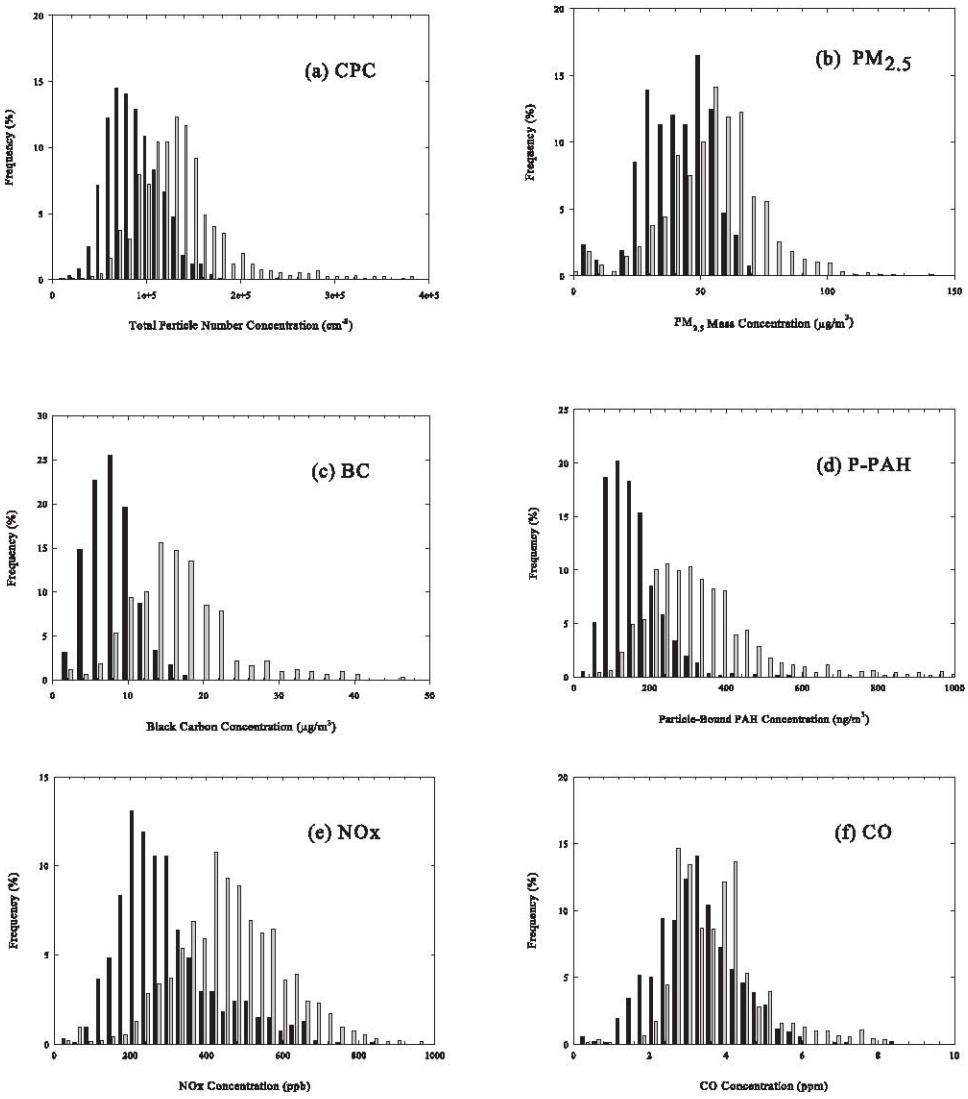


Figure 4. Histogram of measured (a) total particle number, (b) PM-2.5, (c) black carbon, (d) particle bound PAH, (e) NO_x, and (f) CO concentrations inside the exposure enclosure while driving on the I-405 and I-710 freeways under unfiltered mode. Black bars represent I-405. Grey bars represent I-710.

pollutants as discussed in detail later. Since the ventilation conditions were set to a fixed comfortable level for the studied subjects, reasonably stable temperature and relative humidity were achieved for all runs. Concentrations of all measured pollutants were higher on the diesel freeway than on the gasoline freeway. The highest one-minute averaged particle number concentration, 730,000 particles per cm^3, was observed on the I-710 freeway. The highest one-minute averaged PM-2.5 concentration, 150 μg/m3, was also observed on the I-710 freeway on the same day but at a different time.

Table 6. Concentrations of major environmental parameters measured for each subject *.

Date	Fwy	Filter	Subject	Total Particle Number (#/cm^3)	UF particles (#/cm^3)	PM-2.5 (μg/m^3)	PM-10 (μg/m^3)	BC (μg/m^3)	UVPM (μg/m^3)	PB-PAH (ng/m^3)	NOx (ppb)	CO (ppm)	CO_2 (ppm)	Temp (F)	Rh (%)
2006/6/7	710	F	1	4431	7445	n/a	n/a	1.17	0.77	6.88	451	n/a	n/a	n/a	n/a
2006/6/14	405	F	1	1987	3558	n/a	n/a	0.77	0.55	2.50	414	0.95	582	68.09	35.18
2006/6/21	710	U	1	160312	78924	74.6	84.2	11.86	9.48	231.79	413	3.33	599	66.46	36.34
2006/6/28	405	U	1	77860	35874	n/a	n/a	4.60	3.82	104.64	448	3.21	583	64.87	39.62
2006/7/5	710	F	1	2703	3163	15.5	17.8	0.84	0.66	4.92	339	2.72	575	68.18	37.28
2006/7/12	405	U	1	65203	37822	29.9	33.3	5.76	4.78	110.61	199	2.63	596	67.13	37.29
2006/7/19	710	U	1	90240	63074	57.8	66.1	11.75	9.35	176.12	399	5.69	729	69.71	40.97
2006/7/26	405	F	1	1544	2494	15.0	18.3	0.19	0.00	3.42	n/a	6.43	579	71.15	39.72
2006/8/2	710	F	1	2997	3306	11.2	17.6	0.86	0.73	5.07	292	3.26	595	68.46	43.42
2006/8/9	710	U	1	100291	69152	64.3	70.9	8.09	6.67	161.23	408	4.69	588	70.67	36.75
2006/8/16	405	F	1	2214	1978	8.2	15.1	0.86	0.61	2.68	220	2.17	606	61.40	44.51
2006/8/23	405	U	1	77709	43353	50.1	63.5	6.91	6.00	139.89	231	3.25	612	63.87	40.92
2006/9/6	710	U	1	146238	87259	64.0	67.9	14.62	11.61	352.57	466	4.39	631	67.73	37.74
2006/9/13	405	F	1	1666	687	10.1	11.2	0.74	0.64	4.22	155	2.39	584	64.86	42.13
2006/9/20	405	U	1	106683	40965	51.6	53.9	5.50	4.70	118.74	207	3.33	598	66.70	39.09
2006/9/27	710	F	1	3742	1429	9.1	10.2	0.81	0.71	7.41	466	3.96	642	73.12	30.07
2006/10/4	710	U	1	147764	80769	42.2	50.7	10.68	8.75	234.79	379	2.80	656	66.84	37.92
2006/10/4	710	U	2	147764	80769	42.2	50.7	10.68	8.75	234.79	379	2.80	656	66.84	37.92
2006/10/11	405	U	1	96786	41252	n/a	n/a	5.11	4.29	94.62	170	n/a	n/a	n/a	n/a
2006/10/11	405	U	2	96786	41252	n/a	n/a	5.11	4.29	94.62	170	n/a	n/a	n/a	n/a
2006/10/18	405	F	1	2288	771	8.4	14.9	1.65	1.32	5.50	n/a	n/a	n/a	n/a	n/a
2006/10/18	405	F	2	2288	771	8.4	14.9	1.65	1.32	5.50	n/a	n/a	n/a	n/a	n/a
2006/10/25	710	F	1	3949	1534	18.3	26.5	0.76	0.65	6.88	457	2.90	724	64.63	41.65
2006/10/25	710	F	2	3949	1534	18.3	26.5	0.76	0.65	6.88	457	2.90	724	64.63	41.65
2006/11/1	405	F	1	2460	935	9.8	12.5	0.84	0.66	4.49		2.00	599	63.27	41.86
2006/11/8	710	U	1	123313	74567	61.7	71.3	13.70	11.14	293.23	502	3.22	646	67.12	32.62
2006/11/15	405	U	1	90835	42387	29.0	30.3	6.30	5.40	11.98	245	3.19	602	65.18	36.29
2006/11/29	710	F	1	4635	1854	n/a	n/a	0.71	0.62	6.92	494	1.61	580	76.18	22.41
2006/12/6	405	U	1	89592	53394	72.8	84.4	8.52	9.80	109.54	448	3.86	675	70.99	22.98
2006/12/13	405	F	1	2329	1092	8.6	10.8	1.30	1.13	5.41	351	3.64	657	74.13	26.64
2006/12/20	710	U	1	175071	134985	45.1	49.8	9.96	8.41	198.45	551	2.66	607	76.35	20.19
2006/12/27	710	F	1	2911	1006	3.1	7.2	0.39	0.29	3.12	n/a	0.86	569	74.24	26.61

Table 6. Concentrations of major environmental parameters measured for each subject (cont.).

Date	Fwy	Filter	Subject	Total Particle Number (#/cm^3)	UF particles (#/cm^3)	PM-2.5 (µg/m^3)	PM-10 (µg/m^3)	BC (µg/m^3)	UVPM (µg/m^3)	PB-PAH (ng/m^3)	NOx (ppb)	CO (ppm)	CO_2 (ppm)	Temp (F)	Rh (%)
2007/1/9	405	F	1	2084	870	4.2	6.3	1.01	0.77	6.00	285	2.14	623	73.32	21.45
2007/1/16	710	F	1	4991	1908	3.2	5.8	1.21	0.98	7.86	510	3.12	616	75.21	13.48
2007/1/23	405	U	1	76982	56128	27.8	33.2	6.25	5.36	144.69	309	1.75	620	71.46	20.14
2007/1/30	710	U	1	129156	99288	n/a	n/a	9.07	7.36	242.77	421	2.73	613	74.40	27.48
2007/2/6	405	U	1	68130	52306	71.6	84.1	6.53	5.42	123.18	247	3.01	657	72.98	30.22
2007/2/6	405	U	2	68130	52306	71.6	84.1	6.53	5.42	123.18	247	3.01	657	72.98	30.22
2007/2/13	710	F	1	4924	2183	n/a	n/a	0.69	0.61	7.17	444	2.35	627	72.19	28.54
2007/2/13	710	F	2	4924	2183	n/a	n/a	0.69	0.61	7.17	444	2.35	627	72.19	28.54
2007/2/20	405	F	1	2116	887	4.3	13.7	0.54	0.43	3.95	174	2.05	595	72.66	31.15
2007/2/20	405	F	2	2116	887	4.3	13.7	0.54	0.43	3.95	174	2.05	595	72.66	31.15
2007/2/27	710	U	1	46951	48952	n/a	n/a	5.27	4.51	145.97	245	1.17	614	83.47	18.15
2007/2/27	710	U	2	46951	48952	n/a	n/a	5.27	4.51	145.97	245	1.17	614	83.47	18.15
2007/3/6	710	F	1	4164	1709	4.6	12.6	1.09	0.89	8.14	551	2.73	709	75.31	24.33
2007/3/6	710	F	2	4164	1709	4.6	12.6	1.09	0.89	8.14	551	2.73	709	75.31	24.33
2007/3/13	710	U	1	100028	75698	36.4	42.7	7.74	6.29	174.53	391	3.81	733	72.70	27.99
2007/3/13	710	U	2	100028	75698	36.4	42.7	7.74	6.29	174.53	391	3.81	733	72.70	27.99
2007/3/20	405	U	1	76705	52071	32.5	36.0	5.13	4.37	110.89	216	2.82	614	72.09	31.36
2007/3/20	405	U	2	76705	52071	32.5	36.0	5.13	4.37	110.89	216	2.82	614	72.09	31.36
2007/3/27	405	F	1	1623	542	n/a	n/a	0.68	0.67	3.11	126	2.41	589	71.12	27.58
2007/3/27	405	F	2	1623	542	n/a	n/a	0.68	0.67	3.11	126	2.41	589	71.12	27.58
2007/4/4	710	U	1	106208	84727	92.1	102.5	12.28	10.03	239.12	405	3.47	607	73.22	31.22
2007/4/11	405	F	1	1865	550	n/a	n/a	1.18	0.53	5.28	162	2.53	566	70.90	32.46
2007/4/18	710	F	1	2379	842	n/a	n/a	0.35	0.22	3.30	289	3.62	579	69.55	18.41
2007/4/25	405	U	1	67494	50293	27.0	30.5	4.98	4.33	126.51	284	2.44	636	73.11	30.10
2007/5/1	405	U	1	62341	35423	41.2	45.7	4.01	3.57	80.71	238	3.05	624	72.75	33.21
2007/5/1	405	U	2	62341	35423	41.2	45.7	4.01	3.57	80.71	238	3.05	624	72.75	33.21
2007/5/8	710	U	1	78838	62053	26.0	30.5	9.28	7.50	n/a	666	5.13	639	78.22	18.02
2007/5/8	710	U	2	78838	62053	26.0	30.5	9.28	7.50	n/a	666	5.13	639	78.22	18.02
2007/5/15	710	F	1	6347	2328	9.6	11.3	1.05	0.62	n/a	523	2.86	667	65.61	37.93
2007/5/15	710	F	2	6347	2328	9.6	11.3	1.05	0.62	n/a	523	2.86	667	65.61	37.93
2007/5/22	405	F	1	2088	906	n/a	n/a	0.68	0.43	n/a	213	2.27	663	70.45	35.04
2007/5/22	405	F	2	2088	906	n/a	n/a	0.68	0.43	n/a	213	2.27	663	70.45	35.04

Table 6. Concentrations of major environmental parameters measured for each subject (cont.).

Date	Fwy	Filter	Subject	Total Particle Number (#/cm^3)	UF particles (#/cm^3)	PM-2.5 (µg/m^3)	PM-10 (µg/m^3)	BC (µg/m^3)	UVPM (µg/m^3)	PB-PAH (ng/m^3)	NOx (ppb)	CO (ppm)	CO_2 (ppm)	Temp (F)	Rh (%)
2008/3/26	405	F	1	2805	1048	n/a	n/a	0.89	0.68	9.30	274	2.58	826	67.74	41.95
2008/3/26	405	F	2	2805	1048	n/a	n/a	0.89	0.68	9.30	274	2.58	826	67.74	41.95
2008/4/23	710	F	1	6221	2417	n/a	n/a	1.89	1.64	n/a	479	n/a	n/a	n/a	n/a
2008/4/23	710	F	2	6221	2417	n/a	n/a	1.89	1.64	n/a	479	n/a	n/a	n/a	n/a
2008/4/30	710	U	1	77256	69818	n/a	n/a	5.71	4.74	282.48	463	2.43	656	74.14	n/a
2008/4/30	710	U	2	77256	69818	n/a	n/a	5.71	4.74	282.48	463	2.43	656	74.14	n/a
2008/5/7	405	U	1	75818	45440	n/a	n/a	3.77	3.24	190.16	174	1.27	691	69.63	n/a
2008/5/7	405	U	2	75818	45440	n/a	n/a	3.77	3.24	190.16	174	1.27	691	69.63	n/a
2008/5/13	405	U	1	67133	45705	n/a	n/a	4.33	3.70	194.67	175	1.50	609	71.47	31.57
2008/5/20	405	F	1	2185	814	n/a	n/a	1.03	0.92	10.18	195	3.13	635	73.81	33.77
2008/5/27	710	F	1	5456	2084	n/a	n/a	1.45	1.28	17.73	442	n/a	n/a	n/a	n/a
2008/6/3	710	U	1	110290	79291	n/a	n/a	7.19	5.77	394.03	437	3.23	621	72.31	32.58

*Determined from mean values for each subject under each exposure condition; thus, runs with 2 subjects have double the weight of runs with one subject. See text for description of statistical differences. “Total particle number” refers to CPC readings, and “UF particles” refers to SMPS measurements less than 100 nm. Table 6a in the Appendix has comparable information listed by runs. Measurement of PM-10 and PM-2.5 by DustTrak instruments experienced many "lost memory" situations and the PM-2.5 instrument malfunctioned in 2008 and had to be sent back to the factory for repair. Values for both PM-10 and PM-2.5 were removed from analysis when PM-2.5 was significantly larger than PM-10 to enable a valid comparison of the two measurements.

Note, under filtered conditions, particle number concentrations in each SMPS size bin were very low and highly variable. These concentrations should be interpreted with caution. The accuracy of CPC measurements under these conditions is ±10%. Relative standard deviations for UF particles under "filtered" conditions were >60% (see Table 7a).

Exposures

Tables 7a, 7b, and 7c show the mean and standard deviation, median, and geometric mean for key environmental measurements for each freeway and filter condition, respectively. The statistics in Table 7, oriented toward analyses of health effects, are based on 2-hr average concentrations for each subject. Thus, they do not necessarily correspond with atmospheric-science-oriented statistics, based on minute-by-minute concentration measurements, presented later in this section. In "treatment"-type analyses of 2-hr-average measurements, the filter main effect was highly significant for every variable representing PM, as expected. The freeway main effect and the freeway•filter interaction were expected to be significant for PM variables that relate closely to truck exhaust, for which unfiltered concentrations should have been higher on I-710 than I-405. That was true for particle count (averaging 37% higher on I-710), and for mass concentrations of black carbon (89% higher) and PB-PAH (69% higher). For particulate variables not as closely related to truck exhaust – PM-10 and PM-2.5 mass concentrations - the freeway effect and interaction were non-significant, and mean unfiltered exposure levels on I-710 averaged only slightly higher than on I-405.

Variables representing pollutant gases, "background" particulate pollution in the 24 hours before exposure, and in-vehicle climate conditions ideally would not have differed by freeway or by filter status. That was true for the background particulate variables, PM-10 (measured in Long Beach, the monitoring location closest to both freeway routes) and PM-2.5 (data not available from Long Beach, therefore obtained from downtown Los Angeles). For those two variables, the largest differences in means across different exposure conditions were less than 20%. For CO, the filter effect was non-significant but the freeway effect approached statistical significance (P = 0.066), with I-710 averaging roughly 20% higher than I-405. This could relate to more traffic on I-710, and/or to higher background CO concentrations along the I-710 corridor, which has more upwind pollution sources and may experience more atmospheric stagnation than the I-405 corridor. For NOx, again the filter effect was non-significant, but the freeway effect was highly significant, with about a 90% increase on I-710 compared to I-405. Again, this could relate to more traffic and/or to more background pollution. For temperature in the van, the filter effect was non-significant and the freeway effect was modestly significant (P = 0.036), with averages near 70º F (21^{o} C) on I-405 and 72º F (22^{o} C) on I-710. This could reflect generally warmer daytime weather farther inland, not completely controlled by van air conditioning. Similarly, relative humidity averaged near 33% on I-405 and 29% on I-710 (P for freeway effect = 0.02).

Note that we have emphasized UFP, because the principal a priori hypothesis was that UFP exposure would be associated with acute effects. In fact UFP measurements showed the most highly statistically significant associations with measured biological changes. BC and PAH showed weaker relationships, and were not specifically addressed in the a priori hypothesis. That does not necessarily mean that they are less important in causing health effects: the weaker associations could be due to larger measurement errors.

Ultrafine Particle Size Distributions on Freeways

Typical time-resolved UF particle size distributions measured inside the exposure chamber on the

two freeways are shown as contour plots in Figure 5, where x-axis presents the time at which data were collected, y-axis is the particle size on a log scale and the color intensity indicates normalized particle number concentration ($dN/dLogD_p$) for a given size at a given time. The same concentration scale was used for both freeways. Data were collected when the vehicle ventilation system was set to air conditioning on and recirculation off. These ventilation settings were chosen to ensure subjects' comfort inside the exposure chamber. The same ventilation settings were used for all runs.

In general, particle number concentrations were lower on the I-405 freeway than on the I-710 freeway as indicated by more green color in Figure 5b than in Figure 5a. For both freeways, hot spots usually occurs around 10-30 nm corresponding to a primary nuclei mode. There are periods between hot spots when particle number concentrations are lower on both freeways. This happens due to the fact that each two-hour exposure run typically consisted of two or three round trip loops on the freeway. The lower concentration periods correspond to the turning points at which we drove off the freeways to use local

Table 7a. Mean (standard deviation) of environmental measurements, by freeway and filter condition, averaged for all subjects.*

Variable	Units	I-405 unfilt.	I-405 filt.	I-710 unfilt.	I-710 filt.
Total Particle Number	count/cm^3	77845 (12734)	2115 (356)	107515 (36585)	4498 (1252)
UF particles	count/cm^3	45205 (6672)	1120 (760)	76097 (18906)	2283 (1397)
PM-2.5	µg/m³	44.5 (17.6)	8.1 (3.3)	51.4 (19.6)	9.8 (5.7)
PM-10	µg/m³	50.8 (21.3)	13.1 (3.2)	58.5 (21.0)	14.5 (7.0)
BC	µg/m³	5.38 (1.24)	0.88 (0.36)	9.26 (2.81)	0.99 (0.42)
UVPM	µg/m³	4.72 (1.47)	0.68 (0.32)	7.55 (2.20)	0.79 (0.38)
PB-PAH	ng/m^3	118.97 (42.89)	5.17 (2.36)	233.23 (70.91)	7.17 (3.33)
NOx	ppb	244 (82)	224 (82)	436 (109)	455 (77)
CO	ppm	2.68 (0.77)	2.59 (1.13)	3.37 (1.24)	2.72 (0.75)
CO_2	ppm	630 (33)	634 (78)	644 (44)	641 (56)
Temperature	deg F	69.98 (3.19)	69.70 (3.71)	73.09 (5.20)	70.69 (4.26)
Rel. Humidity	%	32.50 (5.77)	34.65 (6.65)	29.41 (8.21)	30.44 (9.15)
Prior 24 hr Amb. PM-10	µg/m³	30.0 (9.2)	26.2 (8.6)	30.1 (8.3)	29.6 (9.7)
Prior 24 hr Amb. PM-2.5	µg/m³	24.0 (5.6)	21.7 (7.7)	21.5 (10.1)	23.6 (12.2)

*Determined from mean values for each subject under each exposure condition; thus, runs with 2 subjects have double the weight of runs with one subject. “Total particle number” refers to CPC readings, and “UF particles” refers to SMPS measurements. See text for description of statistical differences.

Table 7b. Median of environmental measurements, by freeway and filter condition, averaged for all subjects.*

Variable	Units	I-405 unfilt.	I-405 filt.	I-710 unfilt.	I-710 filt.
Total Particle Number	count/cm^3	76705	2116	100291	4431
UF particles	count/cm^3	45440	887	75698	2084
PM-2.5	µg/m³	41.2	8.4	45.1	9.6
PM-10	µg/m³	45.7	13.7	50.7	12.6
BC	µg/m³	5.13	0.84	9.28	0.86
UVPM	µg/m³	4.37	0.66	7.50	0.66
PB-PAH	ng/m^3	110.89	4.49	234.79	6.92
NOx	ppb	231	213	413	461
CO	ppm	3.01	2.39	3.23	2.86
CO_2	ppm	620	599	639	627
Temperature	deg F	71.46	70.90	72.70	72.19
Rel. Humidity	%	31.57	35.04	31.22	28.54
Prior 24 hr Amb. PM-10	µg/m³	29.9	23.9	30.5	29.8
Prior 24 hr Amb. PM-2.5	µg/m³	24.3	19.8	19.9	20.6

Table 7c. Geometric mean of environmental measurements, by freeway and filter condition, averaged for all subjects.*

Variable	Units	I-405 unfilt.	I-405 filt.	I-710 unfilt.	I-710 filt.
Total Particle Number	count/cm^3	76916	2086	101130	4323
UF particles	count/cm^3	44733	973	74134	2034
PM-2.5	µg/m³	41.6	7.5	48.0	8.1
PM-10	µg/m³	47.1	12.7	55.0	13.0
BC	µg/m³	5.3	0.81	8.8	0.9
UVPM	µg/m³	4.6	0.67	7.2	0.7
PB-PAH	ng/m^3	106.9	4.73	223.7	6.6
NOx	ppb	234	211	423	448
CO	ppm	2.54	2.42	3.12	2.59
CO_2	ppm	629	630	643	638
Temperature	deg F	69.91	69.60	72.92	70.57
Rel. Humidity	%	31.97	34.02	28.23	29.01
Prior 24 hr Amb. PM-10	µg/m³	n/a	n/a	n/a	n/a
Prior 24 hr Amb. PM-2.5	µg/m³	n/a	n/a	n/a	n/a

n/a = not available

streets to get back on the freeway in the opposite direction. In Figure 5a, a second mode around 60-100 nm usually occurs suggesting bi-modal size distributions on the I-710 freeway. This is in agreement with previous observations inside passenger vehicles on the I-710 freeway (Zhu et al., 2007). On the I-405 freeway, the primary mode was also around 10-30 nm, but the second mode seems broader and less obvious. These plots can be used later with time resolved health indicators to study the acute effect of UF particles in different size ranges.

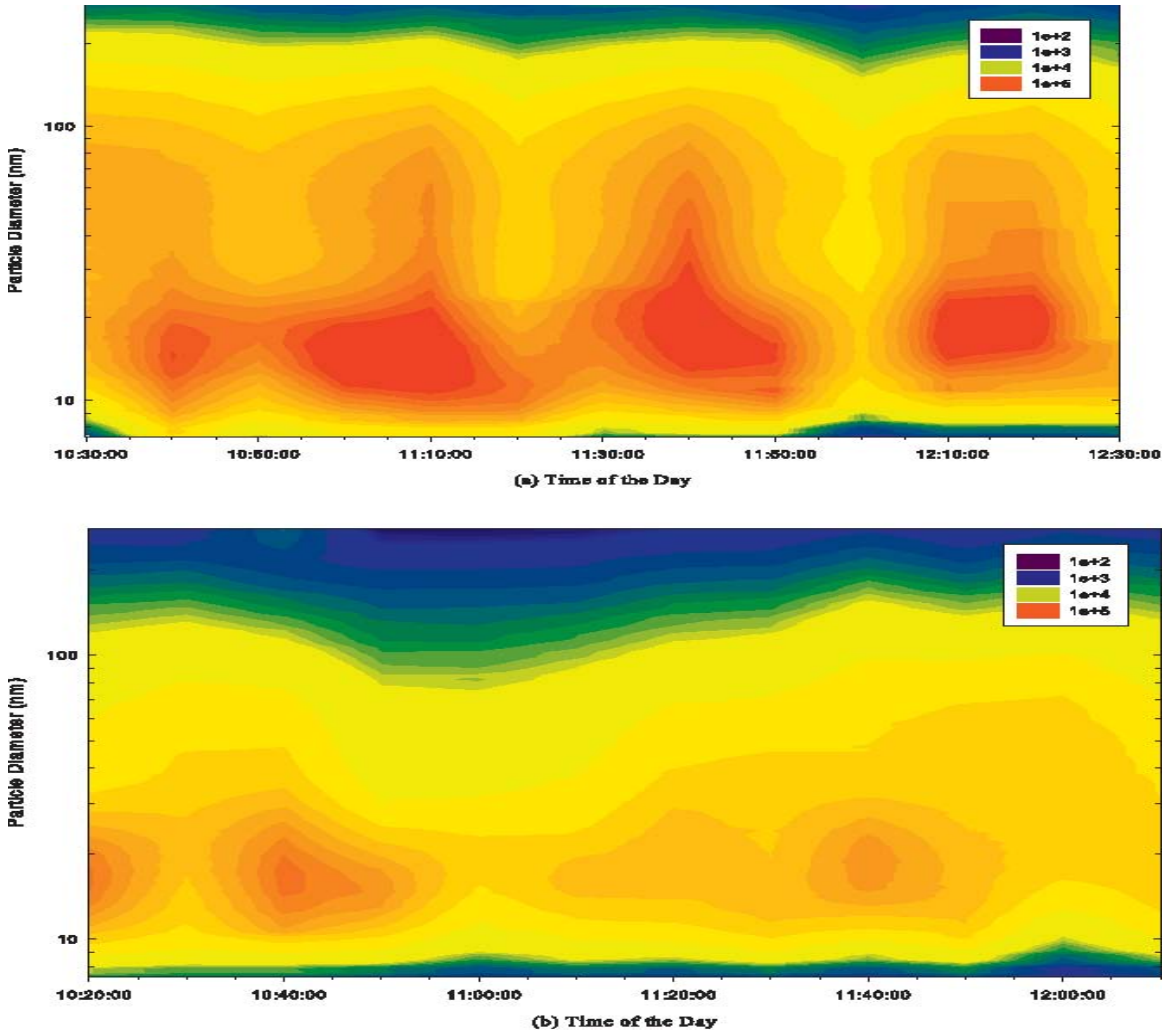


Figure 5. Typical contour plot of UF particle number based size distribution during two hours exposure runs under unfiltered mode on the (a) I-710 and (b) I-405 Freeways.

Exposure Contrast

Large exposure contrasts are critical to the success of health effects studies. In this study, the innovative design of the mobile exposure system and the selection between a gasoline and a diesel dominated freeway led to good exposure contrasts as discussed in the following sections.

I-405 vs. I-710 Freeways. As shown in Tables 6 and 7, and Figures 4 and 5, higher pollutant concentrations were observed on the I-710 freeway, which has much more diesel traffic. This is in agreement with a previous study that reported a linear relationship between number of diesel trucks and particle number concentrations on freeways [Westerdahl, et al, 2005]. The UF particle size distribution characteristics on the two freeways became even clearer when averaged data were

plotted and fitted to bi-modal lognormal size distributions in Figures 6a and 6b for unfiltered and filtered conditions, respectively. The data for each freeway were fitted to the sum of two lognormal distributions using modified SigmaPlot 2000 lognormal six parameter fitting procedure [SigmaPlot, 2000]. For each mode, the geometric mean diameter, μ_g, and the geometrical standard deviation, σ_g, are also shown in Figures 6a and 6b. Error bars in Figure 6a indicate one-standard-deviations. Error bars in Figure 6b were not included because they were relatively large compared to the mean thus making it difficult to distinguish between the two freeways. For both freeways under both filtered and unfiltered conditions, a primary mode around 17-20 nm and a secondary mode around 50-55 nm were observed. The geometrical standard deviations for each mode were also comparable. These size distribution characteristics are consistent as indicated by the smooth error bars in Figure 6a. They were also in good agreement with previously reported in-cabin particle size distributions observed with passenger vehicles on the same freeways [Zhu, et al, 2007]. Thus, subjects who underwent exposure runs with unfiltered air were exposed to similar levels of UF particles found inside passenger vehicles under normal driving conditions.

The primary difference between the two freeways is the particle concentration. In fact, if we multiply the I-405 freeway data with a factor to match up the total particle number within the measured size range on the two freeways, we obtain almost identical size distributions on both freeways. The current finding of higher particle concentrations on I-710 is in contrast to previous studies conducted adjacent to freeways that found that UF particle number concentrations and size distributions were not significantly different between I-405 and I-710 [Zhu, et al, 2002a]. This difference may be due to the fact that near-roadway, as compared to on-roadway, measurements reflect an integrated effect of all the passing vehicles. Although I-710 has a greater fraction of diesel traffic, each heavy duty diesel truck accounts for the space of two to three gasoline vehicles and emits only about two times more UF particles than gasoline engines [Kittelson, et al, 2004]. When both freeways were operating at full capacity, little difference in particle concentrations was observed adjacent to the freeway. However, the data presented here are more likely to be affected by the specific vehicle that the van is following. While diesel trucks were only about 25% of vehicles on I-710, they occupied about 50% of freeway space. Thus, the van had a much greater chance to be within the emission plume of a heavy-duty diesel truck on I-710 than on I-405, which translated into a much stronger signature of diesel emissions. Similar concentration differences have been observed in passenger vehicles on the two freeways [Zhu, et al, 2007].

Filtered vs. Unfiltered Runs

Data presented in Tables 6 and 7 indicate that more than 96% of the particles were removed on a total number basis under filtered conditions for both freeways. Figure 6c presents averaged size-specific filtration system collection efficiency on both the I-710 and I-405 freeways. In general, the filtration system provided about 96% collection efficiency for all measured particle sizes. The collection efficiency is slightly lower on the I-405 and slightly higher on the I-710 for particles less than 20 nm, but the differences are within 2%. For particles larger than 30 nm, the collection efficiencies are very similar for both freeways.

Table 6 and 7 also show significant reduction of PM-2.5 and PM-10 under filtered conditions on these two freeways. The mass concentration of PM-2.5 under filtered condition was 81%

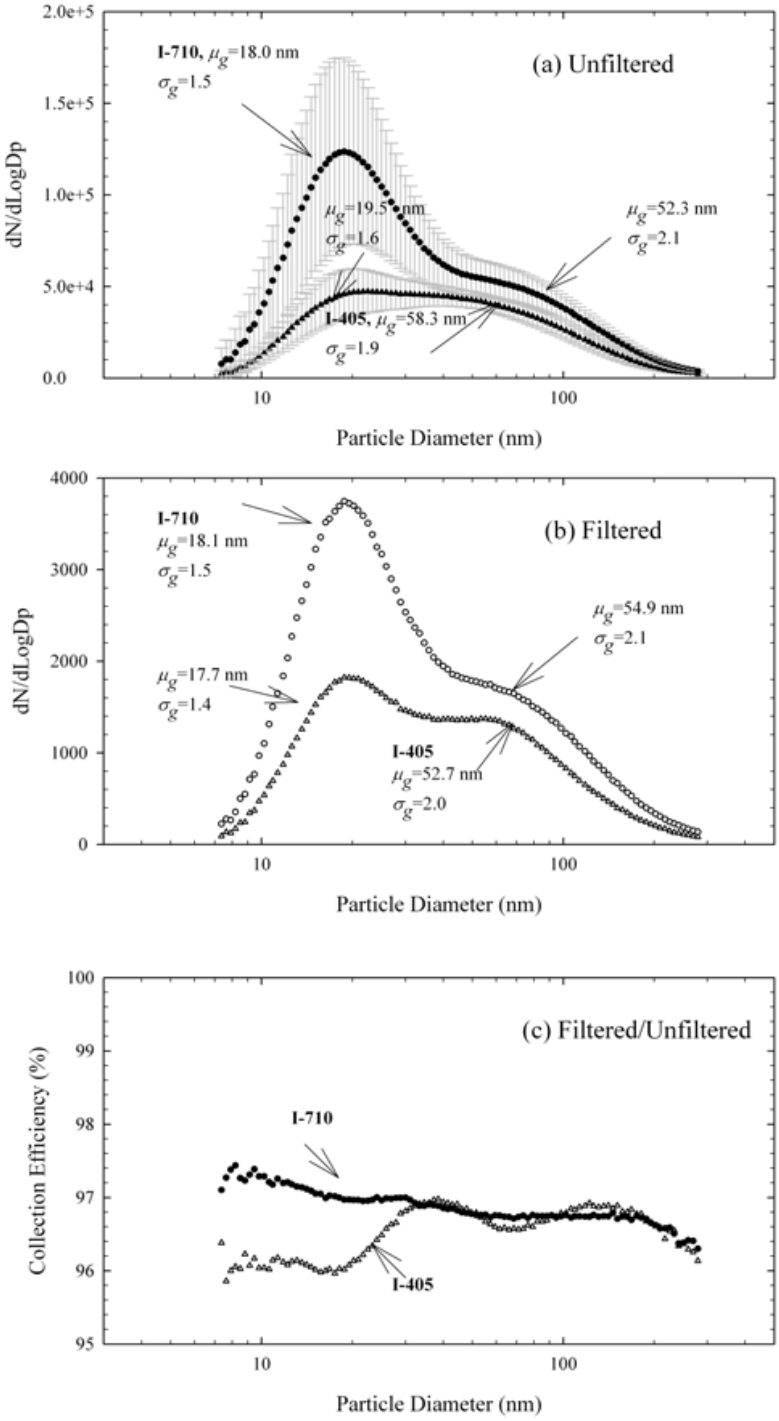


Figure 6. Fitted bi-modal particle size distribution inside the exposure chamber under (a) unfiltered and (b) filtered conditions while driving on the I-710 and I-405 Freeways and (c) size-specific collection efficiencies of the filtration system. Data were averaged for all applicable runs. Error bars indicate one standard deviation.

lower than that unfiltered conditions. The collection coefficient of the filter system to remove larger particle, PM-10 was slightly lower, around 75%. The difference on the collection efficiency for UF particles, PM-2.5 and PM-10 was likely due to larger particles being generated in the exposure enclosure due to vibration and movement of subjects. No significant difference was observed on the removal efficiency of the filtration system on both freeways.

As shown in Tables 6 and 7, black carbon and particle bound PAH concentrations were also reduced to about 5% of unfiltered concentrations. The particle-phase pollutant concentrations were all statistically significantly lower under filtered conditions than unfiltered conditions for each freeway. No significant decreases were observed for gas-phase pollutants on the I-405 freeway. Slight decreases of gas-phase pollutant concentrations were observed on the I-710 freeway, but these differences were not statistically significant. In general, NO_x and CO concentrations were comparable with and without the filter. Thus, the HEPA filtration system works the way we expected in providing excellent PM concentration contrast between exposure and control runs.

Ultrafine Particles vs. Other Pollutants.
The matrix of Pearson correlation coefficients among all pollutants measured on the I-710 and I-405 freeways is shown in Table 8. Each coefficient is based on more than 1200 one-minute observations. In general, non-significant pair-wise correlations between pollutants were observed inside the chamber. Non-significant correlations were observed between particle number concentrations and all other measured pollutants, similar to previously reported data from chasing studies [Pirjola, et al, 2004b. This is in contrast to previously reported strong correlations of normalized particle number, black carbon, and CO concentrations near freeways [Zhu, et al, 2002a; Zhu, et al, 2002b]. The strong correlations reported previously were spatial correlations between traffic pollutants undergoing similar atmospheric dispersion processes. Once the measurements were normalized to their maximum concentration, the main factors that determined their correlations were the wind speed and direction, which were the same for all pollutants. By contrast, correlations presented in Table 8 are temporal correlations. They reflect the source strength of surrounding vehicles on measured pollutants under various driving conditions. These correlations suggest that vehicle and driving conditions affect particle phase and gas phase pollutant emissions differently. They also indicate that no simple surrogate can be used in place of particle number concentrations for estimating commuters' exposure to UF particles.

Westerdahl and colleagues (Westerdahl et al., 2005) measured on-roadway UFP, particle length, BC, PAH, PM2.5, NO, NOx, NO2, CO, and CO2 concentrations on several Los Angeles roadways, including freeways, I-710 (diesel dominated) and I-110 (gasoline only), and surface streets for a total of five days in February and April 2003. In their study, air was pulled into a duct by an inline fan located downstream of all sampling ports. Air entered the duct through a window on the passenger-side, traveled through the manifold, passed the inline fan, and exited through a rear window on the driver's side. Thus, they sampled the freeway air directly. Strong and consistent correlations were reported for data collected on one day (April 16, 2003) between UFP and the following pollutants: particle length, BC, PAH, NO, and CO2. These are all directly emitted primary pollutants. No correlation data were presented between UFP and PM2.5 and NOx. Thus, the strong correlations reported in Westerdahl et al., (2005) reflect the impact of fresh diesel emissions.

In our study, air was sampled inside the exposure chamber after entry into the van via the van's ventilation system. We reported data collected over two years separately for the I-405 and I-710.

The low correlations reported in our study reflect the uncertainty and complexity of how meteorology, season, and driving affect commuters' exposure. Also the exposure chamber, like a vehicles interior space, acts like an averaging vessel to smooth out peaks and valleys of pollutant concentration. This may reduce correlation coefficients for in-vehicle concentration compared to outside freeway air.

Ultrafine Particles vs. PM-2.5.

The non-significant correlations between total particle number concentration and PM-2.5 mass concentration become even clearer in Figure 7. Paired hourly PM-2.5 and total particle number concentrations were plotted for each freeway. R^2 is also given in the figure. While particle number concentrations centered around 130,000 particles/cm^3 for I-710 and 84,000 particles/cm^3 for I-405, corresponding PM-2.5 concentrations ranged from 20 to 120 μg/m^3. The R^2 between particle number concentration and PM-2.5 mass concentrations ranges from 0.003 to 0.1 on the two freeways. These non-significant correlations suggest that there is no simple linear relationship between particle number emission factors and particle mass emission factors from vehicles. It also suggests that particles of different sizes behave differently when they penetrate into the in-cabin environment. In addition, the after-treatment systems may also play a role. For example, if

Table 8. Pearson correlation coefficients among measured pollutants inside the exposure chamber for unfiltered mode, for all subjects. (a) I-405 only and (b) I-710 only.

(a) I-405 freeway

	Total Particle Number	UF particles	PM-2.5	PM-10	BC	UVPM	PB-PAH	NO_x	CO	CO_2
Total Particle Number	1.000									
UF particles	-0.025 (0.920)	1.000								
PM-2.5	0.116 (0.706)	0.226 (0.457)	1.000							
PM-10	0.062 (0.841)	0.263 (0.385)	0.993 (<.0001)	1.000						
BC	0.277 (0.251)	0.485 (0.035)	0.570 (0.042)	0.603 (0.029)	1.000					
UVPM	0.279 (0.247)	0.453 (0.051)	0.569 (0.043)	0.587 (0.035)	0.943 (<.0001)	1.000				
PB-PAH	-0.245 (0.313)	0.323 (0.178)	0.242 (0.426)	0.297 (0.325)	-0.257 (0.288)	-0.208 (0.394)	1.000			
NO_x	-0.007 (0.977)	0.152 (0.534)	0.374 (0.208)	0.387 (0.191)	0.471 (0.042)	0.574 (0.010)	-0.212 (0.383)	1.000		
CO	0.302 (0.239)	-0.158 (0.546)	0.625 (0.022)	0.584 (0.036)	0.583 (0.014)	0.572 (0.016)	-0.737 (0.001)	0.511 (0.036)	1.000	
CO_2	-0.120 (0.647)	0.417 (0.096)	0.742 (0.004)	0.758 (0.003)	0.013 (0.960)	0.150 (0.567)	0.464 (0.061)	-0.087 (0.741)	-0.356 (0.161)	1.000

(b) I-710 freeway

	Total Particle Number	UF particles	PM-2.5	PM-10	BC	UVPM	PB-PAH	NO_x	CO	CO_2
Total Particle Number	1.000									
UF particles	0.816 (<.0001)	1.000								
PM-2.5	0.280 (0.354)	0.145 (0.637)	1.000							
PM-10	0.278 (0.359)	0.119 (0.698)	0.997 (<.0001)	1.000						
BC	0.674 (0.002)	0.384 (0.105)	0.600 (0.030)	0.594 (0.032)	1.000					
UVPM	0.684 (0.001)	0.405 (0.086)	0.605 (0.028)	0.600 (0.030)	0.999 (<.0001)	1.000				
PB-PAH	0.328 (0.198)	0.246 (0.342)	0.336 (0.312)	0.315 (0.346)	0.305 (0.233)	0.287 (0.265)	1.000			
NO_x	0.192 (0.432)	0.272 (0.260)	-0.466 (0.108)	-0.496 (0.085)	0.297 (0.217)	0.291 (0.226)	0.556 (0.021)	1.000		
CO	0.114 (0.641)	-0.019 (0.940)	-0.178 (0.561)	-0.207 (0.498)	0.485 (0.035)	0.458 (0.049)	0.093 (0.722)	0.595 (0.007)	1.000	
CO_2	-0.147 (0.550)	-0.175 (0.473)	-0.444 (0.128)	-0.419 (0.155)	-0.030 (0.904)	-0.050 (0.840)	-0.169 (0.516)	-0.033 (0.892)	0.318 (0.184)	1.000

*"Total particle number" refers to CPC readings, and "UF particles" refers to SMPS measurements.

a diesel truck is equipped with a particle filter, the soot mode will be removed and nucleation-mode particles will dominate. These particles contribute to higher number concentrations but not mass. On the other hand, if the soot mode is present, it may at least prevent the formation of nucleation mode particles and result in lower number but higher PM-2.5 mass. The poor correlation between particle number concentration and PM-2.5 mass may provide an opportunity to study their health effects separately.

Ultrafine Particles vs. NOx.

Hourly average total particle number concentration is plotted versus NOx concentration for the two freeways in Figure 8. R^2 is also given in the figure. The correlations between particles and NOx are non-significant for the two freeways. For the I-710 freeway, the correlation coefficient is about 0.38 for the I-710 freeway and 0.01 for I-405. This result is much lower than the 0.77-0.88 found in a

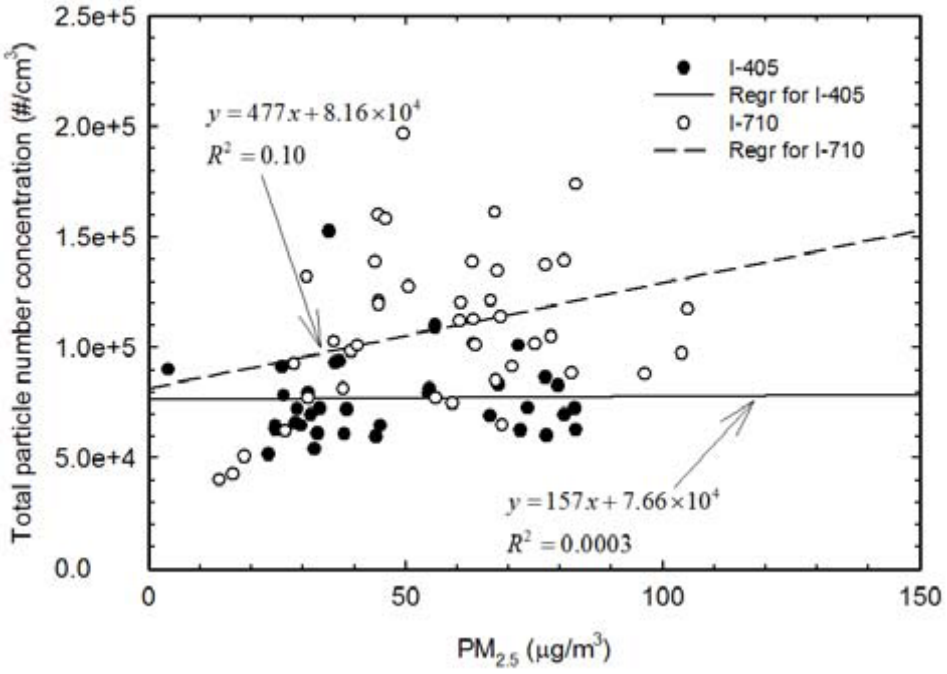


Figure 7. Correlations between PM-2.5 and total particle number concentration on the I-710 and I-405 freeways. Regression curves and equations are also shown.

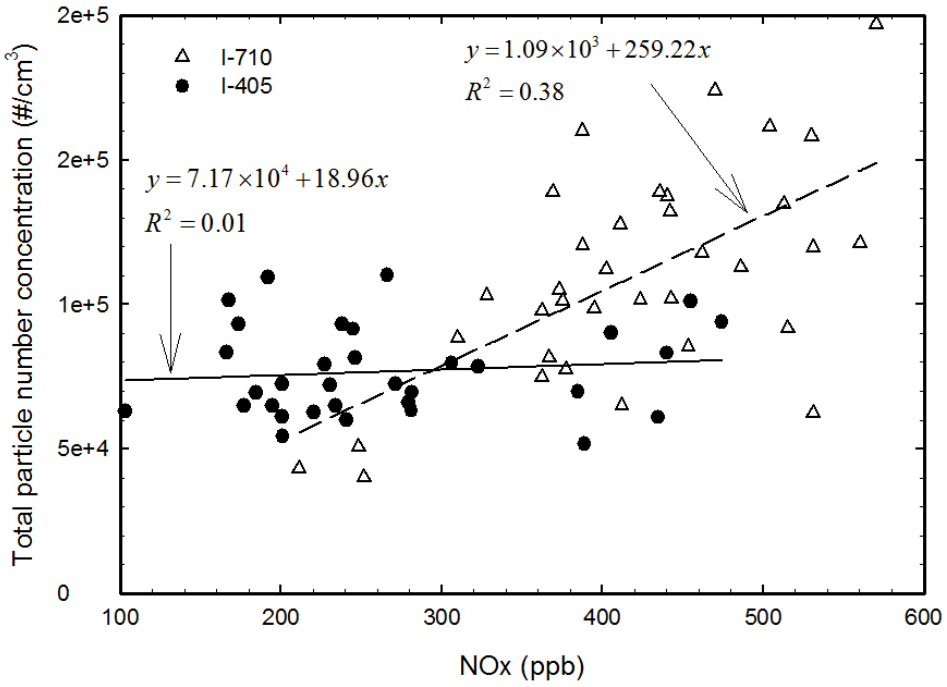


Figure 8. Correlations between NOx and total particle number concentration on the I-710 and I-405 freeways. Regression curves and equations are also shown.

previous study (Beckerman, Jerrett et al. 2008). For I-710, the particle number concentrations centered around 130,000 particles/cm^3 and NOx concentrations ranged from 300 to 600 ppb, while for I-405 the particle number concentrations were around 84,000 particles/cm^3 and corresponding NOx concentrations ranged from 160 to 500 ppb. Note, differences between Table 8 and Figure 8 are due to different averaging periods; run averages were used for Table 8 and hourly averages for Figure 8. Figure 9 compares the traffic volumes on the two freeways. Even though the total traffic volume on I-405 was higher than I-710, the UF particles and NOx emission levels were lower. It is clear that the dominant vehicles on the I-405 freeway were passenger cars, while I-710 freeway had a larger proportion of diesel vehicles. Thus, the likelihood the van was following a diesel vehicle on I-710 freeway was much higher than on the I-405. Emissions from diesel engines are different from those from gasoline vehicles. A previous study pointed out that the NOx emission factor for diesel vehicle was about 40±3 g/kg fuel in 2006, which was much higher than that for gasoline vehicle (3.0±0.2 g/kg fuel) (Ban-Weiss et al., 2008). Hence for a freeway with many diesel vehicles, higher concentrations of both UF particles and NOx would be expected.

Factors Affecting UF particles Exposure Levels

Season. Figures 10(a) and 10(b) depict the seasonal trend for average particle number concentration and the top 5% particle number concentration on the two freeways [Zhu et al, 2009]. The error bars represent one standard deviation. In general, UF particle exposures on the I-405 freeway were about 20,000 particles/cm^3 to 84,000 particles/cm^3 lower than that on the I-710 freeway, while the variability of UF particles number concentration on I-405 freeway was much less than the I-710 freeway. The average particle number concentration on the I-710 freeway shows a seasonal pattern during the study period. The highest UF particle concentration of 180,000 particles/cm^3 was observed in December when the temperature was relatively low, while in July when the temperature was the highest the UF particles levels were reduced by 50%. Cold weather can promote the formation of nucleation-mode particles, increasing the number concentration of particles in winter (Kuhn et al., 2005). One exception was February in which the UF particles concentration was much lower than in other months due to low traffic volume.

The highest 5% of particle number concentration measurements, shown in Figure 10(b), illustrate the highest on-road exposure to particle number concentrations, which may be more likely to be linked to health effects. On the I-405, the top 5% of particle number concentrations were about 2 times greater than the average particle number concentrations, while on I-710 there was a factor around 4 between the two parameters. Thus the exposure to higher particle number concentrations is more likely on the I-710 than on the I-405. The seasonal influence for the top 5% of particle number concentrations in Figure 10(b) is similar to the average particle number concentrations shown in Figure 10(a), especially on the I-710, but the variances among different months are much greater on the I-710. For I-710, the highest 5% of particle number concentrations were observed in December and the lowest in February, as with the mean concentrations. For I-405, the top 5% of UF particle concentrations was more even, varying from 100,000 particles/cm^3 to 200,000 particles/cm^3.

The ratio of UF particle to total particle number concentration varied with season dramatically, as

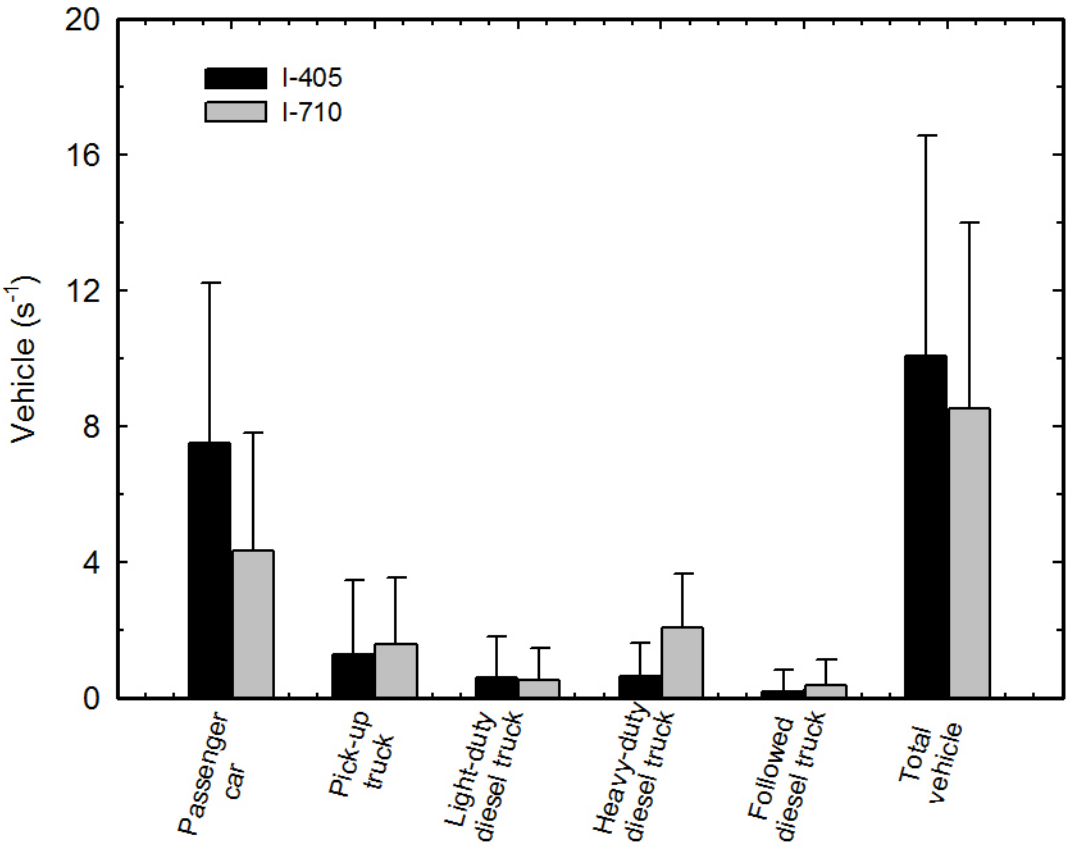


Figure 9. Traffic volume comparisons for I-405 and I-710 freeways. Bars indicate one standard deviation.

shown in Figure 11. In general, UF particles constituted 36 to76% of total particle number concentrations on the I-405, and 56% to 84% on the I-710. As expected, the I-710 freeway had a higher fraction of UF particles than I-405 since there are many more diesel vehicles running on the I-710.

The ratios for all CPC measurements shown in Figure 11(a) followed a similar trend on both freeways. For example, on I-405 from September to November the UF fraction was about 40% of total particle number concentration, but the UF particle fraction increased to about 80% in February. Thus, even though average particle number concentration did not vary much by month on I-405 (as shown in Figure 10(a)), the proportion of UF particles was much higher in February than in September. The UF particle/total particle ratios for the top 5% of CPC measurements, shown in Figure 11(b), also show lower UF particle fractions in summer and fall and higher UF particle fractions in winter and spring. In January and February, the UF particle fractions for the top 5% of CPC measurements for the I-405 were higher than that for the I-710, which was likely due to the relatively low traffic during this period on I-710.

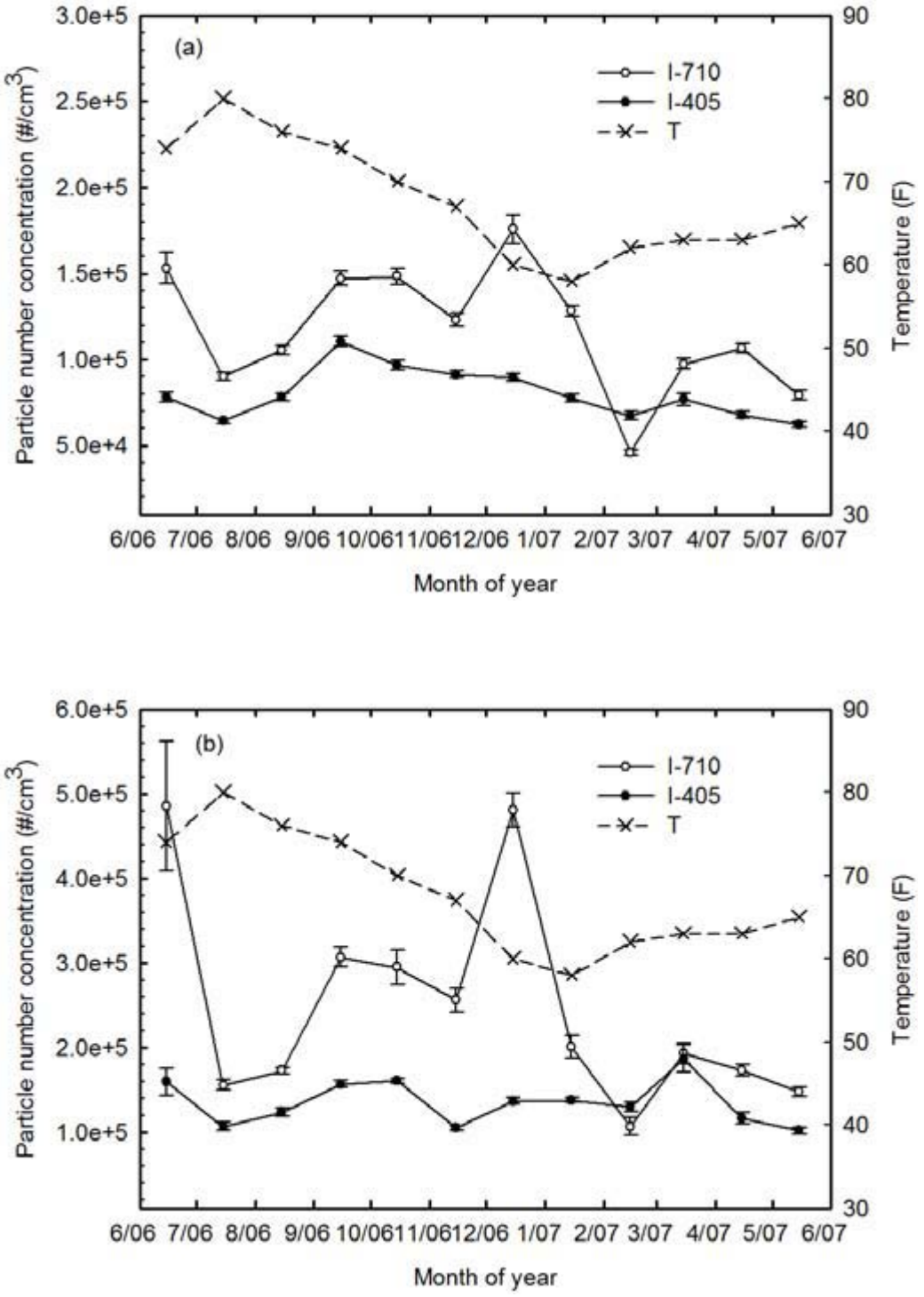
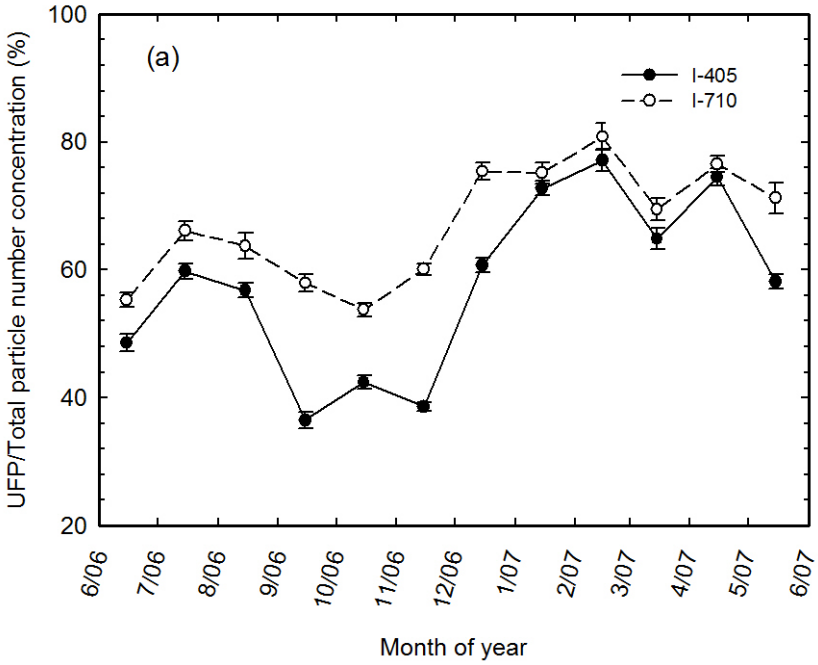


Figure 10. Seasonal variation of (a) mean total particle number concentration measured by the CPC, and (b) the top 5 % of CPC measurements on the I-710 and I-405 freeways from June 2006 to May 2007. T is average ambient temperature (F).



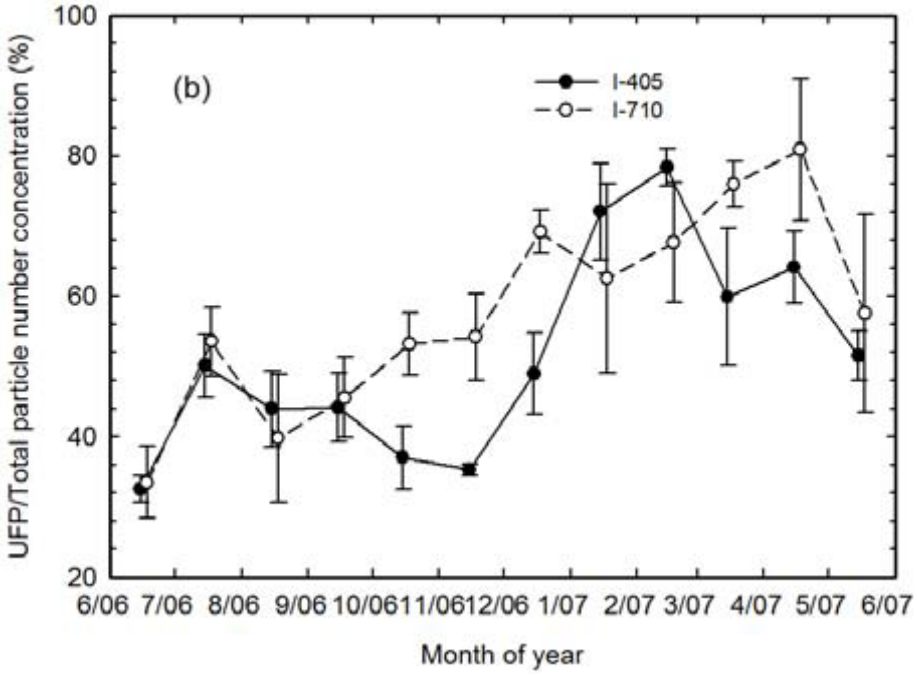


Figure 11. Seasonal variation of the ratios of UF particles to total particle number concentrations for (a) all CPC measurement, and (b) top 5 % of CPC measurements on I-710 and I-405 freeway from June 2006 to May, 2007.

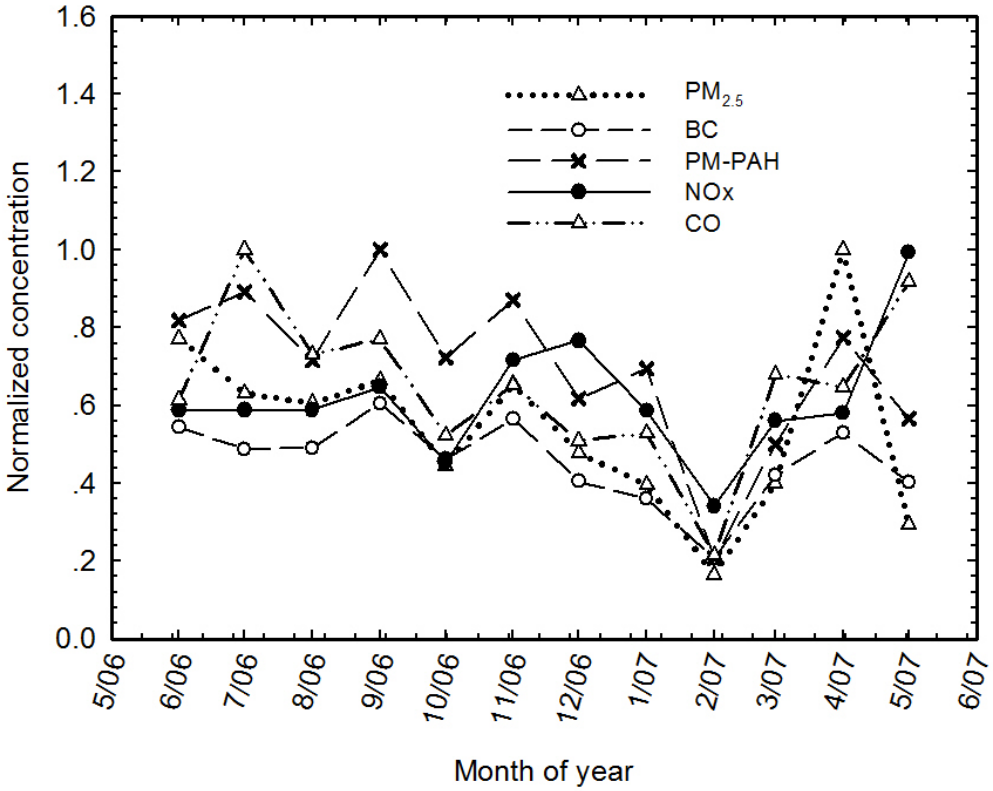


Figure 12. Seasonal variation of vehicle-related air pollutants on I-710 freeway from June 2006 to May 2007. Normalized concentrations are the fraction of pollutant concentrations based on the following concentrations: (1) PM-2.5: 100 μg m^{-3}, (2) BC: 30 μg m^{-3}, (3) PM-PAHs: 400 ng m^{-3}, (4) NO_x: 700 ppb, and (5) CO: 5.5 ppm.

Figure 12 is a time series of the normalized concentrations of PM-2.5, NOx, black carbon, PAH, and CO on I-710. The normalized concentrations are pollutant concentrations expressed as a fraction of the following concentrations: (1) PM-2.5: 100 μg m^{-3}, (2) BC: 30 μg m^{-3}, (3) PM-PAHs: 400 ng m^{-3}, (4) NO_x: 700 ppb, (5) CO: 5.5 ppm. These air pollutants showed strong correlations with each other on a monthly basis over the period June 2006 to May 2007, as monthly concentration averages were determined by the large-scale meteorological variability as well as seasonal variability in source strengths. Generally, the pollution levels were quite constant with a certain deviation, except for the lowest concentrations clustered in February. This trend is quite different from that found in a previous study by Marshall et al., who reported that transport and dispersion are slower on average during the winter because of the weaker incident solar radiation, so that the levels of motor-emitted pollutants such as CO and benzene were higher in winter than summer (Marshall, et al., 2003). But after examining the meteorological data, there was little variation of weather conditions, especially average wind speed, during the measurement period. So the observed seasonal differences in air pollutant concentration are more likely due to the differences in emission source strength. The average traffic volume in July 2006 was about 2.5 fold greater than that in February 2007, but the wind speed in July was about 0.8 km/h more than that in February. The low concentrations during February shown in

Figure 10 and Figure 12 are most likely due to the low traffic density during that month.

In summary, both the total particle number concentration and the UF particles fraction showed a seasonal trend. High particle number concentration was observed in winter time when the temperature was relatively low. The low temperature in winter also resulted in higher UF particle fraction. Gas phase pollutant concentrations were quite constant and did not show a seasonal trend like UF particles. One exception was February in which the UF particles and the gas phase pollutant concentrations were much lower than in other months due to low traffic volume.

Traffic.

Figure 13 presents total particle number concentrations as a function of how many vehicles were surrounding the research van [Zhu et al, 2009]. Four types of vehicles were considered: passenger car, pick-up trucks, light-duty diesel vehicle and heavy-duty diesel vehicle. The frequency of surrounding vehicles that were ahead of the research van, indicated by the gray bars, demonstrates that there were more gasoline vehicles than diesel vehicles on two freeways. More than 50% of the time during the test there were more than five passenger cars, one pick-up, one light duty diesel vehicle and one to three heavy-duty diesel vehicles ahead of the research van. A clear increase of total particle number concentrations with surrounding heavy-duty diesel vehicles was observed in Figure 13(d). But no strong relationship was observed between total particle number concentrations and the other three types of vehicles. When there was no surrounding heavy–duty diesel vehicle, the average particle number concentration was about 75,000 particles/cm^3. When the surrounding heavy-duty diesel vehicles were more than three, particle number concentrations inside the test chamber were more than 120,000 particles/cm^3. But this relationship was not observed for other types of vehicles, as shown in Figures 13 (a), (b) and (c). Therefore, particle concentrations inside the research van were more closely related to the presence and number of heavy-duty diesel vehicles than other types.

Lane

Particle number concentrations were grouped by the lane in which the research van was traveling to characterize the air pollutant levels as a function of lane position, see Figure 14 [Zhu et al, 2009]. The frequencies that the research van was driven in each lane are shown as gray bars. As shown in Figure 2 the direction of I-710 was generally north to south and I-405 was mostly north-west to south-east. Lanes were numbered, 1 to 8, from upwind (west side) to downwind (eastside) for both freeways. Wind direction in Los Angeles is consistent all around the year. In the daytime, the sea breeze comes from the Pacific Ocean. During the study period, the wind direction was generally from the west-south-west (230~280 degrees from north). Thus, lane number increases in the downwind direction. The boundary lanes in both directions were excluded since they experienced more frequent on and off ramp traffic, not typical for the middle lanes.

The frequencies of particle measurements in the top 5% were similar for each lane on both freeways, which indicates a similar opportunity to be exposed to high particle number concentrations for each lane. Figures 14 (c) and (d) depict the top 5% of particle measurements on each lane for the two freeways. For I-405, the average particle number concentrations on each lane ranged from 70,000 to 80,000 particles/cm^3, while top 5% of particle number concentrations varied from 120,000 to 140,000 particles/cm^3. The small variation in both parameters demonstrated that there was little difference in exposure to particulate matter for a given lane. But the variation by lanes was much greater on I-710 than on I-405, as shown in Figure 14 (b) and (d). This may be due in part to the wind being nearly

perpendicular to the I-710 and at a more acute angle to the I-405. Figure 14 (b) shows the average particle number concentrations in the middle lanes were lower than at the sides. The lowest was observed on lane 6, while the highest was on lanes 1 and 8. A possible explanation was the high density of diesel vehicles on both side lanes, emitting high concentrations of particulate matter and other associated air pollutants that dispersed

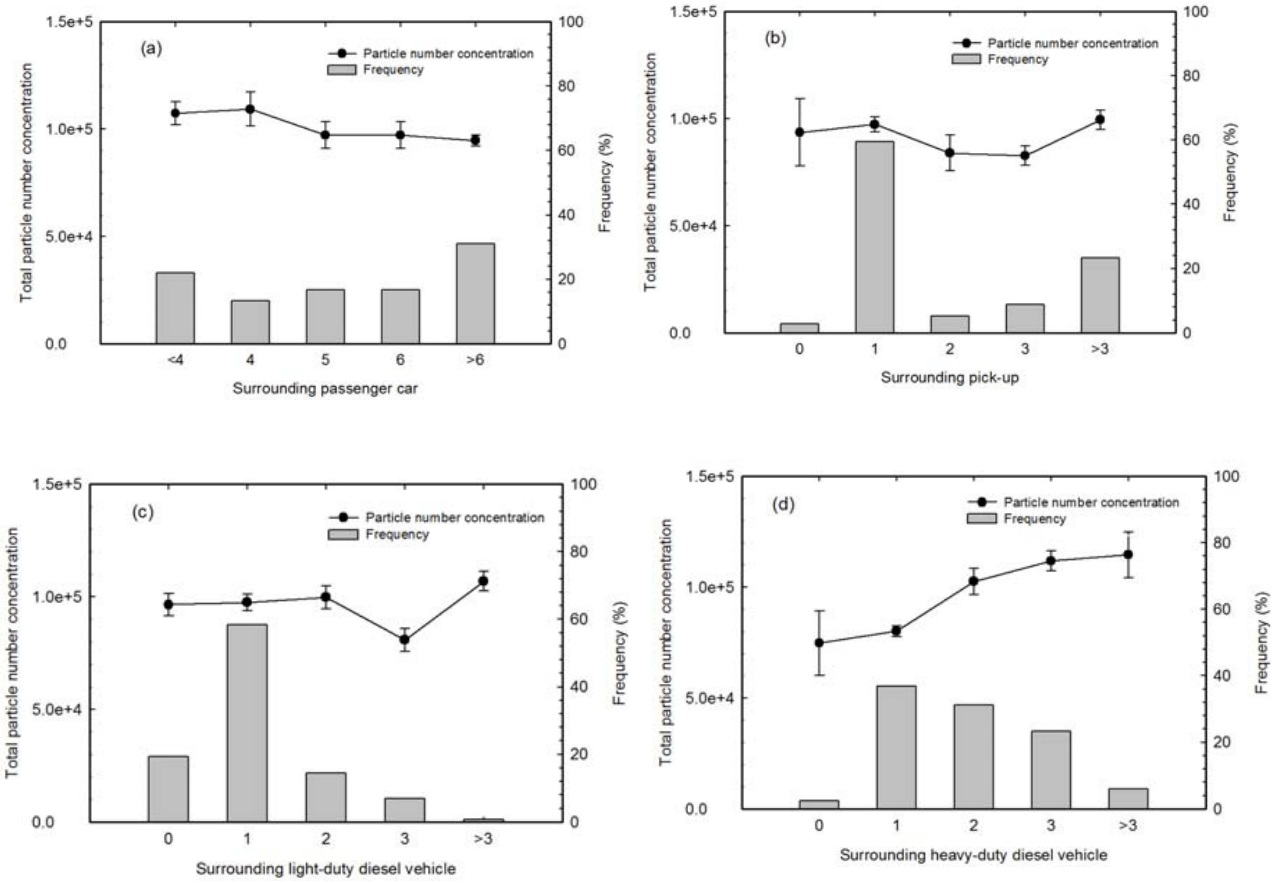


Figure 13. Total particle number concentrations by surrounding vehicle density for four vehicle types. Bars indicate standard error. Gray bars present the frequency that a given number of vehicles were surrounding the test van.

to the nearby lane. Under California Vehicle Code (CVC 21655) heavy duty trucks are restricted to the right hand two lanes on freeways such as I-405 and I-710.

The effect of relative lane position on the ratios of UF particles to total particle number concentrations is shown in Figure 15, in which the ratios for all particle number measurements are shown in Figure 15 (a) and those for top 5% CPC measurements in Figure 15 (b). The overall UF particle fractions on I-710 were 6% to 22% higher than that on I-405, but the trends of UF particle fraction on the lanes along wind direction were different. For I-405, the UF particle fraction was relatively high at the both sides and low in the middle. But I-710 had higher UF particle fraction on the downwind lanes than the

upwind. When one considers the top 5% of particle number measurements, shown in Figure 15 (b), the UF particle fractions on both freeways were lower than that in Figure 15 (a), and the trend of UF particle fraction along the wind direction was not observed.

Speed

The influence of driving speeds on air pollutant exposure levels inside the research van is

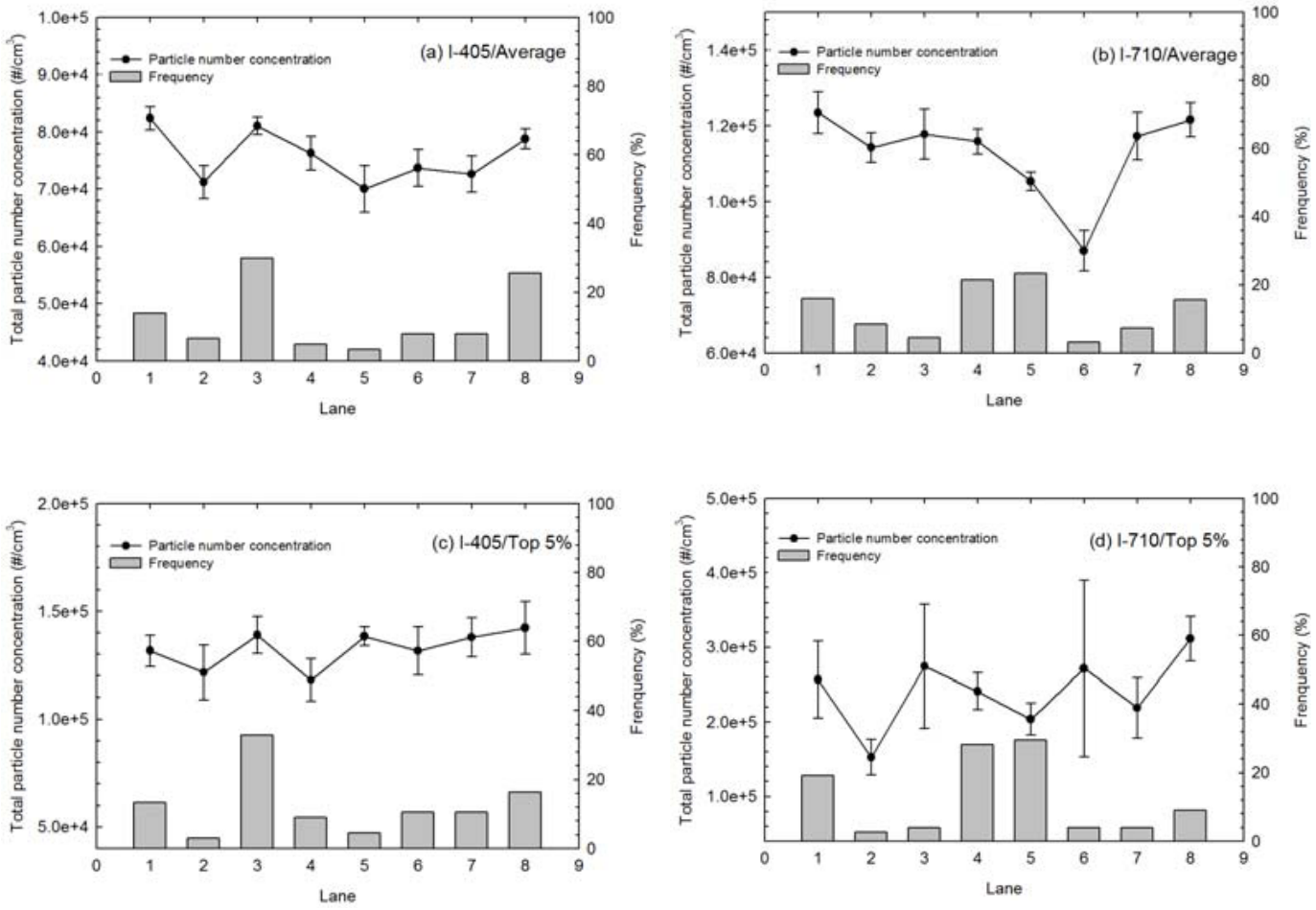


Figure 14. Effect of relative lane position on particle number concentration for (a) all CPC measurements on I-405, (b) all CPC measurements on I-710, (c) top 5% CPC measurements on I-405, and (d) top 5% CPC measurements on I-710. Error bars display one standard error. Gray bars present the frequency that the research van drove on each lane.

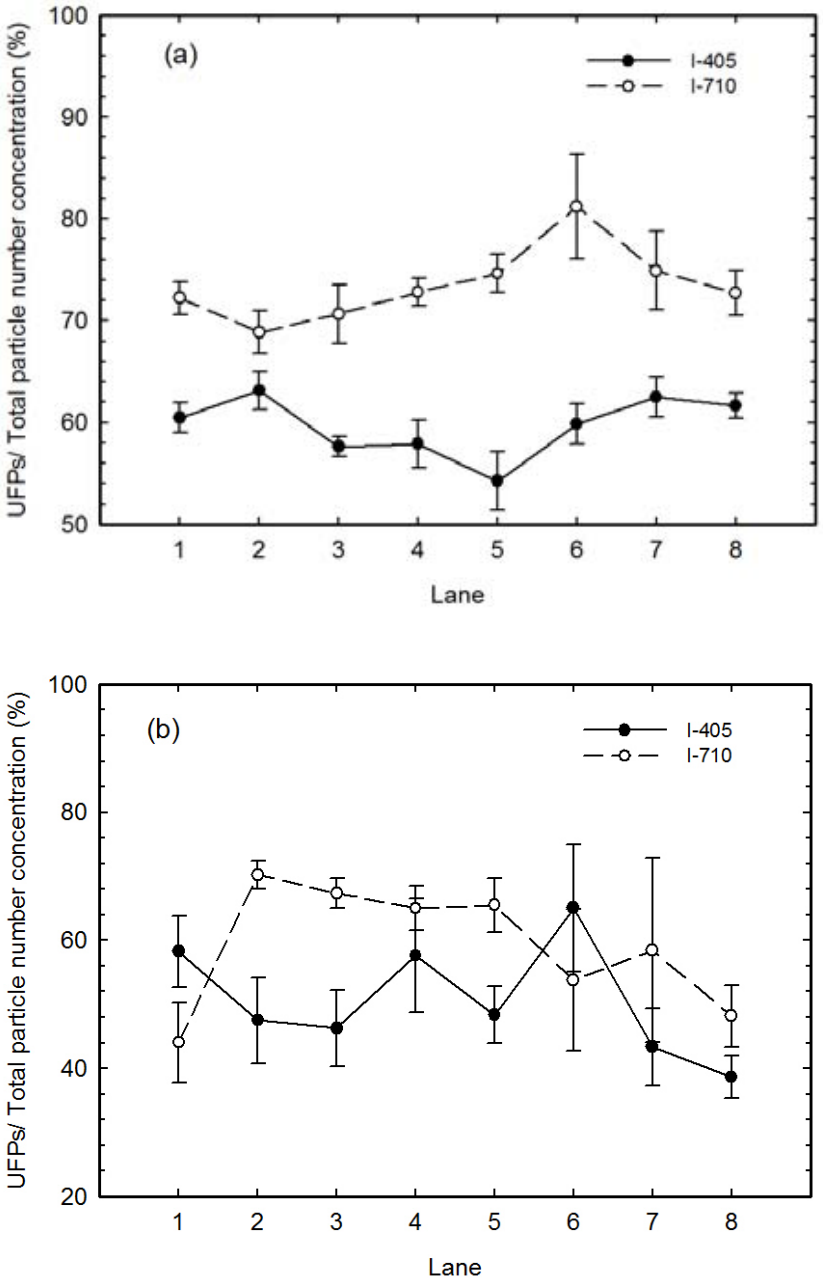


Figure 15. Effect of relative lane position on the ratios of UF particles to particle number concentration for (a) all CPC measurements and (b) top 5% CPC measurements on two freeways. Error bars display one standard error.

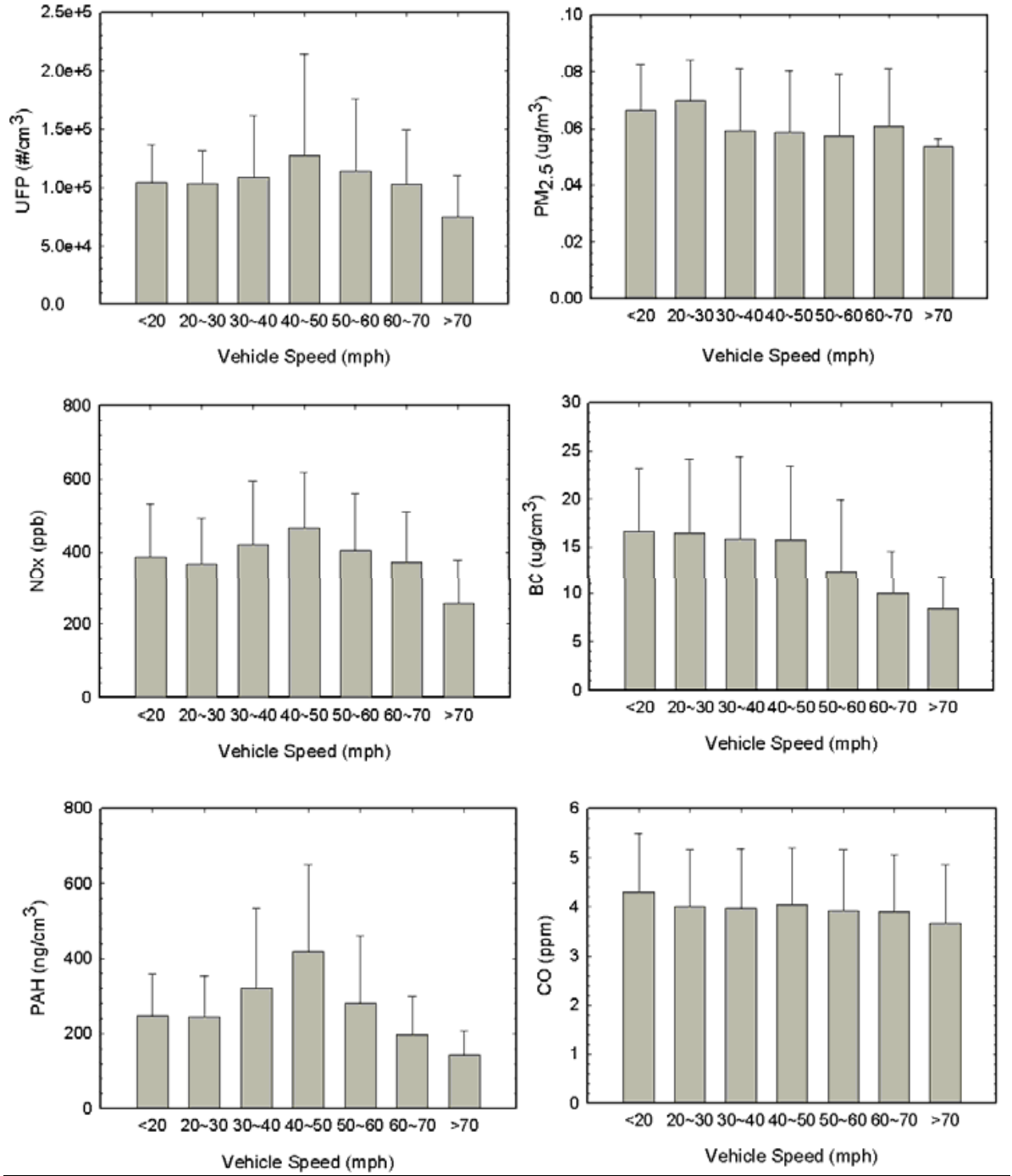


Figure16. Effect of driving speed on air pollutant concentrations. Error bars display one standard error.

shown in the Figure 16 [Zhu et al, 2009]. A peak in particle number concentration of 125,000 particles/cm^3 was associated with the speed of 40 to 50 mph. Particle emissions, especially the

nucleation mode particles, from tail pipes increase with driving speed (Giechaskiel et al., 2005). On the other hand, higher driving speed results in greater turbulent mixing in the van's wake and more rapid dilution of the exhaust stream. 40-50 mph may represent a speed where the combined effect gives the maximum in UF particle concentration. A similar trend was also observed for the NOx and PB-PAH concentration. PM-2.5 and CO concentration didn't change much with driving speed. But for black carbon, the trend was different. When the speed was lower than 50 mph, the in-cabin exposure was constant. But at speeds greater than 50 mph, the concentration decreased with the speed. Black carbon is formed from the incomplete combustion of fuel. High speed increases the combustion efficiency, and reduces black carbon emissions.

In summary, differences in particle concentration across exposure conditions, and similarities in other environmental factors, occurred as expected. Concentrations of the different components of PM varied independently of one another to a considerable extent, so that in principle it would be possible to identify components most strongly associated with cardiopulmonary effects. In practice, the small sample limits our ability to detect real relationships with small effect size, or to associate specific effects with specific PM components.

2. Respiratory Function,and Vital Signs

Most analyses of respiratory function data showed no statistically significant differences between freeways or between filtered and unfiltered exposure conditions. A few showed small statistically significant differences, but not in the patterns that would be expected if freeway PM exposure caused unfavorable effects in unfiltered conditions. Thus, these changes must be attributed to chance variation in unmeasured interfering influences. For the change in FVC from before to after exposure, the freeway•filter interaction was significant; however, the largest mean loss (about 3%, clinically unimportant if observed in an individual) occurred under the cleanest condition – I-405 filtered. For SaO_2 change, the freeway•filter interaction was marginally significant (P = 0.049). The most favorable (albeit clinically trivial) change, +0.65% averaged over all time intervals, occurred in unfiltered exposures on I-405. Other mean changes were +0.04% (I-405 filtered), +0.15% (I-710 unfiltered), and +0.24% (I-710 filtered). Exposure-response analyses showed no statistically significant relationship of changes in respiratory function tests, over any interval, with particle number, PM-2.5, black carbon, PB-PAH, or CO during exposure. Higher temperatures in the van were associated with negative (favorable) changes in respiratory resistance and resonant frequency after exposure.

Pre-exposure diastolic blood pressure (hand-measured in close time proximity to respiratory function tests) showed a positive association with prior 24-hr average PM-10, with slope 3.0 mmHg/(10μg/m^3) (P = 0.007). Thus, an increase from the lowest to the highest observed prior 24-hr PM-10 (14 to 54 μg/m^3) would predict a clinically significant rise of 12 mmHg in diastolic pressure. By contrast, the relationship of pre-exposure diastolic pressure to prior 24-hr average PM-2.5 was not statistically significant, with essentially zero slope. However, PM-2.5 monitoring was farther away from the laboratory and most subjects' residences, and so might have less relevance to their personal exposures. Corresponding analyses of systolic pressure showed non-significant positive slopes. (Automated ambulatory blood pressure measurements are presented in a later section.) The only other statistically significant relationship between pre-exposure respiratory physiology and prior-24-hr PM was for resonant frequency of the respiratory system, which showed a slight decrease in relation to increasing PM-2.5. Decreased resonant frequency is usually associated with decreased respiratory resistance – a favorable change – so this relationship probably is attributable to chance.

3. Symptoms

Total symptom score averaged near 1.1 points before exposure, 2.3 just afterwards, and 1.5 on day two, across all exposure conditions. Thus, a typical subject had one mild symptom before exposure, and one moderate or two mild symptoms after exposure. Although slight, the symptom increases occurred consistently during van rides ($P < 0.0001$ for main effect of time). Averaging across all times, the mean total symptom score for both exposures on I-405 was near 1.6. On I-710 it was near 1.3 unfiltered and 2.1 filtered, giving rise to a marginally significant freeway•filter interaction ($P = 0.04$), most likely attributable to chance. (An increase in symptoms with air filtration would be biologically implausible unless the filter system consistently released a noxious gas while continuing to remove particles; if it had, the symptom increase should have been observed on both freeways.) The only statistically significant pollutant exposure-symptom response relationship was with NOx. The regression slope was +0.0016 per ppb ($P = 0.045$). Thus, an increase in NOx from the lowest to the highest observed 2-hr-average exposure (125 to 695 ppb) would predict an increase by about 1 point, i.e. a mild increase in one symptom.

4. Cardiac arrhythmia and heart rate variability

Twenty-four hour ambulatory electrocardiograms (Holter Monitors).

All 24-hour Holter statistics had skewed distributions and were log-transformed for analysis. A few recordings showed no ectopic beats in 24 hours, so incidence was estimated as 1 in 48 hr (0.02 per hour). The estimate of 0.02 for an observed value of zero was also applied in later analyses of hourly intervals. The 24-hour statistics for mean hourly incidence of ventricular ectopic beats (VEB_hr), percentage of adjacent normal beat-to-beat intervals different by >50 msec (PNN50), root-mean-square of successive differences between adjacent normal intervals (rmsSD), and average standard deviation of normal intervals during successive 5-min segments of the 24-hour recording (ASDNN5 or Magid SD) showed no significant variation that could be related to freeway or filter condition. Yet, the standard deviation of all normal intervals (SDNN or Kleiger SD) showed a significant freeway*filter interaction ($P = 0.02$). On I-405 its geometric mean estimate was 118 msec unfiltered and 123 msec filtered; that is, HRV tended to increase (usually considered favorable) with filtration. However, I-710 showed an opposite trend and geometric means were 128 unfiltered and 116 filtered. These geometric means are well within the normal range; SDNN below 50 msec is associated with high risk of cardiac death [Kleiger et al., 2005]. Therefore, the small magnitude of change, as well as the inconsistent direction of change suggests that there was no clinically important change in HRV detected by time-domain parameters.

Significant time-dependent changes in non-linear measures of HRV were measured for log Poincaré SD1 ($P=0.03$) and SD2 ($P\leq0.001$), recurrence plot analysis, recurrence rate ($P=0.023$), Shannon Entropy ($P=0.001$), Sample Entropy ($P=0.003$), detrended fluctuation analysis (DFA) alpha 2 ($P<0.001$), and log D2 ($P<0.001$). Such changes independent of traffic conditions, filter status, or highway suggest effects related to circadian rhythms. The non-linear HRV parameter DFA alpha 2 ($P=0.004$) was significantly lower with filtration immediately after exposure. This observation is consistent with significantly decreased log VLF power ($P=0.024$) as these parameters are correlated. Yet the clinical significance of this observation remains uncertain as the physiological correlates of these parameters are not well understood. A parameter that changed with filtration was REC or the recurrence rate and this was reported to be lower with filtered road air ($P=0.031$). It has been suggested that non-linear indexes of HRV can be used as a more specific indicators of parasympathetic tone, as such a decrease in REC might indicate an increase in parasympathetic tone. Sample entropy tended to be lower on the 405 when compared to the 710, the lower value of

sample entropy indicating a more regular rhythm with less complexity.

A more consistent pattern of significant variation in 24-hr Holter statistics was found with supraventricular ectopic beats (SVEB), expressed as mean hourly incidence (SVEB_hr). From "treatment" analyses, geometric mean SVEB_hr was 2.58 for unfiltered and 2.14 for filtered exposure days on I-405, 2.89 for unfiltered and 2.12 for filtered exposure days on I-710. The main effect of the filtration was significant (P = 0.021), and the estimated effect size represented a 27% decrease in supraventricular ectopic beats on days with filtered exposures, compared to unfiltered exposures.

Ectopic beat incidence varied by more than three orders of magnitude between individuals. The influence of that variation on exposure effects was tested in two ways. First, subjects were classified by medical judgment (without knowledge of exposures) as low, medium, or high in ectopic beat incidence, considering both supraventricular and ventricular ectopic beats. Overall mean SVEB_hr for these groups was 1, 7, and 131 respectively; while overall mean VEB_hr was 3, 20, and 32 respectively. When the analysis of SVEB_hr was repeated with addition of the low-medium-high grouping factor, the filter effect remained significant and the grouping factor was non-significant. That is, the proportionate improvement in supraventricular ectopic beat incidence with air filtration did not vary markedly according to the individual's underlying degree of cardiac irritability. In the second analysis, instead of grouping, each individual's overall mean SVEB_hr was included as a covariate. The freeway effect remained significant and its estimated size was similar to that from the original analysis.

In exposure-response analyses, all PM measures showed significant or near-significant positive relationships to log-transformed SVEB incidence (Table 9), consistent with the significant filter effect in "treatment" analyses. Pollutant gases, temperature, or prior 24-hr PM-10 measured in Long Beach showed no associations with SVEB. There was a near-significant (P = 0.07) negative relationship to prior 24-hr PM-2.5 measured in downtown Los Angeles. Particle number, black carbon and PAH showed statistically significant relationships to SVEB incidence. PM-2.5 and SVEB incidence were positively associated, but the relationship did not reach statistical significance (P=0.07). Figure 17 presents exposure-response plots – specifically, individual differences in log(SVEB_hr) between unfiltered and filtered exposure days, as a function of corresponding individual differences in particle count – separately for each freeway. These plots suggest that the overall positive relationship of particulate pollution to supraventricular ectopic beats was driven primarily by the most intense exposures on I-710, which substantially exceeded the range of exposures on I-405. Each of the exposure measures given in Figure 4 and shown to be positively correlated to supraventricular ectopic beats and statistically significant in Table 9 (i.e. particle number, black carbon and PAH) were also higher on I-710 than I-405. In other exposure-response analyses (not tabulated), ventricular ectopic beat incidence showed significant negative relationships to particle count, PM-2.5, PM-10, black carbon, and temperature, as well as a positive relationship to downtown Los Angeles PM-2.5 in the previous 24 hr (P < 0.05 for all). Figure 18 shows exposure-response plots for VEB_hr, analogous to those for SVEB_hr in Figure 17. Thus, for the subject population as a whole, VEB_hr tended to change in the opposite direction from SVEB_hr, although individual changes as shown in the plots were not meaningfully correlated. Downtown L.A. PM-2.5 also showed a significant positive relationship to mean heart rate. The slightly higher heart rate could have suppressed premature ventricular beats and thus explain the decreased incidence of ventricular premature beats. Particle count and PB-PAH concentration showed marginally significant or marginally non-significant positive relationships to HRV as measured by rmsSD, ASDNN, or SDNN, where negative relationships would

be expected.

Table 9. Results of exposure-response analyses: Hourly incidence of supraventricular ectopic beats (from 24-hr recording) vs. exposure measures*

Exposure Measure	Effect Size	P Value
Particle number	+3.3% per 10,000 particles	<0.01
PM-2.5	+4.4% per 10 μg/m^3	0.07
PM-10	+3,7% per 10 μg/m^3	0.10
Black carbon	+2.2% per μg/m^3	0.04
PB-PAH	+1.1% per 10 ng/m^3	0.02
NOx	+0.014% per ppb	0.47
CO	+10% per ppm	0.10
Temperature	+0.83% per deg F	0.63
Prior 24 hr Ambient PM-10	-4.0% per 10 μg/m^3	0.63
Prior 24 hr Ambient PM-2.5	-1.5% per μg/m^3	0.07

*****Incidence is log-transformed for analysis, so effect-size estimates are expressed as percentages. See Table 7 for relevant exposure data.

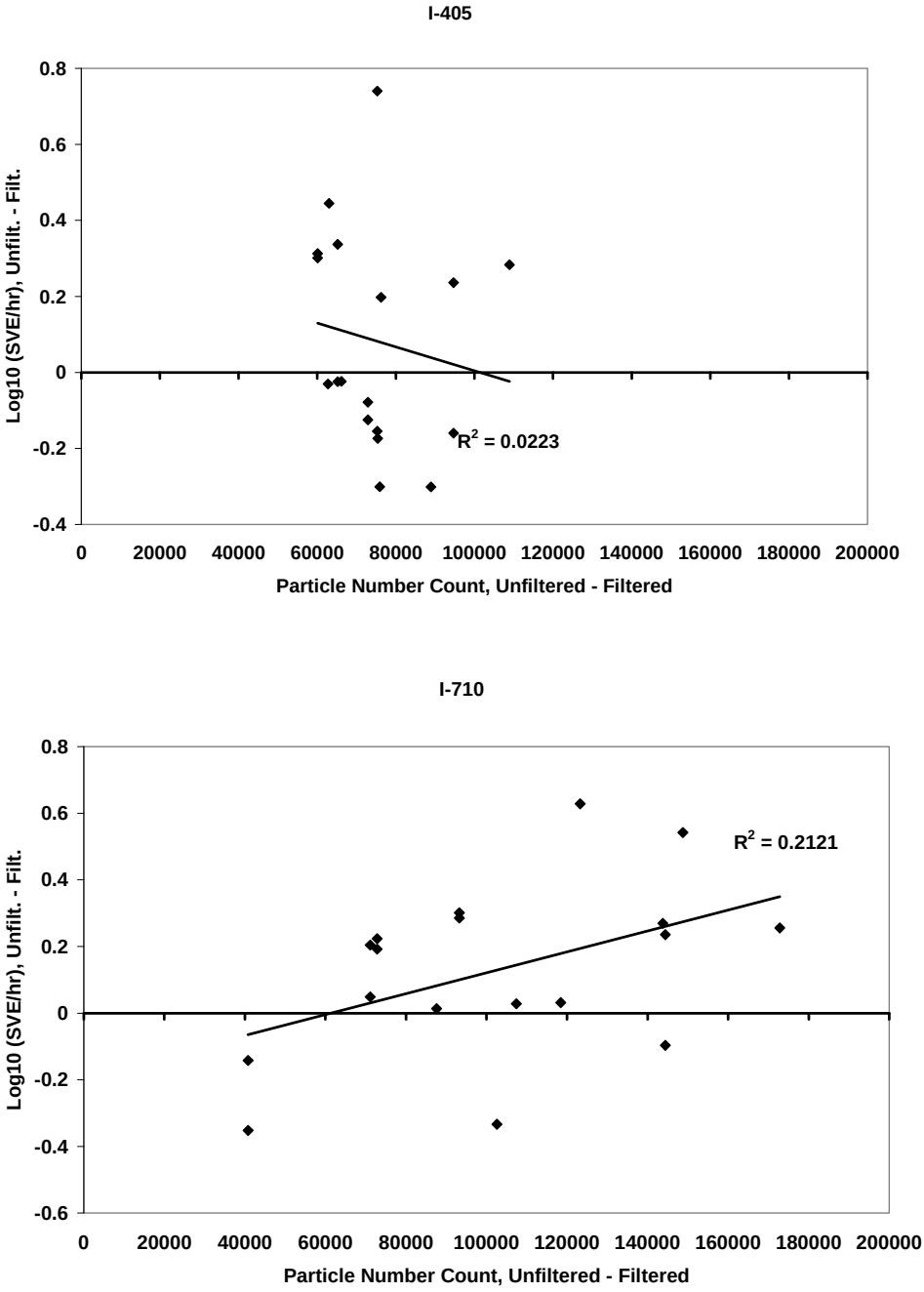


Figure 17. Individual subjects' excess incidence of supraventricular ectopic heartbeats in unfiltered exposure relative to filtered exposure, as a function of the corresponding excess in average particle count during unfiltered relative to filtered exposure. (Ectopic heartbeat incidence is averaged over the entire 24-hr record, then log-transformed to normalize the distribution.) Best-fit regression line and coefficient of determination are shown on each graph. Upper graph: I-405; lower graph: I-710.

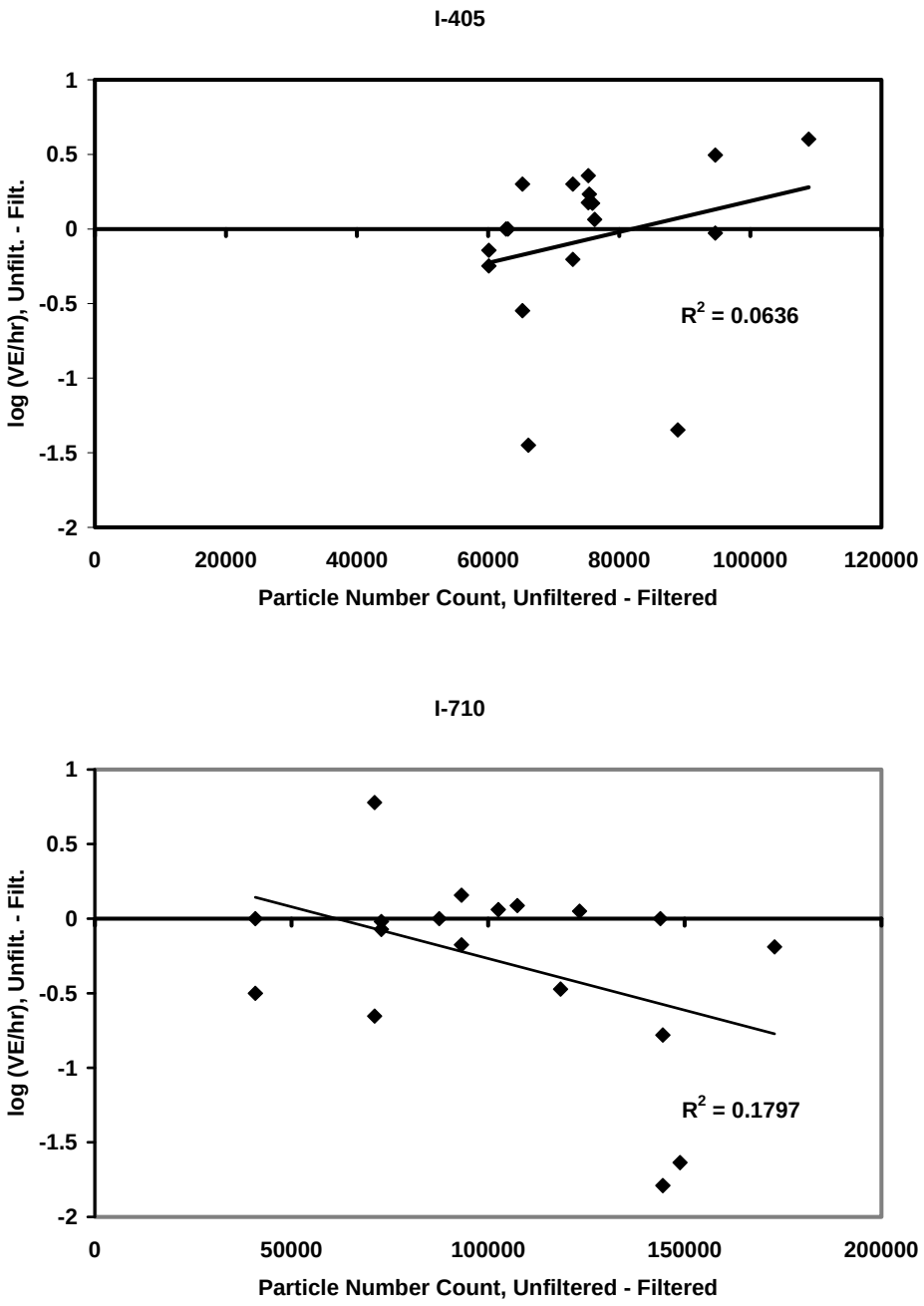


Figure 18. Individual subjects' excess incidence of ventricular ectopic heartbeats in unfiltered exposure relative to filtered exposure, as a function of the corresponding excess in average particle count during unfiltered relative to filtered exposure. (Ectopic heartbeat incidence is averaged over the entire 24-hr record, then log-transformed to normalize the distribution.) Best-fit regression line and coefficient of determination are shown on each graph. Upper graph: I-405; lower graph: I-710.

Heart rate variability is influenced by the underlying rate of breathing. In this study it was not practical to have the subject breathe at a specific frequency for an extended period of time. It would have been of interest to have the subjects breathe in time to a metronome for a 5-minute period before, immediately after and the morning after exposure.

The 24-hr Holter results reported above must be interpreted with caution, in that typically more than 80% of the recording occurred after the freeway exposure (much of it when the subject's activities were unobserved), versus 8-9% during the experimental exposure period, and 4-8% prior to it. To test more specifically for an association of ectopic beats with freeway exposures, additional analyses were performed using Holter statistics for each clock hour, with hours divided into intervals pre-exposure, during exposure, and after exposure. By necessity, "the during-exposure" interval (10:00 through 12:59) included some time before and/or after, since exposure periods usually could not start exactly on the hour. Additional limitations of these analyses relate to the relatively short duration of pre- and during-exposure intervals, which cause the estimates to be somewhat unstable, and

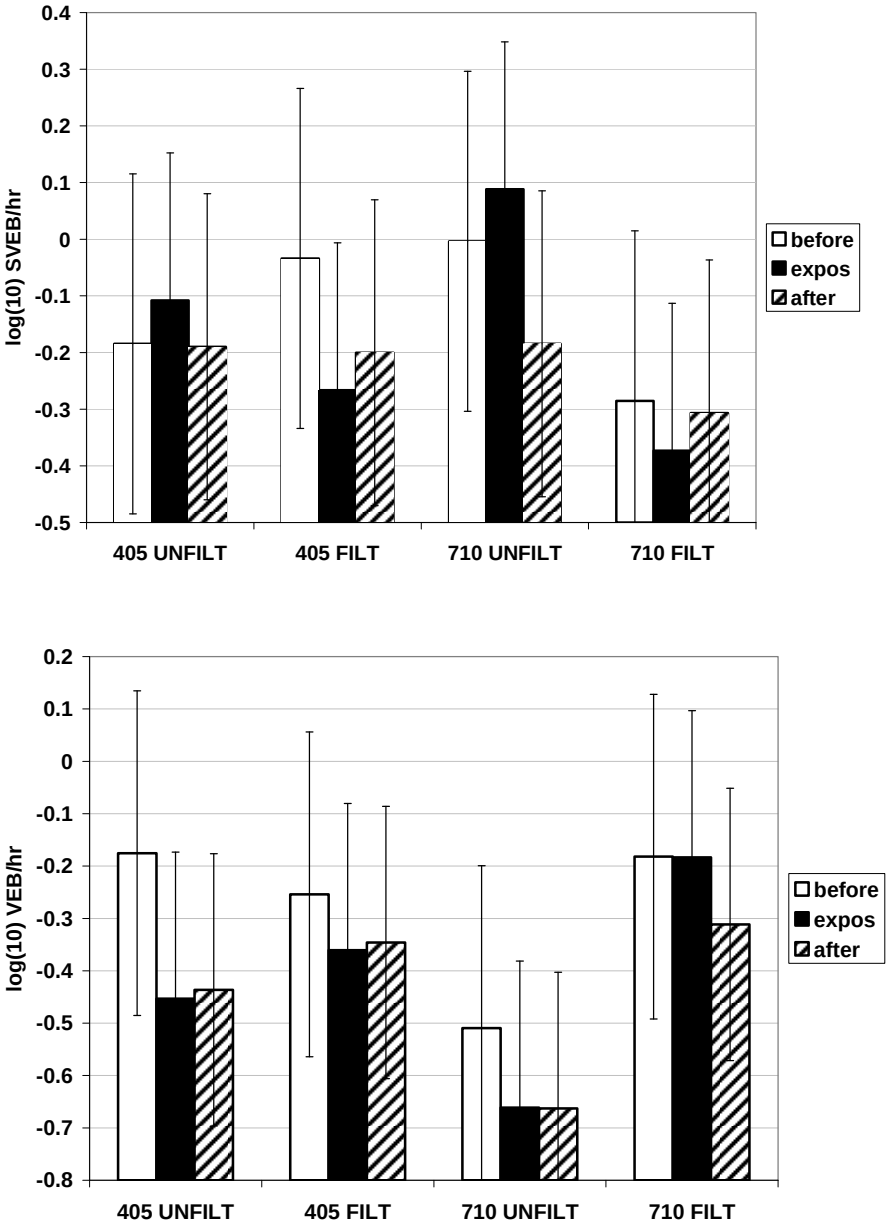


Figure 19. Mean incidence of ectopic heartbeats prior to experimental exposure ('before'), during exposure period ('expos'), and afterwards ('after'), on each freeway in unfiltered ('unfilt') and filtered ('filt') condition. Bar indicates mean estimated by mixed-model analysis of Holter electrocardiogram statistics reported by clock hour; flag indicates standard error, reflecting between-subject and between-hour variation. Upper graph: supraventricular ectopic beats per hour (SVEB/hr), log transformed; lower graph: ventricular ectopic beats per hour (VEB/hr), log transformed.

frequent observations of zero ectopic beats, arbitrarily estimated as 0.02 per hour as described previously. Figure 19 illustrates results for supraventricular and ventricular ectopic beat incidence as judged from hourly Holter statistics. On both freeways, mean incidence of supraventricular ectopics increased during unfiltered exposures, but decreased during filtered-air exposures, relative to pre-exposure. These changes appeared to reverse after exposure. Mean incidence of ventricular ectopics decreased during all exposure periods, compared to pre-exposure, but the decreases were smaller in filtered than in unfiltered air. Statistical tests of the changes during exposure relative to pre-exposure did not show significant effects of filtration. However, analyses restricted to "during-exposure" data showed the main effect of filtration to be significant ($P < 0.05$) both for supraventricular ectopics (positive) and for ventricular ectopics (negative); while analyses restricted to pre-exposure data showed no significant variation. Thus, the hourly analyses tend to corroborate the finding from 24-hr statistics that filtration of van air during freeway rides decreased the incidence of supraventricular ectopic beats.

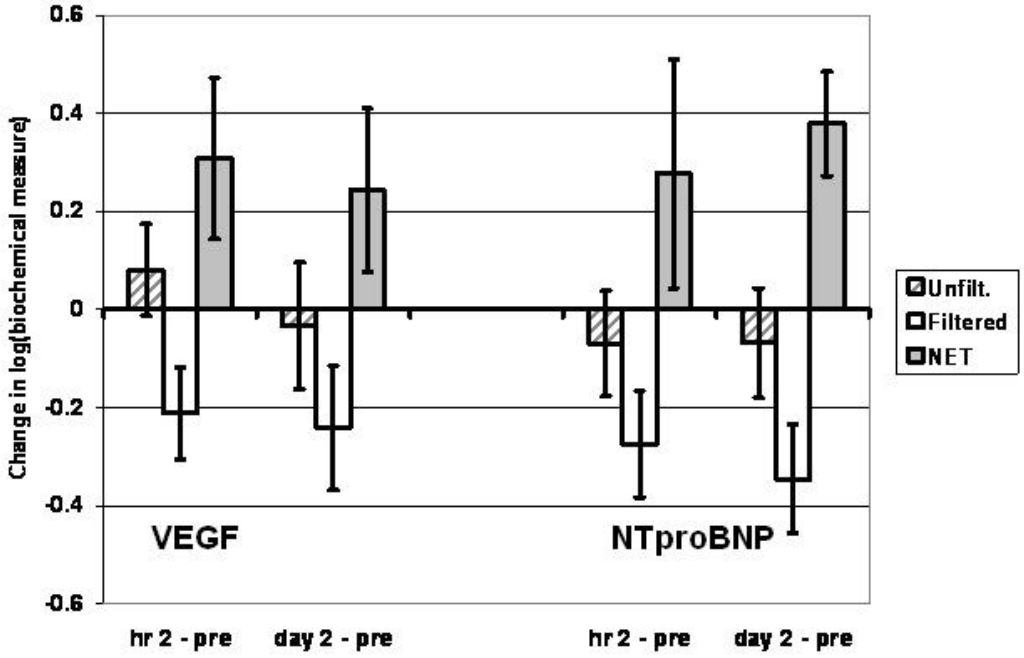


Figure 20.Net change in VEGF and NT-proBNP (both log transformed) between filtered and unfiltered exposures, both freeways pooled. See text for statistical analysis results.

Clinical Relevance of Arrhythmias

The initiation of isolated ectopic beats or sustained arrhythmia is attributed generally to several cellular electophysiological mechanisms. Intrinsic factors (e.g. structural changes, polymorphisms of membrane channels, calcium regulation) or extrinsic factors (e.g. altered autonomic balance, drugs, oxidant stress, endothelin-1, cytokines, NO and catecholamines) can affect the pacemaker current, membrane currents or impulse propagation can increase the likelihood of ectopic beats or sustained arrhythmia. In general, the initiation of an arrhythmia requires: 1) a conditional susceptibility to arrhythmia inherent to myocardium; and 2) a trigger. The presence of longstanding cardiac risk factors such as advancing age and hypertension contribute to such a conditional susceptibility. The conditional susceptibility of the myocardium relates most likely to cellular hypertrophy, intramyocardial fibrosis, attendant changes in membrane properties and possibly altered intracellular calcium dynamics. The subjects who participated in this study are late middle-aged or elderly individuals. While they were generally healthy, subjects were not excluded for hypertension and consequently, the subjects in this study were likely to have conditions capable of initiating an arrhythmia. As shown by the data, the trigger in this study for initiating an increase in supraventricular arrhythmia is exposure to unfiltered road air. While the absolute number of supraventricular ectopic beats initiated in this study is low and likely not to be clinically relevant, it does suggest that such a trigger when applied to hearts with more advanced disease with a greater conditional susceptibility, might yield more complex and sustained arrhythmia. The wide variation in the number ectopic beats for a given individual and the consistency of the magnitude of the magnitude of ectopic beats across all exposures suggests an associated variation in the underlying conditional susceptibility. The small size of the study cohort limits testing the hypothesis that the arrhythmic response to traffic related pollutants is scaled by the magnitude of the baseline ectopic beats so this important clinical question remains unanswered.

The association of an increase in the incidence of atrial ectopic beats and exposure to traffic-related particle number, BC and PAH is consistent with an increase in atrial arrhythmia associated with inhaled particulate matter and pollutant gases described in epidemiological studies. Modulation of autonomic tone is a likely mechanism; however, the observation that NT-proBNP increased relative to unfiltered air exposure implicates intracardiac hemodynamics as another possible mechanism secondary to atrial stretch. NT proBNP is released from stores in the myocardial cells suggesting that the exposure to unfiltered road air increases intracardiac pressure, stretching the atrium and releasing NTproBNP. Stretch channels located on the cardiac cell membrane are also sensitive to stretch and activation of these channels might play a role in the increased frequency of ectopic beats. Of course we did not have a method to detect intramyocardial pressure, but such changes can be considered in the future utilizing non-invasive echocardiography and doppler methods.

The finding of an increase in the supraventricular ectopic beats has a potential public health impact in that supraventricular ectopic beats initiate a wide variety of atrial, AV nodal and atrioventricular arrhythmias. While admittedly speculation, it is plausible that traffic related pollution might initiate clinically important supraventricular arrhythmias. The question might now be asked what percentage of atrial fibrillation is caused by supraventricular ectopic beats triggered by traffic related particles and gases.

Atrial fibrillation is the most common serious arrhythmia in the late middle age and elder population. It contributes to poor quality of life, stroke, myocardial infarction and heart failure. Its incidence is about 1% annually after the age of 65 years, so that the prevalence is quite high among those in their 70s

and 80s. Likewise less serious, yet still debilitating arrhythmia such as atrial tachycardia, AV nodal tachycardia, and AV nodal reciprocating tachycardia are initiated by premature beats. These findings indicate that inhalation of unfiltered road air when compared to breathing filtered air increases premature atrial beats and that the increase is associated with a statistically significant increase in NT pro-BNP. The association with an increase in NT pro-BNP suggests that the mechanism for the increase in atrial arrhythmia relates to an increase in intra-atrial pressure and atrial stretch.

In contrast to the electrocardiographic findings, there were no statistically significant changes in a number of biomarkers intended to inform about inflammation or vascular injury. As such C-reactive protein, fibrinogen, sE-selectin, sVCAM, SICAM, MMP-9, TNF alpha, MCP-1, or interleukins 1b, 6 and 8 were found not to change significantly. Given our relatively small subject population, we cannot exclude the possibility of real but small changes in some of these biomarkers. Decreases in tPAI-1 independent of freeway or filter are probably related to the experimental protocol or circadian variation. Yet, an effect of gaseous pollutants common to all protocols cannot be excluded.

As described in the previous section, N-terminal pro-B-type natriuretic peptide (NT-proBNP) decreased in filtered-air but not in unfiltered-air exposures; that is, PM exposure on freeways was associated with a relative increase. NT pro-BNP is present in greatest concentration in the left ventricle, atria and to some degree in the right ventricle. With stretch NT-proBNP, a biologically inactive molecule, is released into the circulation. Because of NT-proBNP's long half-life it is a sensitive marker of altered heart function and increased intracardiac pressures with associated stretch. The decrease in NT-proBNP during the filtered exposure probably relates to mild volume depletion attendant to the protocol. The absence of a comparable decrease in NT-proBNP in the unfiltered exposure suggests that a counterbalancing effect on cardiac hemodynamics prevented that anticipated decrease in NT-proBNP. Such an effect could have been mediated by endothelin release from the lung with altered cardiac mechanical function and/or increased salt and water retention by the kidney.

In 2006 Sajadieh and colleagues (Sajadieh et al. 2006) studied the association between CRP, NT-pro-BNP and ventricular premature beats in individuals with no apparent heart disease. The variability in the interquartile range for those with ventricular ectopy was 11.7 pmol/L, and for those without ectopy 6.4 pmol/L. Moreover, CRP did not differ between the two groups. This study provides some evidence that ectopic cardiac electrical impulse formation is sensitive to myocardial stretch, even in the absence of overt myocardial pathology or systemic inflammation.

5. Blood Biomarkers.

Vascular endothelial growth factor (VEGF) is a pluripotent growth and permeability factor having significant effect on endothelial function in the lung and systemic vasculature. A similar observation was made with VEGF in that, like NT-proBNP, VEGF decreased after filtered exposure, but did not decrease after unfiltered exposure. A relative increase in VEGF suggests a response of the pulmonary or systemic endothelium to an insult. Yet at this time the fall in VEGF in the filtered exposure cannot be explained. Given the borderline p value, this might be a spurious finding. In contrast to NT-proBNP and VEGF, MPO decreased after exposure to unfiltered air, at hour-2 and at day-2 measurements, yet MPO did not decrease with filtered air. The decrease attributable to unfiltered air averaged between 20% and 30% ($P = 0.02$ for filter effect). One possibility is that

sequestration of white blood cells at sites of vascular injury might have decreased the serum MPO.

The following measures showed no statistically significant variation by freeway, filter condition, or time of measurement: fibrinogen; C-reactive protein; soluble cell-adhesion molecules sE-selectin, vascular cellular adhesion molecule (sVCAM), and intercellular adhesion molecule (sICAM); matrix metallopeptidase 9 (MMP-9); interleukins 1b, 6, and 8; interferon-gamma, tumor necrosis factor alpha; and monocyte chemotactic protein 1 (MCP-1). Tissue plasminogen activator inhibitor 1 (tPAI-1) showed decreases averaging near 30% between pre-exposure and hour-2 measurements, completely reversed by day 2 ($P < 0.001$ for time effect). Interleukin 10 showed a similar pattern, with decreases at hour 2 averaging near 20%. Because those changes did not vary significantly by freeway or filter condition, they are attributable to circadian variation, effects of the exposure protocol per se, or effects of pollutant gases.

Another biochemical measure, which showed statistically significant changes between filtered and unfiltered exposure conditions, myeloperoxidase (MPO), decreased after exposure to unfiltered air, but not after exposure to filtered air. The decrease attributable to unfiltered air averaged 10% at hour-2 and 38% at day-2 measurements ($P = 0.03$ for filter effect, difference between hour 2 and day 2 not significant). Vascular endothelial growth factor (VEGF) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) showed patterns of change different from MPO, as illustrated in Figure 20. In filtered air, both NT-proBNP and VEGF showed decreases from pre-exposure to hour 2, not reversed by day 2. In unfiltered air, there was relatively little change. Thus, in effect, exposure to particulate pollution on either freeway was associated with relative increases (P for filter effect = 0.02 for VEGF, 0.03 for NT-proBNP). Differences between freeways, or between hour-2 and day-2 measurements, were non-significant. The estimated mean net increase in either VEGF or NT-proBNP after unfiltered (relative to filtered) exposure was roughly twofold. These may be considered relatively small effects, since both VEGF and NT-proBNP measurements could vary by at least 3 orders of magnitude, either between different subjects or at different times in the same subject. Nevertheless, the changes may be important to health (see next section).

6. Ambulatory Blood Pressure.

Figure 21 shows measurements by ambulatory blood pressure recorders for time intervals before, during, and after exposure, averaged over all subjects and exposure conditions. As indicated in the Methods section, an undetermined but fairly large percentage of data for the 10:00-12:59 "exposure" interval actually represent measurements shortly after the exposure period ended, and a smaller percentage represent measurements shortly before it began. Differences in systolic pressure, diastolic pressure, and heart rate between time intervals were highly significant ($P < 0.0001$), and consistent with expected circadian

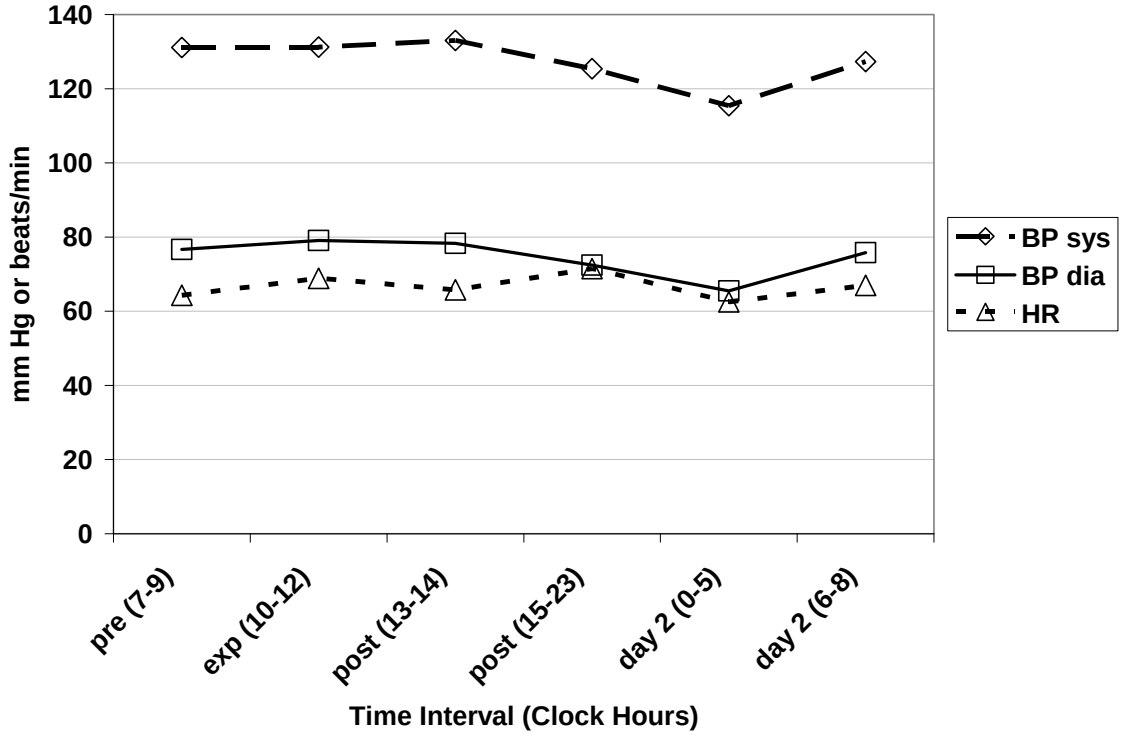


Figure 21. Mean systolic pressure, diastolic pressure, and heart rate as measured by ambulatory blood pressure monitors, for all subjects, all exposure studies. Differences between time intervals were significant, P < 0.001. Differences related to exposure atmosphere were non-significant. BP sys is systolic blood pressure; BP dia is diastolic blood pressure; and HR is heart rate.

variation. Main and interactive effects of freeway and filter condition were non-significant for all three variables. Exposure-response analyses of ambulatory blood pressure data showed no significant relationship of systolic or diastolic blood pressure during and immediately following exposure with any pollutant measured during exposure, nor of pre-exposure blood pressure with prior 24-hour ambient PM-10 or PM-2.5 .

Mean blood pressures measured at the base laboratory were similar to those from the ambulatory recorders. Systolic pressure measured at the laboratory showed no significant changes relatable to exposure conditions. For diastolic pressure change before to after exposure, the freeway*filter interaction was marginally significant (P = 0.049), but the pattern did not suggest a consistently unfavorable effect of PM. Mean changes from pre-exposure (in mmHg), averaged over all 3 measurements after exposure, were +1.7 for unfiltered and +0.3 for filtered exposures on I-405, which could suggest a protective effect of filtration. However, a larger difference in the opposite direction was observed on I-710, where mean changes were -0.5 unfiltered and +3.3 filtered. While noise levels and stress of a two-hour ride on a Los Angeles freeway could affect HRV and blood biomarkers, these stresses were, as far as we know, the same for the filtered and unfiltered runs and the only difference between the two was the exposure to freeway particulate.

7. Comparison with previous studies

In a general sense, this study's results reinforce findings from a wide range of earlier epidemiologic and panel studies that indicate unfavorable acute cardiovascular effects from exposure to traffic emissions. The most directly comparable results come from previous studies with simultaneous monitoring of on-road exposures and health responses. Current results also reinforce those findings in a general sense, but differ in specifics.

The most closely comparable earlier studies in terms of population [Adar et al., 2007ab] involved retirement-home residents on recreational day trips in St. Louis, traveling by bus. The most closely comparable earlier studies in terms of health measures [Riediker et al., 2003, 2004] involved North Carolina State Highway Patrol officers in patrol cars on freeways in the Raleigh-Durham metropolitan area. All these earlier studies lacked filtered-air controls and double-blinding (although neither subjects nor staff members measuring their responses were concurrently aware of exposure levels). Exposure measurement techniques were not directly comparable, but typical PM-2.5 mass concentrations were probably at least 50% lower in previous studies than in the current study. No ultrafine particle data are available for comparison. Despite apparently lower exposure levels, responses were more marked in the earlier studies. The elderly St. Louis panel showed a significant association of increased PM exposure with decreased HRV, not observed in the current study. They tended to be older and less healthy than our subjects, and so might have been more susceptible to cardiac electrophysiologic disturbances. Also, because their subjects were in a familiar recreational setting with friends and neighbors; the level of psychological stress experienced during the experiment may have been lower when compared to our subjects who were in an unfamiliar, non-recreational setting with a small group of relative strangers. Although our subjects were accustomed to freeway travel, and their exposure experience resembled normal freeway travel, it undoubtedly evoked unusual psychological stress, as evidenced by the fact that one volunteer had to be withdrawn early for that reason. Cardiovascular responses to lower levels of stress, as presumably experienced by the other subjects, might either enhance or mask effects of PM exposure. The comparatively young and healthy North Carolina patrolmen were monitored at work in an occupation subject to high stress, but in highly familiar circumstances without unusual acutely stressful episodes. They exhibited increased supraventricular ectopic beat incidence, similar to that seen in our subjects, but with changes in blood biochemistry different from those observed in our subjects.

Recent studies by Delfino et al. [2008,2009] of a panel of elderly Southern Californians with cardiovascular disease history provide some points of comparison with our study. They indicated that ultrafine particle mass, particulate EC, and primary (not secondary) particulate OC personal-exposure measurements were more closely associated with unfavorable acute responses (systemic inflammatory changes and blood platelet activation) than were personal exposures to PM-2.5. Although the population, exposure circumstances, and experimental endpoints were different, these studies tend to corroborate our results pointing to UFP, and perhaps more specifically to EC and/or PAH (a component of primary particulate OC), as the most likely causes of acute cardiovascular effects.

A recently study on the effects of diesel emissions on HRV involved 16 adult volunteers exposed to filtered air and two levels of diluted diesel emission (DE) in 2-h sessions (Peretz et al., 2008). HRV and related parameters were used as indicators of health effects. No ultrafine particle number concentrations were measured. Only fine PM concentrations and gaseous phase pollutants such as NOx and CO were controlled. Even though the exposure levels used in the Peretz's study were

much higher (100 μg/m^3 and 200 μg/m^3), no consistent effect on the autonomic control of the heart was observed, which is similar to the findings of the present study. It should be noted, the subjects in Peretz's study were younger and without pre-existing cardiovascular disease.

In the present study the health endpoints were most strongly associated with particle number, rather than PM mass. As such the findings of Samet and colleagues (Samet et al. 2009) who recently examined the effect of inhaled concentrated ultrafine PM in a cohort of healthy non-smoking subjects might be relevant. While the magnitude of the exposure to particle number was similar between the studies, as was the timing of the measurement of health effects; there were significant differences between the studies. Some of these differences related to the age of the subjects, and the source of the ultrafine particles might account for some of the differences in the studies. The subjects in the present study were much older. The average age of the subjects in the present study was 71±5 years, whereas the subjects in the Chapel Hill concentrated ultrafine PM study ranged from 18 to 35 years. Moreover, in their study the ultrafine PM was concentrated from the Chapel Hill, NC airshed, The mean particle concentrations during the concentrated ultrafine PM exposures ranged from 40,848 to 205,684 particles/cm^3, with a group mean and standard deviation of 120,662 ± 48,232 particles/cm^3, and filtered air particle concentrations were <10 particles/cm^3, and compared favorably to the concentrations of ultrafine PM measured in the present study that is 77,000 on I-405, and 107,000 on I-710.

The study by Samet et al. (Samet et al. 2009) concluded that exposure to ultrafine PM induced mild pulmonary inflammatory and prothrombotic responses, and changes in heart rate variability and cardiac repolarization. Ultrafine PM has been shown to alter cardiac sympathovagal balance in aged adult subjects (Timonen et al. 2006). As shown in this study and the Chapel Hill ultrafine CAPs study exposure to ultrafine PM did not change time-domain measures of HRV. Yet, exposure to concentrated Chapel Hill ultrafine PM increased frequency-domain HRV. Further analysis of the ambulatory ECGs investigating frequency-domain and non-linear measures of HRV are underway currently and should soon be available. When exposed to Chapel Hill ultrafine PM the QT interval showed increased variability that was independent of heart rate variability. These findings suggest that the changes in cardiac repolarization are real and not merely a reflection of changes in RR interval. Likewise, repolarization characteristics are also being examined and should soon be available.

Review of the literature discloses numerous clinical and biological effects associated with ultrafine PM exposure. At the cellular and tissue level these include: modulation of the expression of adhesion molecules in human leukocytes (Frampton et al. 2006), oxidative DNA damage in mononuclear cells (Vinzents et al. 2005), impairment of phagocytic function in alveolar macrophages (Lundborg et al. 2006, Lundborg et al. 2007), lung inflammation (Andre et al. 2006), altered hemostasis (Nemmar et al. 2006) and augmentation of myocardial ischemia-reperfusion injury (Cozzi et al. 2006). In contrast to animal studies, previous studies in man have shown no to minimal evidence of clinical pulmonary effects of ultrafine PM, yet these same studies have shown evidence of subclinical pulmonary responses. For example, ultrafine CAP exposure resulted in a modest elevation in the inflammatory chemokine IL-8 recovered in the bronchioaveolar lavage fluid (Samet et al. 2009). Pietropaoli and colleagues (Pietropaoli et al. 2004) demonstrate impairment of CO diffusing capacity in response to exposure to ambient ultrafine PM, and controlled exposure to synthetic carbon ultrafine PM changed heart rate variability and cardiac repolarization (Frampton et al. 2004). Moreover, increased levels of ultrafine PM was associated with increased frequency of atrial and ventricular runs in subjects with

established coronary artery disease (Berger et al. 2006). Therefore, the findings described in this study are consistent biochemical evidence of vascular and hemodynamic changes but without clinically measurable pulmonary effects.

In summary, this study and previous investigations of on-road exposures and acute responses have shown subtle cardiovascular effects associated with particles from vehicle exhaust, which would not be clinically important if observed in an individual, but may be important to public health if experienced by large populations including some high-risk individuals. This study, which (unlike its predecessors) includes filtered-air control conditions and double-blinding, seems to have shown generally less marked effects despite more intense exposures. Chance differences in susceptibility of individual participants in each study might explain differences in results. Alternatively, the earlier studies may have suggested more (or larger) effects because they measured the sum of effects caused by PM exposure and effects caused by other environmental factors that co-vary with PM exposure. Our study, comparing exposure against control conditions, should have measured only the effects caused by various measures of PM, including number concentration, UF number concentration, PM-2.5 and PM-10 mass concentrations, black carbon, and particle bound PAH.

8. Publications resulting from this study

Linn, W.S., Zhu, Y., Cascio, W.E., Webb, T.L., and Hinds, W.C. Effects of motor vehicle exhaust exposure in healthy late middle age volunteers riding on Los Angeles freeways: Methodology and preliminary results. Am. J. Respir. Crit. Care Med. 2007; 175: A544. [Presented, American Thoracic Society 2007 International Conference, San Francisco, May 2007.]

Zhu, Y., Fung, D., Eiguren-Fernandez, A., and Hinds, W.C. An On-Freeway Exposure and Measurement System for Freeway Aerosol Health Effects Study. Presented, American Association for Aerosol Research Conference, Reno, NV, September 2007.

Zhu, Y., Fung, D.C., Kennedy, N., Hinds, W.C. and Eiguren-Fernandez, A, "Measurements of Ultrafine Particles and Other Vehicular Pollutants inside a Mobile Exposure System on Los Angeles Freeways." J. Air & Waste Mgmt. Assoc, 58:424-434 (2008).

Cascio, W. E., Katwa, L.C., Linn, W.S., Stram, D.O. Zhu, Y., Cascio, J.L., and Hinds, W.C. Effects of Vehicle Exhaust in Aged Adults Riding on Los Angeles Freeways. Am. J. Respir. Crit. Care Med. 2009; 179: A1175. [Presented, American Thoracic Society 2009 International Conference, San Diego, May 2009.]

Zhu, Y, Qunfang, Z., Fung, DC, Kennedy, NJ, and Hinds, WC; "Analysis of Factors Affecting Concentrations of Ultrafine Particles and Associated Pollutants on Freeways." Submitted to Atmospheric Environment. 2009.

D. CONCLUSIONS

A nine-passenger van was modified with a HEPA filter unit to facilitate an on-freeway PM health effects study. The filtration system removed more than 96% of particles when the flow through the

filtration system was switched from bypass mode to filter mode while the vehicle was traveling on the freeways. Thus, the filtration system provided a large PM exposure contrast while keeping gas-phase pollutant concentrations about the same. Pollutant concentrations were monitored in human subjects' breathing zone, which included concentration and size distribution of fine and UF particles and the concentration of CO, BC, particle-bound PAHs, PM-2.5 mass, and NOx. Average total particle number concentration observed inside the enclosure when the filtration system was in bypass mode (unfiltered air) was 77,800 and 107,500 particles/cm^3 on the I-405 and the I-710 freeway, respectively. The highest one-minute particle number concentration observed was over 730,000 particles/cm^3. Bimodal size distributions were typical for both freeways with the first mode around 12–20 nm and the second mode around 50–100 nm. BC and particle-bound PAH concentrations were more than two times greater on I-710 than on the I-405 freeway, presumably because of greater diesel emissions. Non-significant correlations were observed between total particle number concentration and gas phase vehicular pollutants on freeways.

Like most previous controlled studies of exposure to real and simulated ambient particulate pollution, this investigation found little evidence for acute respiratory changes, and subtle but possibly important evidence for acute cardiac and vascular changes. In our relatively healthy late middle-aged and elderly adult volunteers, two-hour exposures to PM on freeways were associated with increased incidence of atrial arrhythmia ($p<0.05$), but with generally stable or decreased incidence of ventricular arrhythmia and generally stable heart rate variability. Concurrent relative increases in NT pro-BNP (a blood biomarker for intracardiac pressure or myocardial stretch) suggest that increased intra-atrial pressure triggered atrial arrhythmia. The blood biomarker VEGF, an indicator of vascular injury/repair also decreased 30% on average in filtered compared to unfiltered air ($P<0.05$). Rhythm effects appeared to relate to PAH ($P=0.02$) , black carbon ($P=0.04$), and the UF particle fraction, in that particle count was more strongly correlated with arrhythmia incidence ($p=0.01$), than PM-2.5 and PM-10 mass concentrations ($p=0.07$ and 0.10 respectively). Whether particle count, black carbon concentration, PAH concentration, or some other closely associated but unmeasured component is most responsible for the effect cannot be determined from this study. Particle count showed the strongest statistical correlation, consistent with the *a priori* hypothesis; however, we cannot rule out the possibility that a truly stronger causal association with another component (e.g. black carbon or PAH) would appear weaker statistically because the effective dose of the other component was measured less accurately by the monitoring instruments. The same problem applies to most if not all studies of ambient traffic-related pollution, in which multiple components with presumably different relative levels of health risk are closely associated with one another. Here, in contrast to most other health studies, rigorous double-blind conditions and filtered-air controls rule out other traffic-related stresses or pollutant gases as causes of the PM-associated cardiovascular effects.

Because sustained arrhythmias can be triggered by premature atrial beats, traffic exposure might play a role in their occurrence - a risk that could be mitigated by filtering PM from the air in the passenger cabin. Original equipment automotive cabin air filters appear to be moderately effective in removing UF particles, but less effective against slightly larger particles [Zhu et al, 2007; Qi et al., 2008]. Judging from the results presented here, careful use of existing cabin air filters in heavy traffic may be effective in reducing health risk, and efforts to improve cabin air filter technology may be indicated.

The observation of a relative increase in VEGF and NT pro-BNP supports speculation that inhalation of ultrafine and other traffic-related PM components produces mild sub-clinical pulmonary injury, an

increase in pulmonary vascular constriction and an increase of pulmonary pressures with attendant increases in right heart pressure. The increase in the right atrial pressure then causes the release of NT pro-BNP and increases atrial stretch and the excitability of the atrium resulting in more frequent premature atrial beats. Future studies will need to be designed to correlate the biochemical indicators of hemodynamic changes to those of actual measurements made non-invasively by echocardiographic imaging of atrial volume, Doppler assessment of intra-atrial and pulmonary pressures and systemic vascular resistance as determined by impedance cardiography

This study did not show substantial differences in the immediate or acute health response between gasoline automobile traffic and diesel truck traffic. However, important differences cannot be ruled out, because the study had relatively limited power to detect them. The limitations relate to the small number of subjects, variability of air pollutant exposures between subjects (including considerable overlap in concentration ranges for most pollutants between I-710 and I-405), and uncertainty about ambient pollution exposures prior to the experimental periods. Prior exposures could be estimated only crudely, by reference to ambient monitoring stations near subjects' residences. Prior 24-hour-average ambient PM exposure estimates showed no obvious associations with health measures that could have confounded the measurements of response to experimental exposures. Even so, confounding cannot be excluded, given that prior ambient exposure effects could have operated on longer time scales, and/or monitoring station measurements might give poor estimates of personal exposures. The experimental results support the possibility of increased risk from diesel, as opposed to gasoline, exhaust exposure, in that exposure-response relationships were most significant with respect to particulate components emitted more by diesel engines – ultrafine particles, black carbon, and particle-bound PAHs.

In summary this study documents a health risk associated with freeway travel. In our relatively healthy late middle-aged adult volunteers, exposures to particulate matter on freeways were associated with increased incidence of atrial arrhythmia, but with generally stable or decreased incidence of ventricular arrhythmia and generally stable time-domain measures of heart rate variability. Concurrent relative increases in NT pro-BNP suggest that increased intra-atrial pressure triggered atrial arrhythmia. Effects appeared to relate to the ultrafine particulate fraction, in that particle count was strongly correlated with arrhythmia incidence, while PM-2.5 and PM-10 mass concentrations were not significantly correlated with arrhythmia incidence. Rigorous double-blind conditions and filtered-air controls in this study rule out other traffic-related stresses or pollutant gases as causes of the particulate matter-associated cardiovascular effects. Because sustained arrhythmias (e.g. atrial fibrillation, AV nodal reentrant tachycardia) are triggered by premature atrial beats, traffic exposure may play a role in their occurrence - a risk that could be mitigated by filtering particulate matter from the vehicle passenger cabins.

E. RECOMMENDATIONS

Results presented here could be confirmed and strengthened by more focused research using more subjects. Such studies should focus on ultrafine particles and the associations found in the present study. The study protocol could be simplified by using passenger vehicles that come equipped with high efficiency filtrations systems. A similar study could be conducted using a more susceptible subject population, such as late middle age subjects with pre-existing cardiovascular conditions.

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XI. List of inventions reported and copyrighted materials produced

Inventions
None

Copyrighted materials produced
Linn WS, Zhu Y, Cascio WE, et al., "Effects of motor vehicle exhaust exposure in healthy elderly volunteers riding on Los Angeles freeways: methodology and preliminary results." Am J Respir Crit Care Med 2007; 175:544.

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Cascio WE, Katwa LC, Linn WS, Stram DO, Zhu Y, Cascio JL, and Hinds WC, "Effects of Vehicle Exhaust in Aged Adults Riding on Los Angeles Freeways." Presented at the 2009 American Thoracic Society (ATS) Meeting, May 2009.

XII. Glossary of Terms, Abbreviations, and Symbols

ASDNN5	Average standard deviation of normal intervals during successive 5-min segments of Holter ECG record
CO	Carbon monoxide
CO2	Carbon dioxide
CPC	Condensation particle counter
CRP	C-reactive protein
ECG	Electrocardiogram
FEV_1	Forced expired volume in 1st second of maximal forced expiration
Fres	Resonant frequency of the respiratory system
FVC	Forced vital capacity (volume of maximal forced expiration)

HEPA	High efficiency air filters for particulate
HRV	Heart rate variability
Kleiger SD	(Same as SDNN)
LDMA	Long differential mobility analyzer
Magid SD	(Same as ASDNN5)
MCP-1	Monocyte chemotactic protein 1
MMF	Maximal midexpriatory flow
MMP-9	Matrix metallopeptidase 9
NT-proBNP	N-terminal pro B-type natriuretic peptide
PAH	Polycyclic aromatic hydrocarbons
PB-PAH	Particle bound polycyclic aromatic hydrocarbons
PM	Particulate matter
PM-10	Particulate matter less than 10 μm
PM-2,5	Particulate matter less than 2.5 μm
PNN50	Percentage of adjacent normal heartbeat intervals different by >50 msec
rmsSD	Root-mean-square of successive differences in intervals between 2 adjacent normal heartbeats
Rrs	Total resistance of the respiratory system measured by forced oscillation at a specified frequency between 5 and 25 Hz
SaO_2	Arterial oxygen saturation (percent oxygemoglobin in arterial blood)
SDNN	Standard deviation of intervals between normal heartbeats
SICAM	Soluble intercellular adhesion molecule
SpO_2	Percent oxyhemoglobin in arterial blood, estimated by pulse oximeter
SVCAM	Soluble vascular cellular adhesion molecule
SVEB	Supraventricular ectopic heartbeats
SVEB_hr	Supraventricular ectopic heartbeats per hour
TSS	Total symptom score
UF	Ultrafine [particles]
VEB_hr	Ventricular ectopic heartbeats per hour
VEGF	Vascular endothelial growth factor

XIII. Appendices

App. A. Selected Tables calculated on the basis of run averages

1. Table A6
2. Table A7
3. Table A8

App. B. Research Team

App. C. Copies of published and submitted peer-reviewed abstracts and papers

Abstracts

Linn, W.S., Zhu, Y., Cascio, W.E., Webb, T.L., and Hinds, W.C. Effects of motor vehicle exhaust exposure in healthy late middle age volunteers riding on Los Angeles freeways: Methodology and preliminary results. Am. J. Respir. Crit. Care Med. 2007; 175: A544. [Presented, American Thoracic Society 2007 International Conference, San Francisco, May 2007.]

Zhu, Y., Fung, D., Eiguren-Fernandez, A., and Hinds, W.C. An On-Freeway Exposure and Measurement System for Freeway Aerosol Health Effects Study. Presented, American Association for Aerosol Research Conference, Reno, NV, September 2007.

Cascio, W. E., Katwa, L.C., Linn, W.S., Stram, D.O. Zhu, Y., Cascio, J.L., and Hinds, W.C. Effects of Vehicle Exhaust in Aged Adults Riding on Los Angeles Freeways. Am. J. Respir. Crit. Care Med. 2009; 179: A1175. [Presented, American Thoracic Society 2009 International Conference, San Diego, May 2009.]

Published and submitted peer-reviewed papers

Zhu, Y., Fung, D.C., Kennedy, N., Hinds, W.C. and Eiguren-Fernandez, A, "Measurements of Ultrafine Particles and Other Vehicular Pollutants inside a Mobile Exposure System on Los Angeles Freeways." J. Air & Waste Mgmt. Assoc, 58:424-434 (2008).

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Table A6. Mean concentrations of environmental parameters measured on each run.

Date	Fwy	Filter	Total Particle Number (#/cm^3)	UF particles (#/cm^3)	PM-2.5 (µg/m^3)	PM-10 (µg/m^3)	BC (µg/m^3)	UVPM (µg/m^3)	PB-PAH (ng/m^3)	NOx (ppb)	CO (ppm)	CO_2 (ppm)	Temp (F)	Rh (%)
2006/6/7	710	F	4431	7445	n/a	n/a	1.17	0.77	6.88	451	n/a	n/a	n/a	n/a
2006/6/14	405	F	1987	3558	n/a	n/a	0.77	0.55	2.50	414	0.95	582	68.09	35.18
2006/6/21	710	U	160312	78924	74.6	84.2	11.86	9.48	231.79	413	3.33	599	66.46	36.34
2006/6/28	405	U	77860	35874	n/a	n/a	4.60	3.82	104.64	448	3.21	583	64.87	39.62
2006/7/5	710	F	2703	3163	15.5	17.8	0.84	0.66	4.92	339	2.72	575	68.18	37.28
2006/7/12	405	U	65203	37822	29.9	33.3	5.76	4.78	110.61	199	2.63	596	67.13	37.29
2006/7/19	710	U	90240	63074	57.8	66.1	11.75	9.35	176.12	399	5.69	729	69.71	40.97
2006/7/26	405	F	1544	2494	15.0	18.3	0.19	0.00	3.42	n/a	6.43	579	71.15	39.72
2006/8/2	710	F	2997	3306	11.2	17.6	0.86	0.73	5.07	292	3.26	595	68.46	43.42
2006/8/9	710	U	100291	69152	64.3	70.9	8.09	6.67	161.23	408	4.69	588	70.67	36.75
2006/8/16	405	F	2214	1978	8.2	15.1	0.86	0.61	2.68	220	2.17	606	61.40	44.51
2006/8/23	405	U	77709	43353	50.1	63.5	6.91	6.00	139.89	231	3.25	612	63.87	40.92
2006/9/6	710	U	146238	87259	64.0	67.9	14.62	11.61	352.57	466	4.39	631	67.73	37.74
2006/9/13	405	F	1666	687	10.1	11.2	0.74	0.64	4.22	155	2.39	584	64.86	42.13
2006/9/20	405	U	106683	40965	51.6	53.9	5.50	4.70	118.74	207	3.33	598	66.70	39.09
2006/9/27	710	F	3742	1429	9.1	10.2	0.81	0.71	7.41	466	3.96	642	73.12	30.07
2006/10/4	710	U	147764	80769	42.2	50.7	10.68	8.75	234.79	379	2.80	656	66.84	37.92
2006/10/11	405	U	96786	41252	n/a	n/a	5.11	4.29	94.62	170	n/a	n/a	n/a	n/a
2006/10/18	405	F	2288	771	8.4	14.9	1.65	1.32	5.50	n/a	n/a	n/a	n/a	n/a
2006/10/25	710	F	3949	1534	18.3	26.5	0.76	0.65	6.88	457	2.90	724	64.63	41.65
2006/11/1	405	F	2460	935	9.8	12.5	0.84	0.66	4.49	n/a	2.00	599	63.27	41.86
2006/11/8	710	U	123313	74567	61.7	71.3	13.70	11.14	293.23	502	3.22	646	67.12	32.62
2006/11/15	405	U	90835	42387	29.0	30.3	6.30	5.40	11.98	245	3.19	602	65.18	36.29
2006/11/29	710	F	4635	1854	n/a	n/a	0.71	0.62	6.92	494	1.61	580	76.18	22.41
2006/12/6	405	U	89592	53394	72.8	84.4	8.52	9.80	109.54	448	3.86	675	70.99	22.98
2006/12/13	405	F	2329	1092	8.6	10.8	1.30	1.13	5.41	351	3.64	657	74.13	26.64
2006/12/20	710	U	175071	134985	45.1	49.8	9.96	8.41	198.45	551	2.66	607	76.35	20.19
2006/12/27	710	F	2911	1006	3.1	7.2	0.39	0.29	3.12	n/a	0.86	569	74.24	26.61
2007/1/9	405	F	2084	870	4.2	6.3	1.01	0.77	6.00	285	2.14	623	73.32	21.45
2007/1/16	710	F	4991	1908	3.2	5.8	1.21	0.98	7.86	510	3.12	616	75.21	13.48
2007/1/23	405	U	76982	56128	27.8	33.2	6.25	5.36	144.69	309	1.75	620	71.46	20.14
2007/1/30	710	U	129156	99288	n/a	n/a	9.07	7.36	242.77	421	2.73	613	74.40	27.48

Table A6. Concentrations of major environmental parameters measured on each run (cont.).

Date	Fwy	Filter	Total Particle Number (#/cm^3)	UF particles (#/cm^3)	PM-2.5 (µg/m^3)	PM-10 (µg/m^3)	BC (µg/m^3)	UVPM (µg/m^3)	PB-PAH (ng/m^3)	NOx (ppb)	CO (ppm)	CO_2 (ppm)	Temp (F)	Rh (%)
2007/2/6	405	U	68130	52306	71.6	84.1	6.53	5.42	123.18	247	3.01	657	72.98	30.22
2007/2/13	710	F	4924	2183	n/a	n/a	0.69	0.61	7.17	444	2.35	627	72.19	28.54
2007/2/20	405	F	2116	887	4.3	13.7	0.54	0.43	3.95	174	2.05	595	72.66	31.15
2007/2/27	710	U	46951	48952	n/a	n/a	5.27	4.51	145.97	245	1.17	614	83.47	18.15
2007/3/6	710	F	4164	1709	4.6	12.6	1.09	0.89	8.14	551	2.73	709	75.31	24.33
2007/3/13	710	U	100028	75698	36.4	42.7	7.74	6.29	174.53	391	3.81	733	72.70	27.99
2007/3/20	405	U	76705	52071	32.5	36.0	5.13	4.37	110.89	216	2.82	614	72.09	31.36
2007/3/27	405	F	1623	542	n/a	n/a	0.68	0.67	3.11	126	2.41	589	71.12	27.58
2007/4/4	710	U	106208	84727	92.1	102.5	12.28	10.03	239.12	405	3.47	607	73.22	31.22
2007/4/11	405	F	1865	550	n/a	n/a	1.18	0.53	5.28	162	2.53	566	70.90	32.46
2007/4/18	710	F	2379	842	n/a	n/a	0.35	0.22	3.30	289	3.62	579	69.55	18.41
2007/4/25	405	U	67494	50293	27.0	30.5	4.98	4.33	126.51	284	2.44	636	73.11	30.10
2007/5/1	405	U	62341	35423	41.2	45.7	4.01	3.57	80.71	238	3.05	624	72.75	33.21
2007/5/8	710	U	78838	62053	26.0	30.5	9.28	7.50	n/a	666	5.13	639	78.22	18.02
2007/5/15	710	F	6347	2328	9.6	11.3	1.05	0.62	n/a	523	2.86	667	65.61	37.93
2007/5/22	405	F	2088	906	n/a	n/a	0.68	0.43	n/a	213	2.27	663	70.45	35.04
2008/3/26	405	F	2805	1048	n/a	n/a	0.89	0.68	9.30	274	2.58	826	67.74	41.95
2008/4/23	710	F	6221	2417	n/a	n/a	1.89	1.64	n/a	479	n/a	n/a	n/a	n/a
2008/4/30	710	U	77256	69818	n/a	n/a	5.71	4.74	282.48	463	2.43	656	74.14	n/a
2008/5/7	405	U	75818	45440	n/a	n/a	3.77	3.24	190.16	174	1.27	691	69.63	n/a
2008/5/13	405	U	67133	45705	n/a	n/a	4.33	3.70	194.67	175	1.50	609	71.47	31.57
2008/5/20	405	F	2185	814	n/a	n/a	1.03	0.92	10.18	195	3.13	635	73.81	33.77
2008/5/27	710	F	5456	2084	n/a	n/a	1.45	1.28	17.73	442	n/a	n/a	n/a	n/a
2008/6/3	710	U	110290	79291	n/a	n/a	7.19	5.77	394.03	437	3.23	621	72.31	32.58

Table A7. Mean (Standard Deviation) of Environmental Measurements, by Freeway and Filter Condition, averaged for all runs.

Variable	Units	I-405 unfilt.	I-405 filt.	I-710 unfilt.	I-710 filt.
Total Particle Number	count/cm^3	78519 (13048)	2090 (343)	113711 (35597)	4275 (1257)
UF particles	count/cm^3	45172 (6750)	1224 (863)	79183 (20201)	2372 (1619)
PM-2.5	µg/m³	43.4 (17.6)	8.6 (3.4)	56.4 (19.5)	9.3 (5.6)
PM-10	µg/m³	49.5 (21.3)	12.9 (3.6)	63.7 (21.0)	13.6 (6.8)
BC	µg/m³	5.55 (1.28)	0.88 (0.35)	9.80 (2.85)	0.95 (0.41)
UVPM	µg/m³	4.91 (1.62)	0.67 (0.32)	7.97 (2.23)	0.76 (0.36)
PB-PAH	ng/m^3	118.63 (44.64)	5.08 (2.35)	240.54 (74.32)	7.12 (3.74)
NOx	ppb	256 (90)	234 (89)	439 (95)	441 (84)
CO	ppm	2.72 (0.78)	2.67 (1.29)	3.48 (1.19)	2.73 (0.88)
CO_2	ppm	624 (32)	624 (68)	638 (44)	626 (54)
Temperature	deg F	69.40 (3.38)	69.45 (4.12)	72.38 (4.85)	71.15 (4.07)
Rel. Humidity	%	32.73 (6.46)	34.88 (7.01)	30.61 (7.80)	29.47 (9.69)
Prior 24 hr Amb. PM-10	µg/m³	30.0 (9.2)	26.2 (8.6)	30.1 (8.3)	29.6 (9.7)
Prior 24 hr Amb. PM-2.5	µg/m³	24.0 (5.6)	21.7 (7.7)	21.5 (10.1)	23.6 (12.2)

Table A8. Pearson correlation coefficients among measured pollutants inside the exposure chamber under unfiltered mode, for all runs. (a) I-405 only; and (b) I-710 only.

(a) I-405 freeway

	Total Particle Number	UF particles	PM-2.5	PM-10	BC	UVPM	PB-PAH	NO_x	CO	CO_2
Total Particle Number	1.000									
UF particles	-0.032 (0.913)	1.000								
PM-2.5	0.236 (0.511)	0.197 (0.586)	1.000							
PM-10	0.164 (0.650)	0.238 (0.508)	0.990 (<.0001)	1.000						
BC	0.316 (0.271)	0.461 (0.097)	0.595 (0.070)	0.632 (0.050)	1.000					
UVPM	0.313 (0.276)	0.458 (0.100)	0.628 (0.052)	0.647 (0.043)	0.947 (<.0001)	1.000				
PB-PAH	-0.311 (0.279)	0.307 (0.287)	0.211 (0.559)	0.272 (0.446)	-0.264 (0.362)	-0.202 (0.489)	1.000			
NO_x	0.059 (0.842)	0.168 (0.565)	0.423 (0.224)	0.438 (0.206)	0.449 (0.107)	0.552 (0.041)	-0.203 (0.486)	1.000		
CO	0.429 (0.144)	-0.201 (0.510)	0.689 (0.027)	0.645 (0.044)	0.584 (0.036)	0.576 (0.039)	-0.683 (0.010)	0.491 (0.088)	1.000	
CO_2	-0.102 (0.741)	0.520 (0.069)	0.684 (0.029)	0.704 (0.023)	0.154 (0.616)	0.308 (0.307)	0.353 (0.236)	0.036 (0.908)	-0.206 (0.500)	1.000

(b) I-710 freeway.

	Total Particle Number	UF particles	PM-2.5	PM-10	BC	UVPM	PB-PAH	NO_x	CO	CO_2
Total Particle Number	1.000									
UF particles	0.799 (0.001)	1.000								
PM-2.5	0.157 (0.665)	0.019 (0.959)	1.000							
PM-10	0.141 (0.697)	-0.015 (0.968)	0.996 (<.0001)	1.000						
BC	0.595 (0.025)	0.276 (0.339)	0.540 (0.108)	0.531 (0.114)	1.000					
UVPM	0.607 (0.021)	0.304 (0.290)	0.544 (0.104)	0.536 (0.110)	0.998 (<.0001)	1.000				
PB-PAH	0.276 (0.362)	0.159 (0.604)	0.284 (0.460)	0.254 (0.510)	0.270 (0.372)	0.248 (0.414)	1.000			
NO_x	0.256 (0.377)	0.336 (0.240)	-0.487 (0.153)	-0.513 (0.130)	0.271 (0.348)	0.275 (0.342)	0.459 (0.115)	1.000		
CO	-0.003 (0.993)	-0.163 (0.577)	-0.129 (0.723)	-0.150 (0.680)	0.433 (0.122)	0.404 (0.152)	0.003 (0.992)	0.431 (0.124)	1.000	
CO_2	-0.253 (0.383)	-0.277 (0.338)	-0.442 (0.201)	-0.412 (0.237)	0.010 (0.973)	-0.011 (0.970)	-0.157 (0.608)	-0.054 (0.855)	0.357 (0.210)	1.000

APPENDIX B. RESEARCH TEAM

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APPENDIX C. **COPIES OF PUBLISHED AND SUBMITTED PEER-REVIEWED PAPERS AND ABSTRACTS**

Three Abstracts associated with ARB project 04-324:

Linn, W.S., Zhu, Y., Cascio, W.E., Webb, T.L., and Hinds, W.C. Effects of motor vehicle exhaust exposure in healthy late middle age volunteers riding on Los Angeles freeways: Methodology and preliminary results. Am. J. Respir. Crit. Care Med. 2007; 175: A544. [Presented, American Thoracic Society 2007 International Conference, San Francisco, May 2007.]

Zhu, Y., Fung, D., Eiguren-Fernandez, A., and Hinds, W.C. An On-Freeway Exposure and Measurement System for Freeway Aerosol Health Effects Study. Presented, American Association for Aerosol Research Conference, Reno, NV, September 2007.

Cascio, W. E., Katwa, L.C., Linn, W.S., Stram, D.O. Zhu, Y., Cascio, J.L., and Hinds, W.C. Effects of Vehicle Exhaust in Aged Adults Riding on Los Angeles Freeways. Am. J. Respir. Crit. Care Med. 2009; 179: A1175. [Presented, American Thoracic Society 2009 International Conference, San Diego, May 2009.]

Abstracts are given below.

Two Papers associated with ARB project 04-324:

Zhu, Y., Fung, D.C., Kennedy, N., Hinds, W.C. and Eiguren-Fernandez, A, "Measurements of Ultrafine Particles and Other Vehicular Pollutants inside a Mobile Exposure System on Los Angeles Freeways." J. Air & Waste Mgmt. Assoc, 58:424-434 (2008).

Zhu, Y, Qunfang, Z., Fung, DC, Kennedy, NJ, and Hinds, WC; "Analysis of Factors Affecting Concentrations of Ultrafine Particles and Associated Pollutants on Freeways." Submitted to Atmospheric Environment. 2009.

Papers follow the abstracts below.

Effects of motor vehicle exhaust exposure in elderly volunteers riding on Los Angeles freeways: Methodology and preliminary results

W.S. Linn, Y. Zhu, W.E. Cascio, T.L. Webb, W.C. Hinds; UCLA & U. of Southern California, Los Angeles, CA; E. Carolina U., Greenville, NC.

BACKGROUND: Exposure to particulate (PM) pollution from motor vehicle traffic has been associated with cardiopulmonary health effects, but specifics are not well understood. OBJECTIVES: Document acute effects of PM exposure in healthy elderly volunteers riding on Los Angeles freeways, and relate effects with specific PM components or sources. EXPERIMENTAL APPROACH: In a 9-passenger van, a 2-person exposure chamber constructed around the 2nd seat allows occupants to breathe either unfiltered outside air, or filtered air free of PM but not of pollutant gases. Instruments in the rear monitor gases and fine/ultrafine PM in the chamber. Healthy elderly volunteers ride in the chamber for 2 h on 4 occasions 1 week apart: once each with filtered and unfiltered air on a diesel-truck-dominated freeway and on a gasoline-car-dominated freeway. Double-blind health assessments include 24-h Holter ECG, cardiac repolarization characteristics, ambulatory blood pressure monitoring, and serial pulmonary function and symptom measurements. PRELIMINARY RESULTS from initial 16 exposures/4 subjects: In unfiltered air, PM count per ml averaged 124000 on the truck freeway vs. 73000 on the car freeway (P<.001). PM(2.5) concentrations averaged 67 vs. 40 mcg/cu m respectively (P<.001). Filters removed >90% of PM but not pollutant gases. No clinically significant physiologic or symptom response was seen in any individual exposure. Small statistically significant (P < .05) increases in mean symptom score, total respiratory resistance, systolic blood pressure, and fingertip pulse oximetry measurements, and small decreases in FEV1 and mean heart rate, were observed at one or more time intervals after exposure regardless of atmosphere. CONCLUSIONS: Double-blind controlled exposure studies with freeway PM are feasible with this system. Observed small physiologic changes may be due to pollutant gases and/or effects of the protocol itself. Larger subject samples will be necessary to evaluate PM exposure-response relationships.

An On-Freeway Exposure and Measurement System for Freeway Aerosol Health Effects Study

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David Fung, Arantzazu Eiguren-Fernandez, and William C. Hinds, University of California Los Angeles, Los Angeles, CA

A novel exposure and air pollution measurement system was developed and used for on-freeway particulate matter (PM) health effects studies. A 9 passenger van was modified with a High-Efficiency Particulate Air (HEPA) filtration system that can deliver filtered or unfiltered air to an exposure chamber inside the van. More than 97% of particles were removed when the flow through the filter box was switched from bypass mode to filter mode while the vehicle was driving on the freeways. The filtration system thus provides a great PM exposure contrast while keep gas-phase pollutant concentrations the same. State-of-art instruments were used to measure concentration and size distribution of fine and ultrafine particles and the concentration of carbon monoxide (CO), black carbon, particle-bound polycyclic aromatic hydrocarbons (PAH), PM-2.5 mass, and oxides of nitrogen (NOx) in human subjects' breathing zone. The construction and technical details of the van, and summary of data collected in the first 32 two-hour runs on two major Los Angeles freeways, Interstate 405 (mostly gasoline traffic) and Interstate 710 (large proportion of heavy-duty diesel traffic) will be presented. Average total particle number concentration observed inside the enclosure, with unfiltered air, was around 8.4×10^4 and 1.3×10^5 particles cm^{-3} on the I-405 and the I-710 freeway, respectively. Bi-modal size distributions were consistent and similar for both freeways with the first mode around 16-20 nm and the second mode around 50-55 nm. Black carbon and particle-bound PAH concentrations were more than two times greater on the I-710 than on the I-405 freeway. Very weak correlations were observed between total particle number concentration and other vehicular pollutants on the freeways. This system is the first of its kind that is specifically designed for clinical, environmental studies investigating relationships between measures of gas- and particle-phase exposures and short term health response during freeway travel.

Effects of Vehicle Exhaust in Aged Adults Riding on Los Angeles Freeways

W E Cascio, MD[1], L C Katwa, PhD[1], W S Linn, MA[2], D O Stram, PhD[2], Y Zhu, PhD[3], J L Cascio[1] and W C Hinds, ScD[3]. [1]ECU, Greenville, NC, United States; [2]USC, LA, CA, United States and [3]UCLA, LA, CA, United States.

BACKGROUND: Exposure to particle (PM) pollution from vehicles is associated with cardiopulmonary (CP) health effects, but specifics are not understood. OBJECTIVE: Document acute PM effects in volunteers aged >60 riding on Los Angeles freeways; relate effects to specific PM components. METHODS: In a van with selectable filtration, volunteers (N=19, age 71±5 y, 53% male) rode for 2 h each in filtered and unfiltered air on a mostly-truck freeway and a mostly-car freeway. Double-blind health assessments included 24-h ambulatory ECG, blood biochemistry related to CP effects, blood pressure, lung function, and symptoms. RESULTS: Mean unfiltered PM count was 107000/ml (trucks) vs. 78000 (cars); mean PM(2.5) mass was 53 vs. 51 μg/m^3. Filtration reduced PM count >95% but did not remove gases. Most endpoints did not vary significantly by freeway/filter condition. Yet, atrial ectopic beat incidence during/after exposure decreased ~20% on average with filtered versus unfiltered air ($P<0.05$). Between-freeway differences were non-significant, but individual responses related to count ($P=0.01$) more than to mass ($P=0.07$). N-terminal pro B-type natriuretic peptide (NT pro-BNP) decreased ~30% on average in filtered vs. unfiltered air ($P<0.05$). CONCLUSIONS: In relatively healthy aged adults, exposures to PM on freeways were associated with increased atrial arrhythmia and biochemical evidence of increased CP stress. Increased NT pro-BNP suggests that increased intra-atrial pressure triggered atrial arrhythmia. Effects appeared to relate to the ultrafine PM fraction. Double-blind conditions rule out other traffic-related stresses or gases as causes. Because sustained arrhythmias (e.g. atrial fibrillation, AV nodal reentrant tachycardia) are triggered by premature atrial beats, traffic exposure might play a role in their occurrence - a risk that could be mitigated by filtering PM from the passenger cabin.